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(54) CD155 VARIANT IMMUNOMODULATORY PROTEINS AND USES THEREOF

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C12N 15/85	(2006.01)

(52) **U.S. Cl.**

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(57) **ABSTRACT**

Provided herein are immunomodulatory proteins comprising variant CD155 and nucleic acids encoding such proteins. The immunomodulatory proteins provide therapeutic utility for a variety of immunological and oncological conditions. Compositions and methods for making and using such proteins are provided.

Specification includes a Sequence Listing.

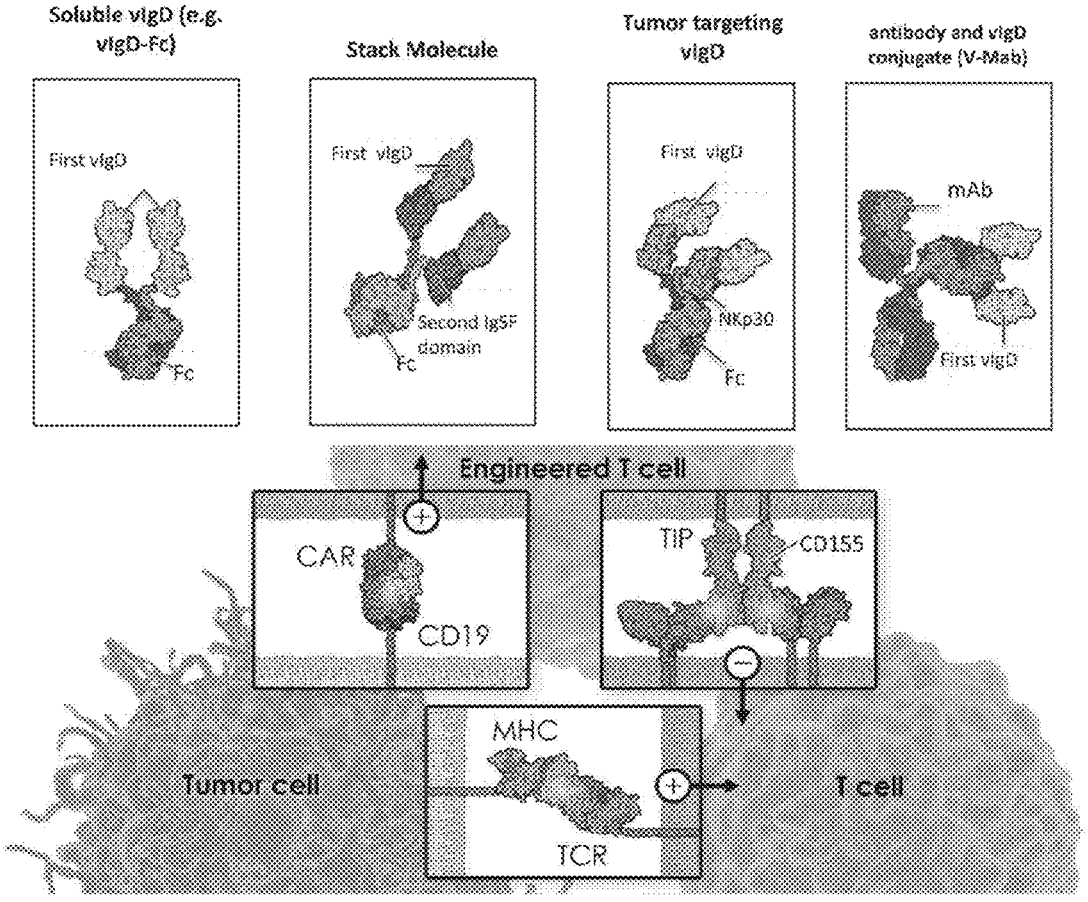
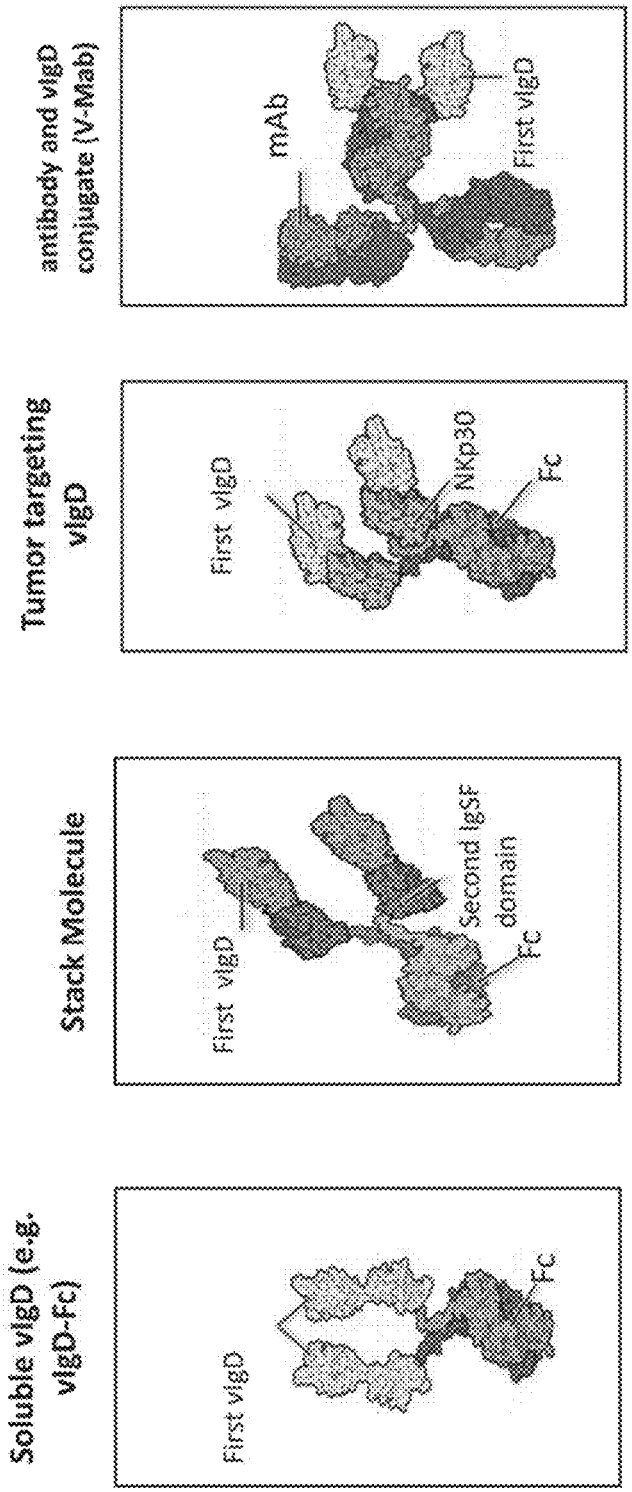


FIG. 1A


Soluble vIgD (e.g. vIgD-Fc)
First vIgD
Fc
Stack Molecule
First vIgD
Second IgSF domain
Fc
Tumor targeting vIgD
First vIgD
NKp30
Fc
antibody and vIgD conjugate (V-Mab)
mAb
First vIgD

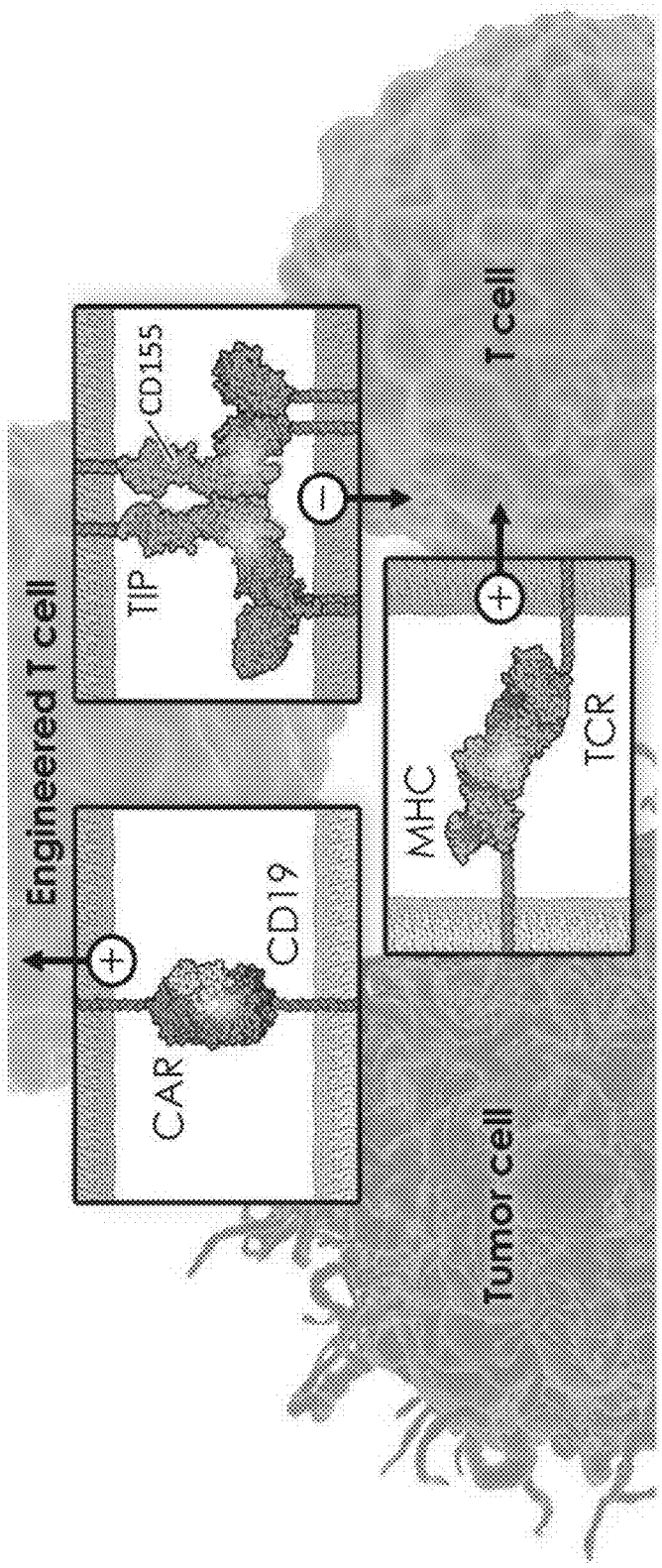

Engineered T cell
CAR
CD19
TIP
CD155
T cell
MHC
TCR
Tumor cell

FIG. 1B

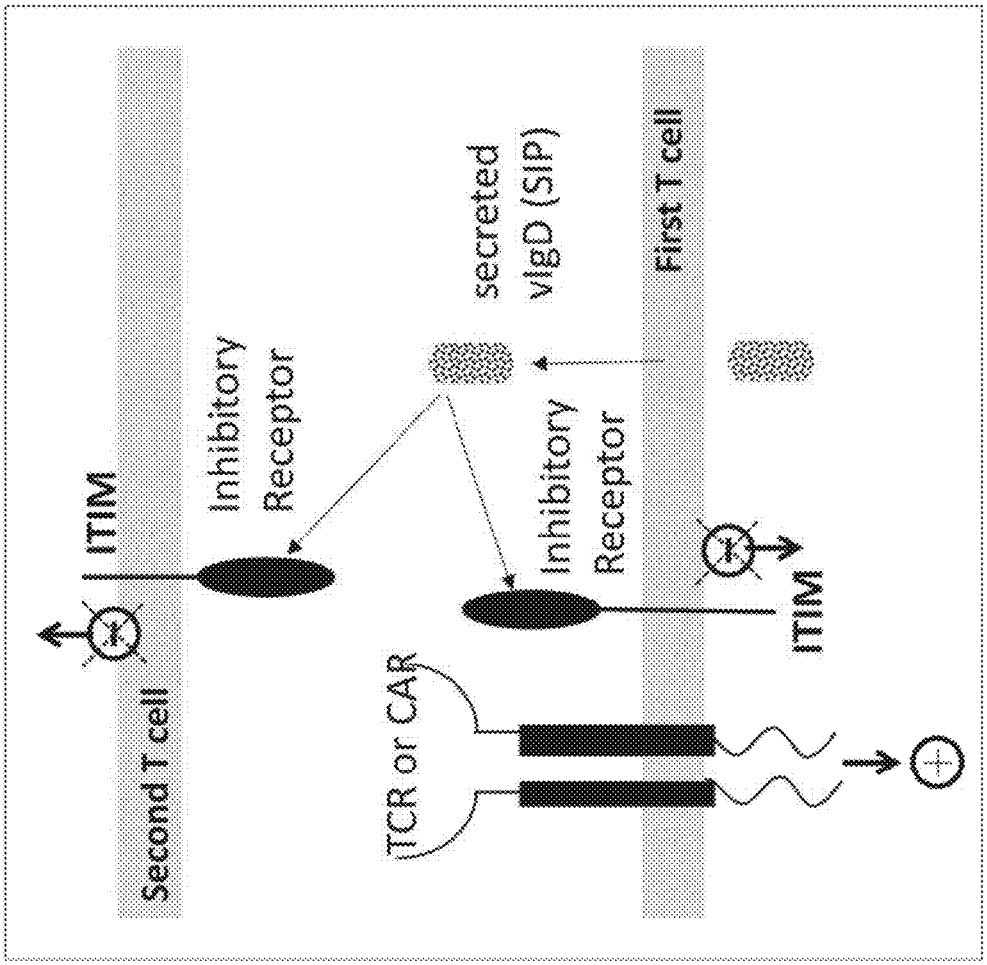

Second T cell
ITIM
Inhibitory Receptor
secreted VlgD (SIP)
First T cell
Inhibitory Receptor
ITIM
TCR or CAR

FIG. 1C

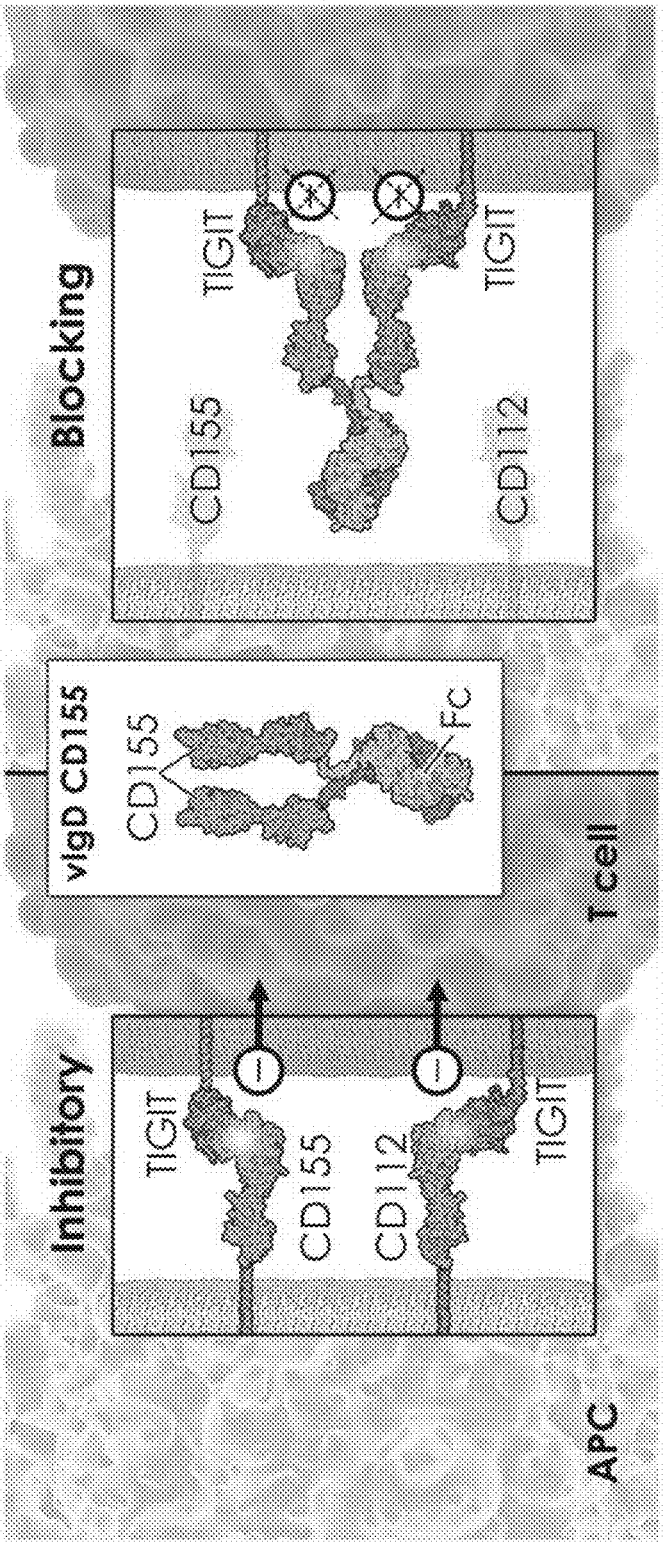

Inhibitory
TIGIT
CD155
CD112
TIGIT
vIgD CD155
CD155
Fc
Blocking
TIGIT
CD155
CD112
TIGIT
T cell
APC

FIG. 2

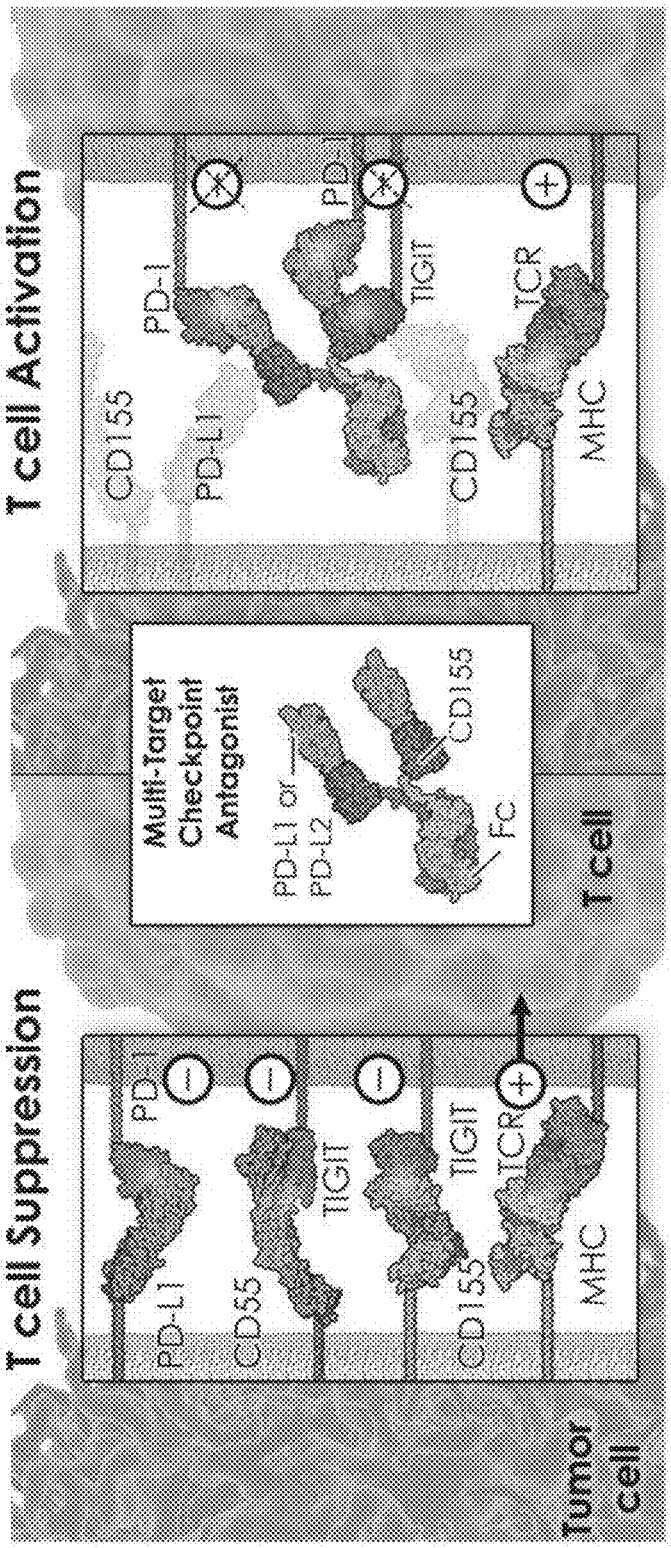

T cell Suppression
T cell Activation
Multi-Target Checkpoint Antagonist
PD-L1 or PD-L2
CD155
Fc
PD-1
PD-L1
CD55
TIGIT
CD155
TCR
MHC
T cell
Tumor cell

FIG. 3

FIG. 4

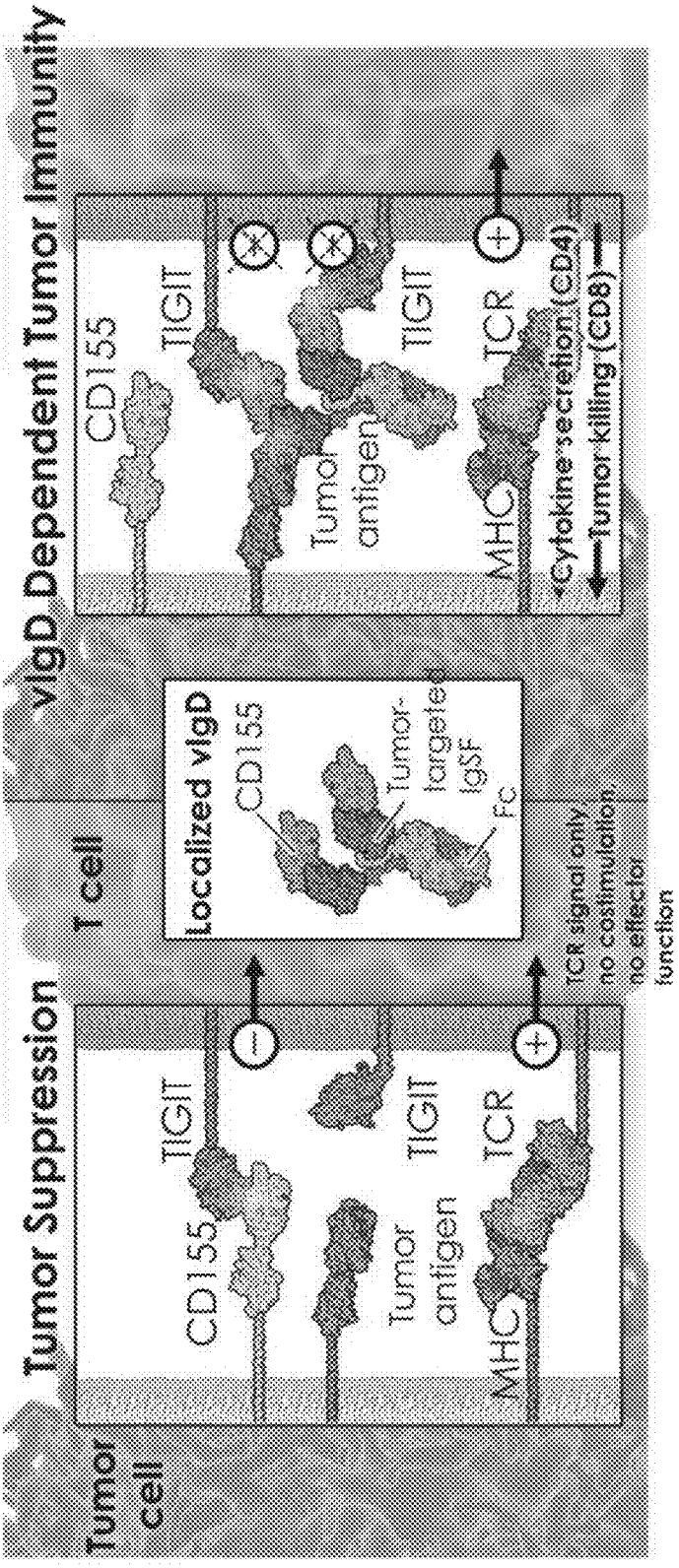
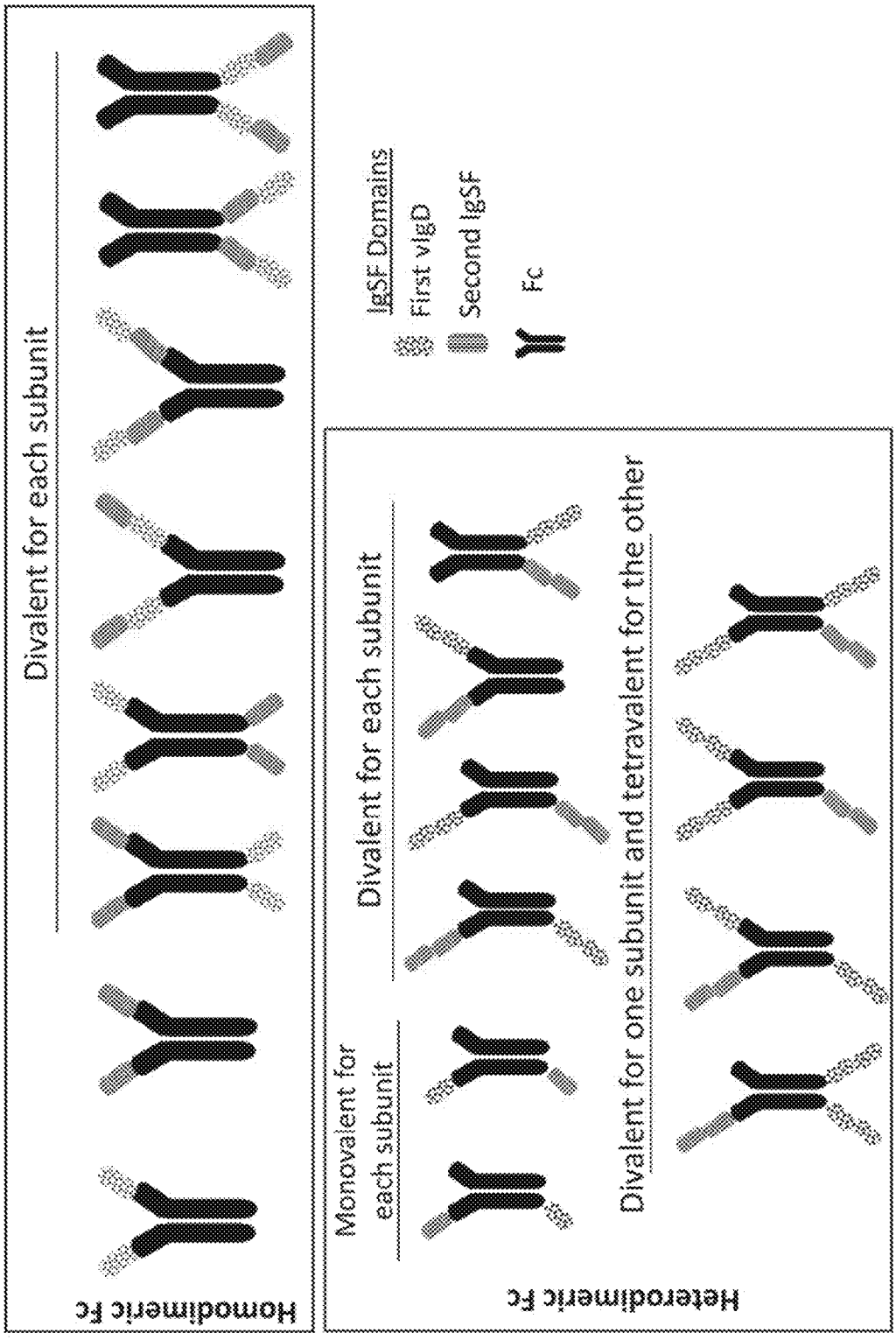

Tumor Suppression
vIgD Dependent Tumor Immunity
Tumor cell
T cell
CD155
TIGIT
Tumor antigen
MHC
TCR
Localized vIgD
CD155
Tumor-targeted IgSF Fc
TCR signal only
no costimulation
no effector function
Cytokine secretion (CD4)
Tumor killing (CD8)

FIG. 5A


Homodimeric Fc
Divalent for each subunit
Heterodimeric Fc
Monovalent for each subunit
Divalent for each subunit
Divalent for one subunit and tetravalent for the other
IgSF Domains
First vIgD
Second IgSF
Fc

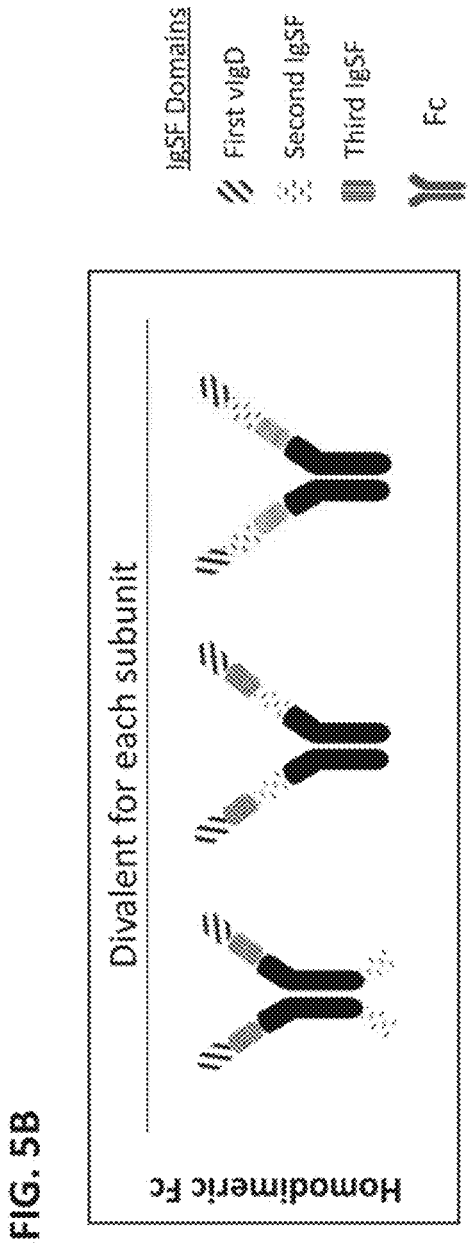
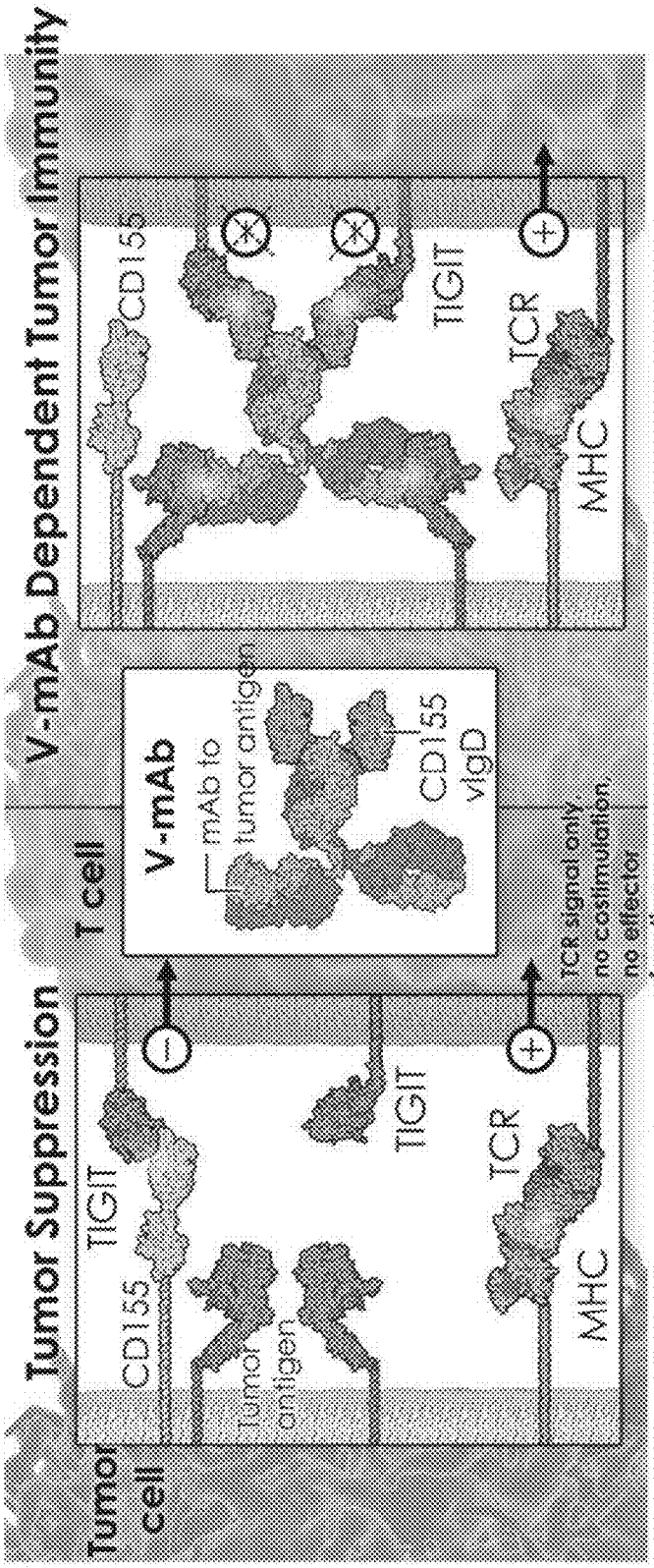

IgSF Domains
First vIgD
Second IgSF
Third IgSF
Fc
Homodimeric Fc
Divalent for each subunit

FIG. 5B

FIG. 6


Tumor Suppression
V-mAb Dependent Tumor Immunity
Tumor cell
T cell
TIGIT
CD155
Tumor antigen
TIGIT
TCR
MHC
V-mAb
mAb to tumor antigen
CD155 vIgD
TCR signal only
no costimulation
no effector function
CD155
TIGIT
TCR
MHC

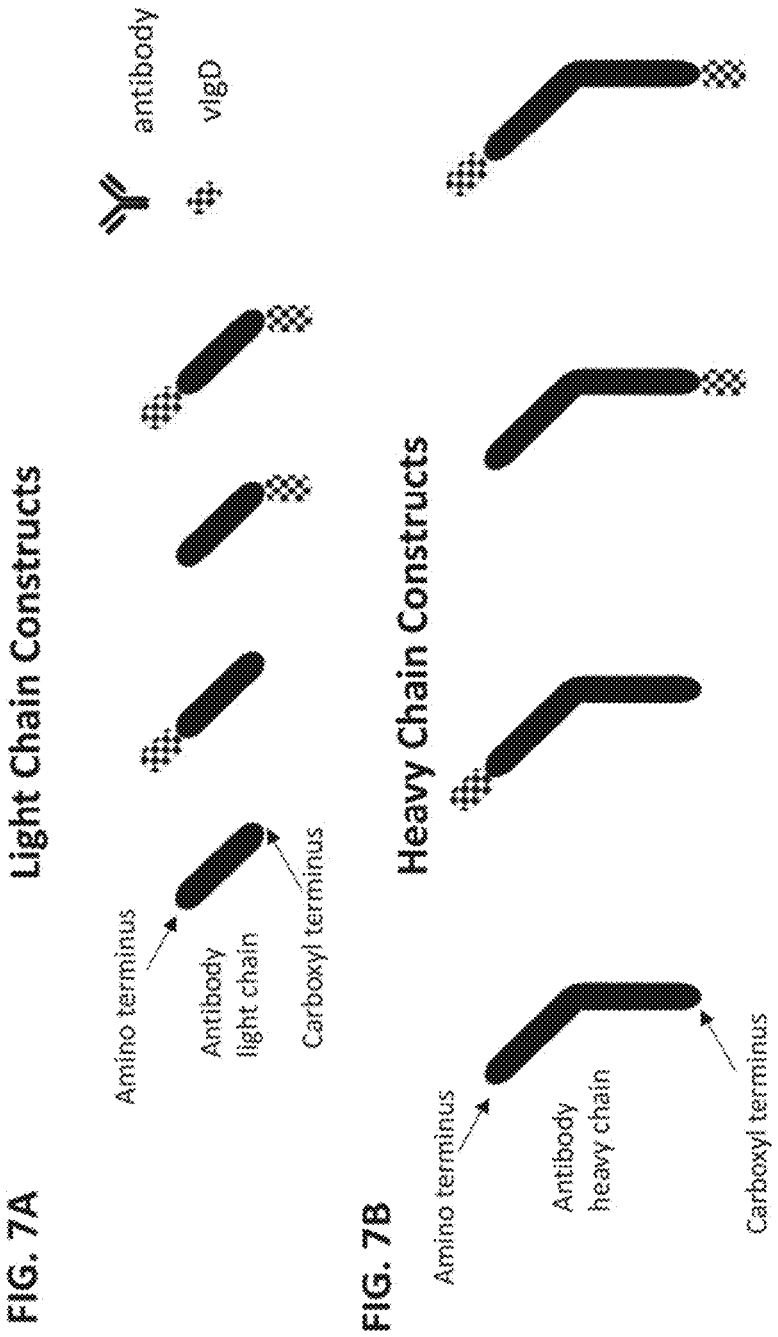

Light Chain Constructs
antibody
vIgD
Amino terminus
Antibody
light chain
Carboxyl terminus
Heavy Chain Constructs
Amino terminus
Antibody
heavy chain
Carboxyl terminus

FIG. 7A

FIG. 7B

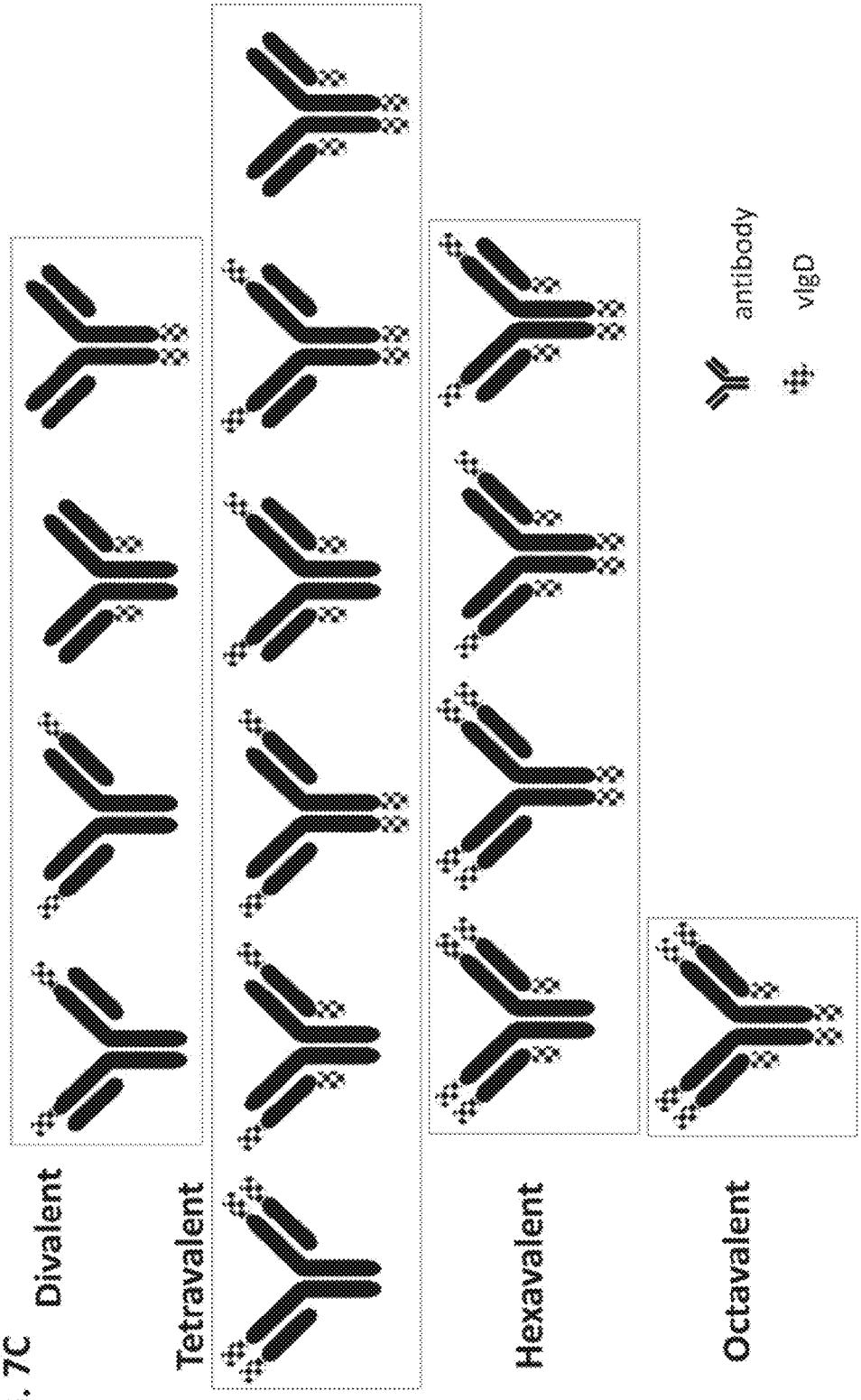

Divalent
Tetravalent
Hexavalent
Octavalent
antibody
vIgD

FIG. 7C

CD155 VARIANT IMMUNOMODULATORY PROTEINS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. application Ser. No. 16/320,981 filed on Jan. 25, 2019 which is a National Stage application under 35 U.S.C. § 371 of International Application No. PCT/US2017/044261, filed on Jul. 27, 2017, which claims priority from U.S. provisional application No. 62/367,822, filed Jul. 28, 2016, U.S. provisional application No. 62/394,696, filed Sep. 14, 2016, U.S. provisional application No. 62/410,839, filed Oct. 20, 2016, U.S. provisional application No. 62/472,558, filed Mar. 16, 2017, and U.S. provisional application No. 62/475,196, filed Mar. 22, 2017, the contents of each of which are hereby incorporated by reference in their entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in XML format. The Sequence Listing is provided as a file entitled 761612000510SEQLIST.xml, created Oct. 13, 2022 which is 2,910,218 bytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

FIELD

[0003] The present disclosure relates to therapeutic compositions for modulating immune response in the treatment of cancer and immunological diseases. In some aspects, the present disclosure relates to particular variants of CD155 that exhibit improved binding, such as improved binding affinity or selectivity, for one or more of the cognate binding partner proteins TIGIT, CD226 or CD96.

BACKGROUND

[0004] Modulation of the immune response by intervening in the processes that occur in the immunological synapse (IS) formed by and between antigen-presenting cells (APCs) or target cells and lymphocytes is of increasing medical interest. Mechanistically, cell surface proteins in the IS can involve the coordinated and often simultaneous interaction of multiple protein targets with a single protein to which they bind. IS interactions occur in close association with the junction of two cells, and a single protein in this structure can interact with both a protein on the same cell (cis) as well as a protein on the associated cell (trans), likely at the same time. Although therapeutics are known that can modulate the IS, improved therapeutics are needed. Provided are immunomodulatory proteins, including soluble proteins or transmembrane immunomodulatory proteins capable of being expressed on cells, that meet such needs.

SUMMARY

[0005] Provided herein are variant CD155 polypeptides. In some embodiments, the variant CD155 polypeptides comprise an IgV domain or a specific binding fragment thereof, an IgC domain or a specific binding fragment thereof, or both, wherein the variant CD155 polypeptide comprises one or more amino acid modifications in an unmodified CD155 or a specific binding fragment thereof corresponding to position(s) selected from 7, 8, 9, 10, 11, 12, 13, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 67, 68, 69, 70, 72, 73, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 87, 88, 89, 90, 91, 92, 94, 95, 96, 97, 98, 99, 100, 102, 104, 106, 107, 108, 110, 111, 112, 113, 114, 115, or 116 with reference to positions set forth in SEQ ID NO: 47. In some embodiments, the amino acid modifications comprise amino acid substitutions, deletions or insertions. In some embodiments, the unmodified CD155 is a mammalian CD155 or a specific binding fragment thereof. In some embodiments, the unmodified CD155 is a human CD155 or a specific binding fragment thereof. In some embodiments, the unmodified CD155 comprises (i) the sequence of amino acids set forth in SEQ ID NO: 47, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO: 47; or (iii) a portion thereof comprising an IgV domain or specific binding fragment thereof.

[0006] In some embodiments of any one of the variant CD155 polypeptides, the specific binding fragment of the IgV domain has a length of at least 50, 60, 70, 80, 90, 100, 110 or more amino acids; or the specific binding fragment of the IgV domain comprises a length that is at least 80% of the length of the IgV domain set forth as amino acids 24-139 of SEQ ID NO: 20. In some embodiments, the variant CD155 polypeptide comprises up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acid modifications, optionally amino acid substitutions, insertions and/or deletions. In some embodiments, the variant CD155 comprises a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 47 or a specific binding fragment thereof. In some embodiments, the variant CD155 polypeptide exhibits altered binding to the ectodomain of TIGIT, CD226 or CD96 compared to the unmodified CD155. In some embodiments, the variant CD155 polypeptide exhibits altered binding to the ectodomain of TIGIT or CD226 compared to the unmodified CD155. In some embodiments, the altered binding is altered binding affinity and/or altered binding selectivity.

[0007] In some embodiments of any one of the variant CD155 polypeptides, the one or more amino acid modifications are selected from G7E, D8G, V9A, V9D, V9I, V9L, V10F, V10G, V10I, V11A, V11E, V11M, Q12H, Q12K, Q12L, A13E, A13R, T15I, T15S, Q16H, P18C, P18F, P18H, P18L, P18S, P18T, P18Y, G19D, F20I, F20S, F20Y, L21S, L21M, G22S, D23A, D23G, D23N, D23Y, S24A, S24P, V25A, V25E, T26M, C29R, Y30C, Y30F, Y30H, Q32L, Q32R, V33M, P34S, N35D, N35F, N35S, M36I, M36R, M36T, E37G, E37P, E37S, E37V, V38A, V38G, T39A, T39S, H40Q, H40R, H40T, V41A, V41M, S42A, S42C, S42G, S42L, S42N, S42P, S42Q, S42T, S42V, S42W, L44P, L44V, T45A, T45G, T45I, T45S, T45Q, T45V, W46C, W46R, A47E, A47G, A47V, R48Q, H49L, H49Q, H49R, G50S, E51G, E51K, E51V, S52A, S52E, S52G, S52K, S52L, S52M, S52P, S52Q, S52R, S52T, S52W, G53R, S54C, S54G, S54H, S54N, S54R, M55I, M55L, M55V, A56V, V57A, V57L, V57T, F58L, F58Y, H59E, H59N, N59R, Q60H, Q60K, Q60P, Q60R, T61A, T61G, T61K, T61M, T61R, T61S, Q62F, Q62H, Q62K, Q62L, Q62M, Q62R, Q62Y, P64S, S65A, S65C, S65G, S65D, S65T, S65Y, S65H, S65N, S65T, S65W, S67A, S67E, S67G, S67H, S67L, S67T, S67V, S67W, E68D, E68G, S69L, S69P, K70E,

K70R, K70Q, L72Q, E73D, E73G, E73R, V75A, V75L, A76E, A76G, A76T, A77T, A77V, R78G, R78K, R78S, L79P, L79Q, L79V, G80D, G80S, A81E, A81P, A81T, A81V, E82D, E82G, L83P, L83Q, R84W, N85D, N85Y, N87T, L88P, R89K, M90I, M90L, M90V, F91S, F91T, F91P, G92A, G92E, G92W, R94H, V95A, E96D, D97G, E98D, E98S, G99D, G99Y, N100Y, T102S, L104E, L104M, L104N, L104P, L104Q, L104T, L104Y, V106A, V1061, V106L, T107A, T107L, T107M, T107S, T107V, F108H, F108L, F108Y, Q110R, G111D, G111R, S112I, S112N, S112V, R113G, R113W, S114N, S114T, V115A, V115M, D116G, or D116N, or a conservative amino acid substitution thereof. In some embodiments, the one or more amino acid modifications are selected from P18S/P64S/F91S, P18S/F91S/L104P, P18L/L79V/F91S, P18S/F91S, P18T/F91S, P18T/S42P/F91S, G7E/P18T/Y30C/F91S, P18T/F91S/G111D, P18S/F91P, P18T/F91S/F108L, P18S/F91S, P18T/T45A/F91S, P18T/F91S/R94H, P18S/Y30C/F91S, A81V/L83P, A13E/P18S/A56V/F91S, P18T/F91S/V115A, P18T/Q60K, S52M, T45Q/S52L/L104E/G111R, S42G, Q62F, S52Q, S42A/L104Q/G111R, S42A/S52Q/L104Q/G111R, S52W/L104E, S42C, S52W, S52M/L104Q, S42L/S52L/Q62F/L104Q, S42W, S42Q, S52L, S52R, L104E, GI IR, S52E, Q62Y, T45Q/S52M/L104E, S42N/L104Q/G111R, S52M/V57L, S42N/S52Q/Q62F, S42A/S52L/L104E/G111R, S42W/S52Q/V57L/Q62Y, L104Q, S42L/S52Q/L104E, S42C/S52L, S42W/S52R/Q62Y/L104Q, T45Q/S52R/L104E, S52R/Q62F/L104Q/G111R, T45Q/S52L/V57L/L104E, S52M/Q62Y, Q62F/L104E/G111R, T45Q/S52Q, S52L/L104E, S42V/S52E, T45Q/S52R/G111R, S42G/S52Q/L104E/G111R, S42N/S52E/V57L/L104E, S42C/S52M/Q62F, S42L, S42A, S42G/S52L/Q62F/L104Q, S42N, P18T/S65A/S67V/F91S, P18F/T39A/T45Q/T61R/S65N/S67L/E73G/R78G, P18T/T45Q/T61R/S65N/S67L, P18F/S65A/S67V/F91S, P18F/T45Q/T61R/S65N/S67L/F91S/L104P, P18S/L79P/L104M, P18S/L104M, L79P/L104M, P18T/T45Q/L79P, P18T/T45Q/T61R/S65H/S67H, P18T/A81E, P18S/D23Y/E37P/S52G/Q62M/G80S/A81P/G99Y/S 112N, A13R/D23Y/E37P/S42P/Q62Y/A81E, A13R/D23Y/E37P/G99Y/S112N, A13R/D23Y/E37P/Q62M/A77V/G80S/A81P/G99Y, P18L/E37S/Q62M/G80S/A81P/G99Y/S112N, P18S/L104T, P18S/Q62H/L79Q/F91S, T45Q/S52K/Q62F/L104Q/G111R, T45Q/S52Q/Q62Y/L104Q/G111R, T45Q/S52Q/Q62Y/L104E/G111R, V57A/T61M/S65W/S67A/E96D/L104T, P18L/V57T/T61S/S65Y/S67A/L104T, P18T/T45Q, P18L/V57A/T61M/S65W/S67A/L104T, T61M/S65W/S67A/L104T, P18S/V41A/S42G/T45G/L104N, P18H/S42G/T45I/S52T/G53R/S54H/V57L/H59E/T61S/S65D/E68G/L104N, P18S/S42G/T45V/F58L/S67W/L104N, P18S/T45I/L104N, P18S/S42G/T45G/L104N/V106A, P18H/H40R/S42G/T45I/S52T/G53R/S54H/V57L/H59E/T61S/S65D/E68G/L104Y/V106L/F108H, E37V/S42G/T45G/L104N, P18S/T45Q/L79P/L104T, P18L/Q62R, A13R/D23Y/E37P/S42L/S52G/Q62Y/A81E, P18L/H49R/L104T/D116N, A13R/D23Y/E37P/Q62M/G80S/A81P/L104T, S65T/L104T, A13R/D23Y/E37P/S52G/V57A/Q62M/K70E/L104T, P18L/A47V/Q62Y/E73D/L104T, H40T/V411M/A47V/S52Q/Q62L/S65T/E73R/D97G/E98S/L104T/D116N, P18L/S42P/T45Q/T61G/S65H/S67E/L104T/D116N, P18S/H40T/V41M/A47V/S52Q/Q62L/S65T/E73R/L104M/V106A, H40T/V41M/A47V/S52Q/Q62L/S65T/E68G/E73R/D97G/E98S/L104T, T45Q/S52E/L104E, T45Q/S52E/Q62F/L104E, P18F/T26M/L44V/Q62K/L79P/F91S/104M/G111D, P18S/T45S/T61K/S65W/S67A/F91S/G111R, P18S/L79P/L104M/T107M, P18S/S65W/S67A/M90V/V95A/L104Q/G111R, P18S/A47G/L79P/F91S/L104M/T107A/R113W, P18T/D23G/S24A/N35D/H49L/L79P/F91S/L104M/G111R, V9L/P18S/Q60R/V75L/L79P/R89K/F91S/L104E/G111R, P18S/H49R/E73D/L79P/N85D/F91S/V95A/L104M/G111R, V11A/P18S/L79P/F91S/L104M/G111R, V11A/P18S/S54R/Q60P/Q62K/L79P/N85D/F91S/T107M, P18T/S52P/S65A/S67V/L79P/F91S/L104M/G111R, P18T/M36T/L79P/F91S/G111R, D8G/P18S/M36I/V38A/H49Q/A76E/F91S/L104M/T107A/R113W, P18S/S52P/S65A/S67V/L79P/F91S/L104M/T107S/R113W, T15I/P18T/L79P/F91S/L104M/G111R, P18F/T26M/L44V/Q62K/L79P/E82D/F91S/L104M/G111D, P18T/E37G/G53R/Q62K/L79P/F91S/E98D/L104M/T107M, P18L/K70E/L79P/F91S/V95A/G111R, V9I/Q12K/P18F/S65A/S67V/L79P/L104T/G111R/S112I, P18F/S65A/S67V/F91S/L104M/G111R, V9I/V10I/P18S/F20S/T45A/L79P/F91S/L104M/F108Y/G111R/S112V, V9L/P18L/L79P/M90I/F91S/T102S/L104M/G111R, P18C/T26M/L44V/M55I/Q62K/L79P/F91S/L104M/T107M, V9I/P18T/D23G/L79P/F91S/G111R, P18F/L79P/M90L/F91S/V95A/L104M/G111R, P18T/M36T/S65A/S67E/L79Q/A81T/F91S/G111R, V9L/P18T/Q62R/L79P/F91S/L104M/G111R, P18S/S65W/S67A/L104Q/G111R, P18T/G19D/M36T/S54N/L79P/L83Q/F91S/T107M/F108Y, V9L/P18L/M55V/S69L/L79P/A81E/F91S/T107M, P18F/H40Q/T61K/Q62K/L79P/F91S/L104M/T107V, P18S/Q32R/Q62K/R78G/L79P/F91S/T107A/R113W, Q12H/P18T/L21S/G22S/V57A/Q62R/L79P/F91S/T107M, V9I/P18S/S24P/H49Q/F58Y/Q60R/Q62K/L79P/F91S/T107M, P18T/W46C/H49R/S65A/S67V/A76T/L79P/S87T/L104M, P18S/S42T/E51G/L79P/F91S/G92W/T107M, V10F/T15S/P18L/R48Q/L79P/F91S/T107M/V115M, P18S/L21M/Y30F/N35D/R84W/F91S/T107M/D116G, P18F/E51V/S54G/Q60R/L79Q/E82G/S87T/M901/F91S/G92R/T107M, Q16H/P18F/F91S/T107M, P18T/D23G/Q60R/S67L/L79P/F91S/T107M/V115A, D8G/V9I/V11A/P18T/T26M/S52P/L79P/F91S/G92A/T107L/V115A, V9I/P18F/A47E/G50S/E68G/L79P/F91S/T107M, P18S/M55I/Q62K/S69P/L79P/F91S/T107M, P18T/T39S/S52P/S54R/L79P/F91S/T107M, P18S/D23N/L79P/F91S/T107M/S114N, P18S/P34S/E51V/L79P/F91S/G111R, P18S/H59N/V75A/L79P/A81T/F91S/L104M/T107M, P18S/W46R/E68D/L79P/F91S/T107M/R113G, V9L/P18F/T45A/S65A/S67V/R78K/L79V/F91S/T107M/S114T, P18T/M55L/T61R/L79P/F91S/V106I/T107M, T15I/P18S/V33M/N35F/T39S/M55L/R78S/L79P/F91S/T107M, P18S/Q62K/K70E/L79P/F91S/G92E/R113W, P18F/F20I/T26M/A47V/E51K/L79P/F91S, P18T/D23A/Q60H/L79P/M90V/F91S/T107M, P18S/D23G/C29R/N35D/E37G/M55I/Q62K/S65A/S67G/R78G/L79P/F91S/L104M/T107M/Q110R, A13E/P18S/M36R/Q62K/S67T/L79P/N85D/F91S/T107M, V9I/P18T/H49R/L79P/N85D/F91S/L104T/T107M, V9A/P18F/T61S/Q62L/L79P/F91S/G111R, D8E/P18T/T61A/L79P/F91S/T107M, P18S/V41A/H49R/S54C/L79S/N85Y/L88P/F91S/L104M/T107M, V 11E/P18H/F20Y/V25E/N35S/H49R/L79P/F91S/T107M/G111R, V11A/P18F/D23A/L79P/G80D/V95A/T107M, P18S/K70R/L79P/F91S/G111R, V9L/V11M/P18S/N35S/S54G/Q62K/L79P/L104M/T107M/V115M, V9L/P18Y/V25A/V38G/M55V/A77T/L79P/M90I/F91S/L104M, V10G/P18T/L72Q/L79P/F91S/T107M, P18S/H59R/A76G/R78S/L79P, V9A/P18S/M36T/S65G/L79P/F91S/L104T/G111R/S112I, P18T/S52A/V57A/Q60R/

Q62K/S65C/L79P/F91T/N100Y/T107M, V11A/P18F/N35D/A47E/Q62K/L79P/F91S/G99D/T107M/S114N, V11A/P18T/N35S/L79P/S87T/F91S, V9D/V11M/Q12L/P18S/E37V/M55I/Q60R/K70Q/L79P/F91S/L104M/T107M, or T15S/P18S/Y30H/Q32L/Q62R/L79P/F91S/T107M.

[0008] In some embodiments of any one of the variant CD155 polypeptides, the variant CD155 polypeptide comprises one IgC domain or two IgC domains or a specific fragment thereof.

[0009] In some embodiments of any one of the variant CD155 polypeptides, the sequence of amino acids set forth in any of SEQ ID NOS: 59-80, 178-274, 1230-1252, 1269, and 1610-1655 or a specific binding fragment thereof, or a sequence of amino acids that exhibits at least 95% sequence identity to any of SEQ ID NOS: 59-80, 178-274, 1230-1252, 1269, and 1610-1655 or a specific binding fragment thereof and that contains the one or more of the amino acid substitutions.

[0010] In some embodiments of any one of the variant CD155 polypeptides, the unmodified CD155 comprises an IgV domain or specific binding fragment thereof comprising (i) the sequence of amino acids set forth in SEQ ID NO: 58 or 155, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO: 58 or 155; or (iii) a portion thereof comprising a specific binding fragment thereof. In some embodiments of any one of the variant CD155 polypeptides, the variant CD155 polypeptide comprises the IgV domain or a specific binding fragment thereof. In some embodiments, the IgV domain or specific binding fragment thereof is the only CD155 portion of the variant CD155 polypeptide.

[0011] In some embodiments of any one of the variant CD155 polypeptides, the variant CD155 polypeptide comprises the sequence of amino acids set forth in any of SEQ ID NOS: 81-102, 156-177, 275-468, 1184-1229, 1270-1271, 1656-1747 or a specific binding fragment thereof, a sequence of amino acids that exhibits at least 95% sequence identity to any of SEQ ID NOS: 81-102, 156-177, 275-468, 1184-1229, 1270-1271, 1656-1747 or a specific binding fragment thereof and that contains the one or more of the amino acid substitutions.

[0012] In some embodiments of any one of the variant CD155 polypeptides, the variant CD155 polypeptide specifically binds to the ectodomain of one or more of TIGIT, CD226 or CD96 with increased affinity compared to the unmodified CD155. In some embodiments, the variant CD155 polypeptide specifically binds to the ectodomain of TIGIT or CD226 with increased affinity compared to the unmodified CD155. In some embodiments, the variant CD155 polypeptide specifically binds to the ectodomain of TIGIT and the ectodomain of CD226 each with increased affinity compared to the unmodified CD155. In some embodiments, the variant CD155 polypeptide specifically binds to the ectodomain of one or more of TIGIT, CD226 or CD96 with increased affinity and specifically binds to the ectodomain of one or more of the other of TIGIT, CD226 or CD96 with decreased affinity compared to the unmodified CD155. In some embodiments, the variant CD155 polypeptide specifically binds to the ectodomain of TIGIT with increased affinity and specifically binds to the ectodomain of CD226 with decreased affinity compared to the unmodified CD155. In some embodiments, the variant polypeptide specifically binds to the ectodomain of TIGIT with increased selectivity compared to the unmodified CD155. In some embodiments, the increased selectivity comprises a greater ratio of binding of the variant polypeptide for TIGIT versus CD226 compared to the ratio of binding of the unmodified CD155 polypeptide for TIGIT versus CD226. In some embodiments, the ratio is greater by at least or at least about 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold or more. In some embodiments, the variant CD155 polypeptide specifically binds to the ectodomain of CD226 with increased affinity and specifically binds to the ectodomain of TIGIT with decreased affinity compared to the unmodified CD155.

[0013] In some embodiments of any one of the variant CD155 polypeptides, the TIGIT is a human TIGIT. In some embodiments, the CD226 is a human CD226. In some embodiments, the CD96 is a human CD96.

[0014] In some embodiments of any one of the variant CD155 polypeptides, the binding activity is altered (increased or decreased) more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold 40-fold or 50-fold compared to the unmodified CD155.

[0015] In some embodiments of any one of the variant CD155 polypeptides, the variant CD155 polypeptide is a soluble protein.

[0016] In some embodiments of any one of the variant CD155 polypeptides, the variant CD155 polypeptide is linked to a multimerization domain. In some embodiments, the multimerization domain is an Fc domain or a variant thereof with reduced effector function.

[0017] In some embodiments of any one of the variant CD155 polypeptides, the variant CD155 polypeptide is linked to a moiety that increases biological half-life of the polypeptide. In some embodiments, the variant CD155 polypeptide is linked to an Fc domain or a variant thereof with reduced effector function. In some embodiments, the Fc domain is mammalian, optionally human; or the variant Fc domain comprises one or more amino acid modifications compared to an unmodified Fc domain that is mammalian, optionally human. In some embodiments, the Fc domain or variant thereof comprises the sequence of amino acids set forth in SEQ ID NO: 56 or SEQ ID NO: 57 or a sequence of amino acids that exhibits at least 85% sequence identity to SEQ ID NO: 56 or SEQ ID NO: 57. In some embodiments, the Fc domain comprises one or more amino acid modifications selected from among E233P, L234A, L234V, L235A, L235E, G236del, G237A, S267K, N297G, V302C, and K447del, each by EU numbering. In some embodiments, the Fe domain comprises the amino acid modification C220S by EU numbering. In some embodiments, the variant CD155 polypeptide is linked to the multimerization domain or Fc indirectly via a linker, optionally a G4S linker.

[0018] In some embodiments of any one of the variant CD155 polypeptides, the variant CD155 polypeptide is a transmembrane immunomodulatory protein that further comprises a transmembrane domain linked to the extracellular domain (ECD) of specific binding fragment thereof of the variant CD155 polypeptide. In some embodiments, the transmembrane domain comprises the sequence of amino acids set forth as residues 344-367 of SEQ ID NO: 20 or a functional variant thereof that exhibits at least 85% sequence identity to residues 344-367 of SEQ ID NO: 20. In some embodiments, the variant CD155 polypeptide further comprises a cytoplasmic signaling domain linked to the trans-

membrane domain. In some embodiments, the cytoplasmic signaling domain comprises the sequence of amino acids set forth as residues 368-417 of SEQ ID NO: 20 or a functional variant thereof that exhibits at least 85% sequence identity to residues 368-417 of SEQ ID NO: 20.

[0019] In some of any of the provided embodiments, the variant CD155 polypeptide modulates a response of an immune cell, such as a T cell. In some embodiments, the response, e.g. T cell response, is increased or is decreased. In some embodiments of any one of the variant CD155 polypeptides, the variant CD155 increases IFN-gamma (interferon-gamma) expression relative to the unmodified CD155 in an in vitro primary T-cell assay. In some embodiments of any one of the variant CD155 polypeptides described herein, the variant CD155 polypeptide increases T cell signaling relative to the unmodified CD155, such as determined using a reporter assay involving a T cell (e.g. Jurkat) engineered with a reporter (e.g. luciferase) operably connected to an IL-2 promoter. In some embodiments of any one of the variant CD155 polypeptides described herein, the variant CD155 polypeptide decreases T cell signaling relative to the unmodified CD155, such as determined using a reporter assay involving a T cell (e.g. Jurkat) engineered with a reporter (e.g. luciferase) operably connected to an IL-2 promoter. In some of any such embodiments, the variant CD155 polypeptide is provided in any of a variety of formats, such as soluble or immobilized (e.g. plate-bound).

[0020] In some embodiments of any one of the variant CD155 polypeptides, the variant CD155 decreases IFN-gamma (interferon-gamma) expression relative to the unmodified CD155 in an in vitro primary T-cell assay. In some embodiments of any one of the variant CD155 polypeptides, the variant CD155 polypeptide is deglycosylated. In some embodiments of any one of the variant CD155 polypeptides described herein, the variant CD155 polypeptide increases T cell signaling relative to the unmodified CD155, such as determined using a reporter assay involving a T cell (e.g. Jurkat) engineered with a reporter (e.g. luciferase) operably connected to an IL-2 promoter. In some embodiments of any one of the variant CD155 polypeptides described herein, the variant CD155 polypeptide decreases T cell signaling relative to the unmodified CD155, such as determined using a reporter assay involving a T cell (e.g. Jurkat) engineered with a reporter (e.g. luciferase) operably connected to an IL-2 promoter. In some of any such embodiments, the variant CD155 polypeptide is provided in any of a variety of formats, such as soluble or immobilized (e.g. plate-bound).

[0021] In some embodiments, provided herein is an immunomodulatory polypeptide comprising the variant CD155 according to any one of the provided embodiments and a second polypeptide comprising an immunoglobulin superfamily (IgSF) domain of an second polypeptide comprising an immunoglobulin superfamily (IgSF) domain of an IgSF family member or an affinity-modified IgSF domain thereof, said affinity-modified IgSF domain comprising one or more amino acid modifications compared to the unmodified or wild-type IgSF domain of the IgSF family member. In some cases, the variant CD155 polypeptide and the second polypeptide are linked directly or are linked indirectly via a linker. In some embodiments, the IgSF domain is affinity modified and exhibits altered binding to one or more of its cognate binding partner(s) compared to the unmodified or wild-type IgSF domain of the IgSF family member. In some embodiments, the IgSF domain exhibits increased binding to one or more of its cognate binding partner(s) compared to the unmodified or wild-type IgSF domain. In some embodiments, the variant CD155 polypeptide is a first variant CD155 polypeptide and the IgSF domain of the second polypeptide is an IgSF domain from a second variant CD155 polypeptide according to any one of the embodiments described herein, wherein the first and second variant CD155 polypeptides are the same or different. In some embodiments, the variant CD155 polypeptide is capable of specifically binding to TIGIT or CD226 and the IgSF domain is capable of binding to a cognate binding partner other than one specifically bound by the variant CD155 polypeptide. In some embodiments, the IgSF domain is from a member of the B7 family.

[0022] In some embodiments, the IgSF domain binds to a ligand expressed on a tumor or is an inflammatory-localizing moiety that binds to a ligand expressed on a cell or tissue associated with an inflammatory environment. In some embodiments, the ligand is B7H6. In some embodiments, the IgSF domain is from NKp30.

[0023] In some embodiments, the IgSF domain of the second polypeptide is an IgSF domain of a ligand that binds to an inhibitory receptor, or is an affinity-modified IgSF domain thereof. In some embodiments, the IgSF domain of the second polypeptide is an affinity-modified IgSF domain and the affinity-modified IgSF domain exhibits increased binding affinity and/or binding selectivity for the inhibitory receptor compared to binding of the unmodified IgSF domain to the inhibitory receptor. In some embodiments, the inhibitory receptor is TIGIT, CD112R, CTLA-4 or PD-1; or the ligand of the inhibitory receptor is CD112, CD80, PD-L1 or PD-L2.

[0024] In some embodiments, the second polypeptide is selected from: (i) a variant CD80 polypeptide comprising an IgSF domain of any of SEQ ID NOS set forth in Table 3, optionally any of the SEQ ID NOs: 896-928, 930-968, 970-1002, 1004-1042, 1044-1116; (ii) a variant PD-L1 polypeptide comprising an IgSF domain of any of SEQ ID NOS set forth in Table 4, optionally any of the SEQ ID NOs: 470-664, 1753-1755, 1757-2031; (iii) a variant PD-L2 polypeptide comprising an IgSF of any of SEQ ID NOS set forth in Table 5, optionally any of the SEQ ID NOs: 31, 667-717, 719-725, 727-794, 796-870, 872-895; (iv) a variant CD112 polypeptide comprising an IgSF domain set forth in any of SEQ ID NOS: 1273-1366, 1368-1609, (v) a sequence of amino acids that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 95%, 97%, 98%, 99% or more sequence identity to any of the SEQ ID NOS in (i)-(iii) and that comprises the amino acid modifications, optionally amino acid substitutions, insertions and/or deletions; or (vi) a specific binding fragment of any of (i)-(v). In some embodiments, the immunomodulatory protein further contains a third polypeptide containing an IgSF domain of an IgSF family member or an affinity-modified IgSF domain thereof, said affinity-modified IgSF domain containing one or more amino acid modifications compared to the unmodified or wild-type IgSF domain of the IgSF family member. In some aspects, the third polypeptide is the same as the first and/or second polypeptide; or the third polypeptide is different from the first and/or second polypeptide. In some embodiments, the third polypeptide is selected from: (i) a variant CD80 polypeptide comprising an IgSF domain set forth in any of SEQ ID NOS: 896-928, 930-968, 970-1002, 1004-1042,

1044-1116; (ii) a variant PD-L1 polypeptide comprising an IgSF domain set forth in any of SEQ ID NOS: 470-664, 1753-1755, 1757-2031; (iii) a variant PD-L2 polypeptide comprising an IgSF domain set forth in any of SEQ ID NOS: 667-717, 719-725, 727-794, 796-870, 872-895; (iv) a variant CD112 polypeptide comprising an IgSF domain set for in any of SEQ ID NOS: 1273-1366, 1368-1609; (v) a sequence of amino acids that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 95%, 97%, 98%, 99% or more sequence identity to any of the SEQ ID NOS in (i)-(iv) and that comprises the amino acid modifications, optionally amino acid substitutions, insertions and/or deletions; or (vi) a specific binding fragment of any of (i)-(v).

[0025] In some embodiments, the IgSF domain or affinity-modified IgSF domain thereof, optionally of the second or third polypeptide is or comprises an IgV domain. In some embodiments, the variant CD155 polypeptide is or comprises an IgV domain.

[0026] In some of any such embodiments, the immunomodulatory protein further contains at least one additional polypeptide containing an IgSF domain of an IgSF family member or an affinity-modified IgSF domain thereof, said affinity-modified IgSF domain containing one or more amino acid modifications compared to the unmodified or wild-type IgSF domain of the IgSF family member.

[0027] In some embodiments according to any one of the immunomodulatory proteins, the immunomodulatory protein is linked to a multimerization domain. In some embodiments, the immunomodulatory protein further comprises a multimerization domain linked to at least one of the variant CD155 polypeptide or the second polypeptide. In some embodiments, the immunomodulatory protein further contains a multimerization domain linked to at least one of the variant CD155 polypeptide, the second polypeptide and/or the third polypeptide. In some embodiments, the multimerization domain is an Fc domain or a variant thereof with reduced effector function. In some embodiments, the immunomodulatory protein is linked to an Fc domain or a variant thereof with reduced effector function. In some embodiments, the Fc domain or variant thereof comprises the sequence of amino acids set forth in SEQ ID NO: 56 or SEQ ID NO: 57 or a sequence of amino acids that exhibits at least 85% sequence identity to SEQ ID NO: 56 or SEQ ID NO: 57. In some embodiments, the multimerization domain promotes heterodimer formation.

[0028] Provided herein is an immunomodulatory protein containing a first variant CD155 polypeptide provided herein in which the multimerization domain is a first multimerization domain and a second variant CD155 polypeptide provided in which the multimerization domain is a second multimerization domain, wherein the first and second multimerization domains interact to form a multimer containing the first and second variant CD155 polypeptide. In some embodiments, the multimerization domain is a first multimerization domain and interacts with a second multimerization domain to form a multimer containing the immunomodulatory protein. In some cases, the immunomodulatory protein is a first immunomodulatory protein and a second immunomodulatory protein is linked directly or indirectly via a linker to the second multimerization domain, wherein the multimer comprises the first and second immunomodulatory protein. In some embodiments, the second immunomodulatory protein is an immunomodulatory protein according to any of the embodiments provided.

[0029] In some embodiments, the multimer is a dimer. In some cases, the immunomodulatory protein is a homodimer. In some aspects, the immunomodulatory protein is a heterodimer.

[0030] In some of any such embodiments, the first and/or second multimerization domain is an Fc domain or a variant thereof with reduced effector function. In some embodiments, the first and second multimerization domain is the same or different.

[0031] In some embodiments, provided herein is a conjugate comprising a variant CD155 according to any one of the provided embodiments, or an immunomodulatory protein according to any one of the provided embodiments, linked to a moiety. In some embodiments, the moiety is a targeting moiety that specifically binds to a molecule on the surface of a cell. In some embodiments, the targeting moiety specifically binds to a molecule on the surface of an immune cell. In some embodiments, the immune cell is an antigen presenting cell or a lymphocyte. In some embodiments, the targeting moiety binds to a molecule on the surface of a tumor. In some embodiments, the moiety is a protein, a peptide, nucleic acid, small molecule or nanoparticle. In some embodiments, the moiety is an antibody or antigen-binding fragment. In some embodiments, the conjugate is divalent, tetravalent, hexavalent or octavalent.

[0032] In some embodiments, provided herein is a nucleic acid molecule encoding a variant CD155 polypeptide according to any one of the provided embodiments or an immunomodulatory protein according to any one of the provided embodiments. In some embodiments, the nucleic acid molecule is a synthetic nucleic acid. In some embodiments, the nucleic acid is cDNA.

[0033] In some embodiments, provided herein is a vector comprising the nucleic acid of any one of the provided embodiments. In some embodiments, the vector is an expression vector. In some embodiments, the vector is a mammalian expression vector or a viral vector.

[0034] In some embodiments, provided herein is a cell comprising the vector according to any one of the provided embodiments. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a human cell.

[0035] In some embodiments, provided herein is a method of producing a variant CD155 polypeptide or an immunomodulatory protein, comprising introducing the nucleic acid molecule according to any one of the provided embodiments or vector according to any one of the provided embodiments into a host cell under conditions to express the protein in the cell. In some embodiments, the method further comprises isolating or purifying the variant CD155 polypeptide or immunomodulatory protein from the cell.

[0036] In some embodiments, provided herein is a method of engineering a cell expressing a variant CD155 polypeptide, comprising introducing a nucleic acid molecule encoding the variant CD155 polypeptide or immunomodulatory protein according to any one of the provided embodiments into a host cell under conditions in which the polypeptide is expressed in the cell.

[0037] In some embodiments, provided herein are engineered cells. In some embodiments, the engineered cell comprises any of the variant CD155 polypeptide described herein, or any of the immunomodulatory protein described herein, or any of the nucleic acid molecules described herein, or any of the vectors described herein. In some embodiments, the variant CD155 polypeptide or immuno-

modulatory protein is encoded by a nucleic acid in the engineered cells described herein containing sequence of nucleotides encoding a signal peptide. In some embodiments, the variant CD155 polypeptide or immunomodulatory protein in the engineered cells described herein does not comprise a transmembrane domain and/or is not expressed on the surface of the cell. In some embodiments, the variant CD155 polypeptide or immunomodulatory polypeptide in the engineered cells described herein is secreted from the engineered cell. In some embodiments, the engineered cell comprises a variant CD155 polypeptide that comprises a transmembrane domain and/or comprises any of the transmembrane immunomodulatory protein described herein. In some embodiments, the variant CD155 polypeptide in the engineered cells described herein is expressed on the surface of the cell.

[0038] In some embodiments, provided herein is an engineered cell, expressing the variant CD155 polypeptide or immunomodulatory protein according to any one of the provided embodiments described herein. In some embodiments, the engineered cell is an immune cell. In some embodiments, the immune cell is an antigen presenting cell (APC) or a lymphocyte. In some embodiments, the engineering cell is a primary cell. In some embodiments, the engineered cell is a mammalian cell. In some embodiments, the engineered cell is a human cell. In some embodiments, the lymphocyte is a T cell. In some embodiments, the APC is an artificial APC. In some embodiments, the engineered cell further comprises a chimeric antigen receptor (CAR) or an engineered T-cell receptor (TCR).

[0039] Also provided herein are infectious agents. In some embodiments, the provided infectious agents comprise a nucleic acid molecule encoding any of the variant CD155 polypeptide described herein, or any of the immunomodulatory proteins described herein. In some embodiments, the encoded variant CD155 polypeptide or immunomodulatory proteins does not comprise a transmembrane domain and/or is not expressed on the surface of a cell in which it is expressed. In some embodiments, the encoded variant CD155 polypeptide or immunomodulatory polypeptide is secreted from a cell in which it is expressed. In some embodiments, the encoded variant CD155 polypeptide comprises a transmembrane domain. In some embodiments, the encoded variant CD155 polypeptide is expressed on the surface of a cell in which it is expressed.

[0040] In some embodiments, the infectious agent is a bacterium or a virus. In some embodiments, the virus is a lentiviral or retroviral construct or a hybrid thereof. In some embodiments, the infectious agent is a virus and the virus is an oncolytic virus. In some embodiments, the oncolytic virus is an adenovirus, adeno-associated virus, herpes virus, Herpes Simplex Virus, Vesticular Stomatic virus, Reovirus, Newcastle Disease virus, parvovirus, measles virus, vesticular stomatitis virus (VSV), Coxsackie virus or a Vaccinia virus. In some embodiments, the virus specifically targets dendritic cells (DCs) and/or is dendritic cell-tropic. In some embodiments, the virus is a lentiviral vector that is pseudotyped with a modified Sindbis virus envelope product.

[0041] In some embodiments, the infectious agents additionally include a nucleic acid molecule encoding a further gene product that results in death of a target cell or that can augment or boost an immune response. In some embodiments, the further gene product is selected from an anticancer agent, an anti-metastatic agent, an antiangiogenic agent, an immunomodulatory molecule, an immune checkpoint inhibitor, an antibody, a cytokine, a growth factor, an antigen, a cytotoxic gene product, a pro-apoptotic gene product, an anti-apoptotic gene product, a cell matrix degradative gene, genes for tissue regeneration or reprogramming human somatic cells to pluripotency.

[0042] In some embodiments, provided herein is a pharmaceutical composition, comprising the variant CD155 polypeptide according to any one of the embodiments described herein, an immunomodulatory protein according to any one of the embodiments described herein, a conjugate according to any one of the embodiments described herein, an engineered cell according to any one of the embodiments described herein, or an infectious agent according to any one of the embodiments described herein. In some embodiments, the pharmaceutical composition further comprises a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is sterile.

[0043] In some embodiments, provided herein is an article of manufacture comprising the pharmaceutical composition according to any one of the embodiments described herein in a vial or container. In some embodiments, the vial or container is sealed.

[0044] In some embodiments, provided herein is a kit comprising the pharmaceutical composition according to any one of the embodiments described herein and instructions for use. In some embodiments, provided herein is a kit comprising the article of manufacture according to any one of the embodiments described herein and instructions for use.

[0045] In some embodiments, provided herein is a method of modulating an immune response, such as increasing or decreasing an immune response, in a subject, comprising administering the pharmaceutical composition according to any one of the embodiments described herein to the subject. In some embodiments, the method comprises administering the engineered cells of any one of the embodiments described herein. In some embodiments, the engineered cells are autologous to the subject. In some embodiments, the engineered cells are allogenic to the subject. In some embodiments, the method further comprises administering to the subject a soluble variant CD155 polypeptide according to any one of the embodiments described herein, an immunomodulatory protein according to any one of the embodiments described herein, or a conjugate according to any one of the embodiments described herein. In some embodiments, the method comprises administering to the subject an infectious agent encoding a variant CD155 polypeptide according to any one of the embodiments described herein.

[0046] In some embodiments, modulating the immune response treats a disease or condition in the subject.

[0047] In some embodiments, the immune response is increased. Various formats of a variant CD155 polypeptide are contemplated for administration to a subject to increase an immune response, such as antagonist formats of a variant CD155. In some cases, such methods are carried out under conditions in which signaling by the inhibitory receptor TIGIT is blocked or attenuated by the administration. In some embodiments of the methods provided herein, a variant CD155 polypeptide or immunomodulatory protein that is soluble is administered to the subject. In some embodiments of the methods provided herein, the soluble immunomodulatory protein is an immunomodulatory Fc fusion

protein. In some embodiments of the methods provided herein, any of the variant CD155 polypeptide described herein, or any of the immunomodulatory protein described herein is administered to the subject. In some embodiments of the methods provided herein, an engineered cell comprising a secretable variant CD155 polypeptide is administered to the subject. In some embodiments, any of the engineered cells described herein is administered to the subject. In some embodiments of the methods provided herein, an infectious agent encoding a variant CD155 polypeptide that is a secretable immunomodulatory protein is administered to the subject, optionally under conditions in which the infectious agent infects a tumor cell or immune cell and the secretable immunomodulatory protein is secreted from the infected cell.

[0048] In some embodiments, the disease or condition is a tumor or cancer. In some embodiments, the disease or condition is selected from melanoma, lung cancer, bladder cancer or a hematological malignancy. In some of any of such embodiments, the variant CD155 is administered in a format that increases an immune response in the subject.

[0049] In some embodiments, the immune response is decreased. Various formats of a variant CD155 polypeptide are contemplated for administration to a subject to decrease an immune response, such as agonist formats of a variant CD155. In some cases, such methods are carried out under conditions in which signaling by the inhibitory receptor TIGIT is activated or stimulated or induced by the administration. In some embodiments of the methods provided herein, an immunomodulatory protein or conjugate comprising a variant CD155 polypeptide linked to a moiety that localizes to a cell or tissue of an inflammatory environment is administered to the subject. In some embodiments, the binding molecule comprises an antibody or an antigen-binding fragment thereof or comprises a second polypeptide comprising a wild-type IgSF domain or variant thereof. In some embodiments of the methods provided herein, any of the immunomodulatory proteins described herein or the conjugates described herein is administered to the subject. In some embodiments of the methods provided herein, a variant CD155 polypeptide that is a transmembrane immunomodulatory protein is administered to the subject. In some embodiments of the methods provided herein, any of the engineered cells comprising a variant CD155 polypeptide that is a transmembrane immunomodulatory protein described herein is administered to the subject. In some embodiments of the methods provided herein, an infectious agent encoding a variant CD155 polypeptide that is a transmembrane immunomodulatory protein is administered to the subject, optionally under conditions in which the infectious agent infects a cell in the subject and the transmembrane immunomodulatory protein is expressed on the surface of the infected cell.

[0050] In some embodiments, the disease or condition is an inflammatory or autoimmune disease or condition. In some embodiments, the disease or condition is an antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, a vasculitis, an autoimmune skin disease, transplantation, a Rheumatic disease, an inflammatory gastrointestinal disease, an inflammatory eye disease, an inflammatory neurological disease, an inflammatory pulmonary disease, an inflammatory endocrine disease, or an autoimmune hematological disease. In some embodiments, the disease or condition is selected from interstitial bowel disease, transplant, Crohn's disease, ulcerative colitis, multiple sclerosis, asthma, rheumatoid arthritis, or psoriasis. In some of any such embodiments, the variant CD155 is administered in a format that decreases an immune response in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0051] FIGS. **1A-1C** depict various formats of the provided variant IgSF domain molecules. FIG. **1A** depicts soluble molecules, including: (1) a variant IgSF domain (vIgD) fused to an Fc chain; (2) a stack molecule containing a first variant IgSF domain (first vIgD) and a second IgSF domain, such as a second variant IgSF domain (second vIgD); (3) a tumor targeting IgSF molecule containing a first variant IgSF domain (vIgD) and an IgSF domain that targets to a tumor antigen, such as an NKp30 IgSF domain; and (4) a variant IgSF domain (vIgD) linked to an antibody (V-mAb). FIG. **1B** depicts a transmembrane immunomodulatory protein (TIP) containing a variant IgSF domain (vIgD) expressed on the surface of a cell. In an exemplary embodiment, the cognate binding partner of the transmembrane bound vIgD is an inhibitory receptor (e.g. TIGIT), and the TIP containing the vIgD (e.g. CD155 vIgD) antagonizes or blocks the negative signaling of the inhibitory receptor, thereby resulting in an activated T cell or effector T cell. In some cases, if clustering of the inhibitory receptor (TIGIT) is proximal to an activating receptor (e.g. CD226) then agonizing activity by the TIP may be realized. FIG. **1C** depicts a secreted immunomodulatory protein (SIP) in which a variant IgSF domain (vIgD) is secreted from a cell, such as a first T cell (e.g. CAR T cell). In an exemplary embodiment, the cognate binding partner of the secreted vIgD is an inhibitory receptor (e.g., TIGIT), which can be expressed by the first cell (e.g., T cell, such as a CAR T cell) and/or on a second cell (e.g. T cell; either endogenous or engineered, such as a CAR T cell). Upon binding of the SIP with its cognate binding partner, the SIP antagonizes or blocks the negative signaling via the inhibitory receptor, thereby resulting in an activated T cell or effector T cell. In all cases, the vIgD can be a V-domain (IgV) only, the combination of the V-domain (IgV) and C-domain (IgC), including the entire extracellular domain (ECD), or any combination of Ig domains of the IgSF superfamily member.

[0052] FIG. **2** depicts an exemplary schematic of the activity of a variant IgSF domain (vIgD) fused to an Fc (vIgD-Fc) in which the vIgD is a variant of an IgSF domain of CD155. As shown, a soluble vIgD of CD155 interacts with its cognate binding partners to block interactions of CD155 or CD112 with TIGIT, thereby blocking the TIGIT inhibitory receptor, and, in some cases, allowing the T cell to differentiate into an effector phenotype.

[0053] FIG. **3** depicts an exemplary schematic of a stack molecule that is a multi-target checkpoint antagonist containing a first variant IgSF domain (first vIgD) that is a PD-L1 or PD-L2 vIgD and a second IgSF domain (e.g. a second vIgD) that binds to a second inhibitory receptor. In the exemplary schematic, the second IgSF domain (e.g. second vIgD) is a CD155 vIgD. As shown, the first vIgD and second vIgD interact with their cognate binding partners to block interactions of PD-L1 or PD-L2 with PD-1 and block interactions of CD155 with TIGIT, respectively, blocking multiple inhibitory receptors.

[0054] FIG. **4** depicts an exemplary schematic of a stack molecule for localizing the variant IgSF (vIgD) to a tumor cell. In this format, the stack molecule contains a first variant

IgSF domain (first vIgD) and a second IgSF domain (e.g. a second vIgD) in which the second IgSF domain (e.g a second vIgD) is a tumor-targeted IgSF domain that binds to a tumor antigen. An exemplary tumor-targeted IgSF domain is an IgSF domain of NKp30, which binds to the tumor antigen B7-H6. In this depiction, the variant IgSF domain (vIgD) is a variant of an IgSF domain of CD155. As shown, binding of tumor-targeted IgSF domain to the surface of the tumor cell localizes the first variant IgSF domain on the tumor cell surface where it can interact with one or more of its cognate binding partners expressed on the surface of an adjacent immune cell (e.g. T cell) to antagonize the inhibitory signal of TIGIT.

[0055] FIG. **5**A depicts various exemplary configurations of a stack molecule containing a first variant IgSF domain (first vIgD) and a second IgSF domain, such as a second variant IgSF domain (second vIgD). As shown, the first vIgD and second IgSF domain are independently linked, directly or indirectly, to the N- or C-terminus of an Fc region. For generating a homodimeric Fc molecule, the Fc region is one that is capable of forming a homodimer with a matched Fc subunit by co-expression of the individual Fc regions in a cell. For generating a heterodimeric Fc molecule, the individual Fc regions contain mutations (e.g. "knob-into-hole" mutations in the CH3 domain), such that formation of the heterodimer is favored compared to homodimers when the individual Fc regions are co-expressed in a cell.

[0056] FIG. **5**B depicts various exemplary configurations of a stack molecule containing a first variant IgSF domain (first vIgD), a second IgSF domain, such as a second variant IgSF domain (second vIgD), and a third IgSF domain, such as a third variant IgSF domain (third vIgD). As shown, the first vIgD, second IgSF, and third IgSF domains are independently linked, directly or indirectly, to the N- or C-terminus of an Fc region. For generating a homodimeric Fc molecule, the Fc region is one that is capable of forming a homodimer with a matched Fc region by co-expression of the individual Fc regions in a cell.

[0057] FIG. **6** depicts an exemplary schematic of the activity of a variant IgSF domain (vIgD)-conjugated to an antibody (V-Mab) in which the antibody (e.g. anti-HER2 antibody) binds to an antigen on the surface of the tumor cell to localize the vIgD to the cell. As shown, binding of the antibody to the surface of the tumor cell localizes the vIgD on the tumor cell surface where it can interact with one or more of its cognate binding partners expressed on the surface of an adjacent immune cell (e.g. T cell) to agonize or antagonize receptor signaling. In an exemplary embodiment as shown, the variant IgSF domain (vIgD) is a variant of an IgSF domain of CD155 that binds, such as has increased affinity for, the inhibitory receptor TIGIT. Binding of the CD155 vIgD to the TIGIT inhibitory receptor antagonizes or blocks the negative signaling of the inhibitory receptor, thereby resulting in an activated T cell or effector T cell. In some cases, if clustering of the inhibitory receptor (TIGIT) is proximal to an activating receptor (e.g. CD226) then agonizing of the inhibitory receptor activity by the TIP may be realized.

[0058] FIGS. **7**A-**7**C depict various exemplary configurations of a variant IgSF-antibody conjugate (V-Mab). FIG. **7**A shows various configurations in which a variant IgSF domain is linked, directly or indirectly, to the N- and/or C-terminus of the light chain of an antibody. FIG. **7**B shows various configurations in which a variant IgSF domain is linked, directly or indirectly, to the N- and/or C-terminus of the heavy chain of an antibody. FIG. **7**C depicts the results V-Mab configurations when a light chain of FIG. **7**A and a heavy chain of FIG. **7**B are co-expressed in a cell.

DETAILED DESCRIPTION

[0059] Provided herein are immunomodulatory proteins that are or comprise variants or mutants of CD155 (also known as Poliovirus receptor or PVR) or specific binding fragments thereof that exhibit activity to bind to at least one target ligand cognate binding partner (also called counter-structure protein). In some embodiments, the variant CD155 polypeptides contain one or more amino acid modifications (e.g. amino acid substitutions, deletions or additions) compared to an unmodified or wild-type CD155 polypeptide. In some embodiments, the one or more amino acid modifications (e.g. substitutions) are in an IgSF domain (e.g. IgV) of an unmodified or wild-type CD155 polypeptide. In some embodiments, the variant CD155 polypeptide and immunomodulatory proteins exhibits altered, such as increased or decreased, binding activity or affinity for at least one cognate binding partner, such as at least one of TIGIT, CD226, or CD96. In some embodiments, the immunomodulatory proteins are soluble. In some embodiments, the immunomodulatory proteins are transmembrane immunomodulatory proteins capable of being expressed on the surface of cells. In some embodiments, also provided herein are one or more other immunomodulatory proteins that are conjugates or fusions containing a variant CD155 polypeptide provided herein and one or more other moiety or polypeptide.

[0060] In some embodiments, the variant CD155 polypeptides and immunomodulatory proteins modulate an immunological immune response, such an increase or decrease an immune response. In some embodiments, the variant CD155 polypeptides and immunomodulatory proteins provided herein can be used for the treatment of diseases or conditions that are associated with a dysregulated immune response.

[0061] In some embodiments, the provided variant CD155 polypeptides modulate T cell activation via interactions with costimulatory and/or coinhibitory signaling molecules. In general, antigen specific T-cell activation generally requires two distinct signals. The first signal is provided by the interaction of the T-cell receptor (TCR) with major histocompatibility complex (MHC) associated antigens present on antigen presenting cells (APCs). The second signal is costimulatory to TCR engagement and is necessary for T cell proliferation, differentiation and/or survival, including, in some cases, to avoid T-cell apoptosis or anergy.

[0062] In some embodiments, under normal physiological conditions, the T cell-mediated immune response is initiated by antigen recognition by the T cell receptor (TCR) and is regulated by a balance of co-stimulatory and inhibitory signals (e.g., immune checkpoint proteins). The immune system relies on immune checkpoints to prevent autoimmunity (i.e., self-tolerance) and to protect tissues from excessive damage during an immune response, for example during an attack against a pathogenic infection. In some cases, however, these immunomodulatory proteins can be dysregulated in diseases and conditions, including tumors, as a mechanism for evading the immune system.

[0063] In some embodiments, among known T-cell costimulatory receptors is CD226, which is the T-cell costimulatory receptor for the ligands CD155 (also known

as the poliovirus receptor, PVR) and CD112 (also known as Nectin-2). CD155 and CD112 are normally expressed on the surface of APCs (e.g. dendritic cells) and, in some cases, are overexpressed in tumors. Binding of CD155 or CD112 ligands by CD226, and engagement of CD226, enhances immune responses. In some aspects, CD226 is expressed on NK cells and T cells, including CD4+ and CD8+ T cells, whereby engagement of CD226 can promote Th1 differentiation and/or NK cell activation. However, CD155 and CD112 ligands can also bind to the inhibitory T-cell receptor TIGIT (T cell immunoreceptor with Ig and ITIM domains) to inhibit or down-modulate immune responses. TIGIT, which also can be expressed on NK cells and T cells, can suppress or inhibit the cytolytic activity of NK cells, T cell proliferation and/or proinflammatory cytokine production. In some embodiments, the receptor CD96, which binds the ligand CD155 but not CD112, also can exhibit an opposing effect to CD226 to suppress NK cell activity. Thus, CD226 and TIGIT or CD96 may play opposing roles in immune responses to modulate pro-inflammatory or anti-inflammatory response which, in some cases, are associated with a number of diseases and conditions.

[0064] In some embodiments, enhancement or suppression of the activity of CD226, TIGIT and/or CD96 receptors has clinical significance for treatment of inflammatory and autoimmune disorders, cancer, and viral infections. In some cases, however, therapies to intervene and alter the immunomodulatory effects of such receptors are constrained by the spatial orientation requirements as well as size limitations imposed by the confines of the immunological synapse. In some aspects, existing therapeutic drugs, including antibody drugs, may not be able to interact simultaneously with the multiple target proteins involved in modulating these interactions. In addition, in some cases, existing therapeutic drugs may only have the ability to antagonize but not agonize an immune response. Additionally, pharmacokinetic differences between drugs that independently target one of these receptors can create difficulties in properly maintaining a desired blood concentration of such drug combinations throughout the course of treatment.

[0065] In some embodiments, the provided variant CD155 polypeptides or immunomodulatory proteins modulate (e.g. increase or decrease) immunological activity induced or associated with one or more of the costimulatory receptor TIGIT, CD226, and CD96. Thus, in some embodiments, the provided polypeptides overcome these constraints by providing variant CD155 with altered (e.g. increased or decreased) binding affinities to TIGIT, CD226 and/or CD96, thereby agonizing or antagonizing the complementary effects of costimulation by receptors. Methods of making and using these variant CD155 are also provided.

[0066] All publications, including patents, patent applications scientific articles and databases, mentioned in this specification are herein incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, including patent, patent application, scientific article or database, were specifically and individually indicated to be incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

[0067] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

I. Definitions

[0068] Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

[0069] The terms used throughout this specification are defined as follows unless otherwise limited in specific instances. As used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms, acronyms, and abbreviations used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Unless indicated otherwise, abbreviations and symbols for chemical and biochemical names is per IUPAC-IUB nomenclature. Unless indicated otherwise, all numerical ranges are inclusive of the values defining the range as well as all integer values in-between.

[0070] The term "affinity modified" as used in the context of an immunoglobulin superfamily domain, means a mammalian immunoglobulin superfamily (IgSF) domain having an altered amino acid sequence (relative to the corresponding wild-type parental or unmodified IgSF domain) such that it has an increased or decreased binding affinity or avidity to at least one of its cognate binding partners (alternatively "counter-structures") compared to the parental wild-type or unmodified (i.e., non-affinity modified) IgSF control domain. Included in this context is an affinity modified CD155 IgSF domain. In some embodiments, the affinity-modified IgSF domain can contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more amino acid differences, such as amino acid substitutions, in a wildtype or unmodified IgSF domain. An increase or decrease in binding affinity or avidity can be determined using well known binding assays such as flow cytometry. Larsen et al., American Journal of Transplantation, Vol 5: 443-453 (2005). See also, Linsley et al., Immunity, Vol 1: 793-801 (1994). An increase in a protein's binding affinity or avidity to its cognate binding partner(s) is to a value at least 10% greater than that of the wild-type IgSF domain control and in some embodiments, at least 20%, 30%, 40%, 50%, 100%, 200%, 300%, 500%, 1000%, 5000%, or 10000% greater than that of the wild-type IgSF domain control value. A decrease in a protein's binding affinity or avidity to at least one of its cognate binding partner is to a value no greater than 90% of the control but no less than 10% of the wild-type IgSF domain control value, and in some embodiments no greater than 80%, 70% 60%, 50%, 40%, 30%, or 20% but no less than 10% of the wild-type IgSF domain control value. An affinity-modified protein is altered in primary amino acid sequence by substitution, addition, or deletion of amino acid residues. The term "affinity modified IgSF domain" is not to be construed

as imposing any condition for any particular starting composition or method by which the affinity-modified IgSF domain was created. Thus, the affinity modified IgSF domains of the present invention are not limited to wild type IgSF domains that are then transformed to an affinity modified IgSF domain by any particular process of affinity modification. An affinity modified IgSF domain polypeptide can, for example, be generated starting from wild type mammalian IgSF domain sequence information, then modeled in silico for binding to its cognate binding partner, and finally recombinantly or chemically synthesized to yield the affinity modified IgSF domain composition of matter. In but one alternative example, an affinity modified IgSF domain can be created by site-directed mutagenesis of a wild-type IgSF domain. Thus, affinity modified IgSF domain denotes a product and not necessarily a product produced by any given process. A variety of techniques including recombinant methods, chemical synthesis, or combinations thereof, may be employed.

[0071] The term "allogeneic" as used herein means a cell or tissue that is removed from one organism and then infused or adoptively transferred into a genetically dissimilar organism of the same species. In some embodiments of the invention, the species is murine or human.

[0072] The term "autologous" as used herein means a cell or tissue that is removed from the same organism to which it is later infused or adoptively transferred. An autologous cell or tissue can be altered by, for example, recombinant DNA methodologies, such that it is no longer genetically identical to the native cell or native tissue which is removed from the organism. For example, a native autologous T-cell can be genetically engineered by recombinant DNA techniques to become an autologous engineered cell expressing a transmembrane immunomodulatory protein and/or chimeric antigen receptor (CAR), which in some cases involves engineering a T-cell or TIL (tumor infiltrating lymphocyte). The engineered cells are then infused into a patient from which the native T-cell was isolated. In some embodiments, the organism is human or murine.

[0073] The terms "binding affinity," and "binding avidity" as used herein means the specific binding affinity and specific binding avidity, respectively, of a protein for its counter-structure under specific binding conditions. In biochemical kinetics avidity refers to the accumulated strength of multiple affinities of individual non-covalent binding interactions, such as between CD155 and its counter-structures TIGIT, CD226, and/or CD96. As such, avidity is distinct from affinity, which describes the strength of a single interaction. An increase or attenuation in binding affinity of a variant CD155 containing an affinity modified CD155 IgSF domain to its counter-structure is determined relative to the binding affinity of the unmodified CD155, such as an unmodified CD155 containing the native or wild-type IgSF domain, such as IgV domain. Methods for determining binding affinity or avidity are known in art. See, for example, Larsen et al., American Journal of Transplantation, Vol 5: 443-453 (2005). In some embodiments, a variant CD155 of the invention (i.e. a CD155 protein containing an affinity modified IgSF domain) specifically binds to TIGIT, CD226, and/or CD96 measured by flow cytometry with a binding affinity that yields a Mean Fluorescence Intensity (MFI) value at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% greater than a wild-type CD155 control in a binding assay.

[0074] The term "biological half-life" refers to the amount of time it takes for a substance, such as an immunomodulatory polypeptide comprising a variant CD155 of the present invention, to lose half of its pharmacologic or physiologic activity or concentration. Biological half-life can be affected by elimination, excretion, degradation (e.g., enzymatic) of the substance, or absorption and concentration in certain organs or tissues of the body. In some embodiments, biological half-life can be assessed by determining the time it takes for the blood plasma concentration of the substance to reach half its steady state level ("plasma half-life"). Conjugates that can be used to derivatize and increase the biological half-life of polypeptides of the invention are known in the art and include, but are not limited to, polyethylene glycol (PEG), hydroxyethyl starch (HES), XTEN (extended recombinant peptides; see, WO2013130683), human serum albumin (HSA), bovine serum albumin (BSA), lipids (acylation), and poly-Pro-Ala-Ser (PAS), polyglutamic acid (glutamylation).

[0075] The term "chimeric antigen receptor" or "CAR" as used herein refers to an artificial (i.e., man-made) transmembrane protein expressed on a mammalian cell comprising at least an ectodomain, a transmembrane, and an endodomain. Optionally, the CAR protein includes a "spacer" which covalently links the ectodomain to the transmembrane domain. A spacer is often a polypeptide linking the ectodomain to the transmembrane domain via peptide bonds. The CAR is typically expressed on a mammalian lymphocyte. In some embodiments, the CAR is expressed on a mammalian cell such as a T-cell or a tumor infiltrating lymphocyte (TIL). A CAR expressed on a T-cell is referred to herein as a "CAR T-cell" or "CAR-T." In some embodiments the CAR-T is a T helper cell, a cytotoxic T-cell, a natural killer T-cell, a memory T-cell, a regulatory T-cell, or a gamma delta T-cell. When used clinically in, e.g. adoptive cell transfer, a CAR-T with antigen binding specificity to the patient's tumor is typically engineered to express on a T-cell obtained from the patient. The engineered T-cell expressing the CAR is then infused back into the patient. The CAR-T is thus often an autologous CAR-T although allogeneic CAR-T are included within the scope of the invention. The ectodomain of a CAR comprises an antigen binding region, such as an antibody or antigen binding fragment thereof (e.g. scFv), that specifically binds under physiological conditions with a target antigen, such as a tumor specific antigen. Upon specific binding a biochemical chain of events (i.e., signal transduction) results in modulation of the immunological activity of the CAR-T. Thus, for example, upon specific binding by the antigen binding region of the CAR-T to its target antigen can lead to changes in the immunological activity of the T-cell activity as reflected by changes in cytotoxicity, proliferation or cytokine production. Signal transduction upon CAR-T activation is achieved in some embodiments by the CD3-zeta chain ("CD3-z") which is involved in signal transduction in native mammalian T-cells. CAR-Ts can further comprises multiple signaling domains such as CD28, 41BB or OX40, to further modulate immunomodulatory response of the T-cell. CD3-z comprises a conserved motif known as an immunoreceptor tyrosine-based activation motif (ITAM) which is involved in T-cell receptor signal transduction.

[0076] The term "collectively" or "collective" when used in reference to cytokine production induced by the presence of two or more variant CD155 of the invention in an in vitro assay, means the overall cytokine expression level irrespec-

tive of the cytokine production induced by individual variant CD155. In some embodiments, the cytokine being assayed is IFN-gamma, such as in an in vitro primary T-cell assay.

[0077] The term "cognate binding partner" (used interchangeably with "counter-structure") in reference to a polypeptide, such as in reference to an IgSF domain of a variant CD155, refers to at least one molecule (typically a native mammalian protein) to which the referenced polypeptide specifically binds under specific binding conditions. In some aspects, a variant CD155 containing an affinity modified IgSF domain specifically binds to the counter-structure of the corresponding native or wildtype CD155 but with increased or attenuated affinity. A species of ligand recognized and specifically binding to its cognate receptor under specific binding conditions is an example of a counter-structure or cognate binding partner of that receptor. A "cognate cell surface binding partner" is a cognate binding partner expressed on a mammalian cell surface. A "cell surface molecular species" is a cognate binding partner of ligands of the immunological synapse (IS), expressed on and by cells, such as mammalian cells, forming the immunological synapse.

[0078] As used herein, "conjugate," "conjugation" or grammatical variations thereof refers the joining or linking together of two or more compounds resulting in the formation of another compound, by any joining or linking methods known in the art. It can also refer to a compound which is generated by the joining or linking together two or more compounds. For example, a variant CD155 polypeptide linked directly or indirectly to one or more chemical moieties or polypeptide is an exemplary conjugate. Such conjugates include fusion proteins, those produced by chemical conjugates and those produced by any other methods.

[0079] The term "competitive binding" as used herein means that a protein is capable of specifically binding to at least two cognate binding partners but that specific binding of one cognate binding partner inhibits, such as prevents or precludes, simultaneous binding of the second cognate binding partner. Thus, in some cases, it is not possible for a protein to bind the two cognate binding partners at the same time. Generally, competitive binders contain the same or overlapping binding site for specific binding but this is not a requirement. In some embodiments, competitive binding causes a measurable inhibition (partial or complete) of specific binding of a protein to one of its cognate binding partner due to specific binding of a second cognate binding partner. A variety of methods are known to quantify competitive binding such as ELISA (enzyme linked immunosorbent assay) assays.

[0080] The term "conservative amino acid substitution" as used herein means an amino acid substitution in which an amino acid residue is substituted by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

[0081] The term, "corresponding to" with reference to positions of a protein, such as recitation that nucleotides or amino acid positions "correspond to" nucleotides or amino acid positions in a disclosed sequence, such as set forth in the Sequence listing, refers to nucleotides or amino acid positions identified upon alignment with the disclosed sequence based on structural sequence alignment or using a standard alignment algorithm, such as the GAP algorithm. For example, corresponding residues can be determined by alignment of a reference sequence with the sequence of wild-type CD155 set forth in SEQ ID NO:47 (ECD domain) or set forth in SEQ ID NO: 58 or 155 (IgV domain) by structural alignment methods as described herein. By aligning the sequences, one skilled in the art can identify corresponding residues, for example, using conserved and identical amino acid residues as guides.

[0082] The terms "decrease" or "attenuate" "or suppress" as used herein means to decrease by a statistically significant amount. A decrease can be at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%.

[0083] The terms "derivatives" or "derivatized" refer to modification of a protein by covalently linking it, directly or indirectly, to a composition so as to alter such characteristics as biological half-life, bioavailability, immunogenicity, solubility, toxicity, potency, or efficacy while retaining or enhancing its therapeutic benefit. Derivatives of immunomodulatory polypeptides of the invention are within the scope of the invention and can be made by, for example, glycosylation, pegylation, lipidation, or Fc-fusion.

[0084] As used herein, "domain" (typically a sequence of three or more, generally 5 or 7 or more amino acids, such as 10 to 200 amino acid residues) refers to a portion of a molecule, such as a protein or encoding nucleic acid that is structurally and/or functionally distinct from other portions of the molecule and is identifiable. For example, domains include those portions of a polypeptide chain that can form an independently folded structure within a protein made up of one or more structural motifs and/or that is recognized by virtue of a functional activity, such as binding activity. A protein can have one, or more than one, distinct domains. For example, a domain can be identified, defined or distinguished by homology of the primary sequence or structure to related family members, such as homology to motifs. In another example, a domain can be distinguished by its function, such as an ability to interact with a biomolecule, such as a cognate binding partner. A domain independently can exhibit a biological function or activity such that the domain independently or fused to another molecule can perform an activity, such as, for example binding. A domain can be a linear sequence of amino acids or a non-linear sequence of amino acids. Many polypeptides contain a plurality of domains. Such domains are known, and can be identified by those of skill in the art. For exemplification herein, definitions are provided, but it is understood that it is well within the skill in the art to recognize particular domains by name. If needed appropriate software can be employed to identify domains.

[0085] The term "ectodomain" as used herein refers to the region of a membrane protein, such as a transmembrane protein, that lies outside the vesicular membrane. Ectodomains often comprise binding domains that specifically bind to ligands or cell surface receptors, such as via a binding

domain that specifically binds to the ligand or cell surface receptor. The ectodomain of a cellular transmembrane protein is alternately referred to as an extracellular domain.

[0086] The terms "effective amount" or "therapeutically effective amount" refer to a quantity and/or concentration of a therapeutic composition of the invention, including a protein composition or cell composition, that when administered ex vivo (by contact with a cell from a patient) or in vivo (by administration into a patient) either alone (i.e., as a monotherapy) or in combination with additional therapeutic agents, yields a statistically significant decrease in disease progression as, for example, by ameliorating or eliminating symptoms and/or the cause of the disease. An effective amount may be an amount that relieves, lessens, or alleviates at least one symptom or biological response or effect associated with a disease or disorder, prevents progression of the disease or disorder, or improves physical functioning of the patient. In the case of cell therapy, the effective amount is an effective dose or number of cells administered to a patient by adoptive cell therapy. In some embodiments the patient is a mammal such as a non-human primate or human patient.

[0087] The term "endodomain" as used herein refers to the region found in some membrane proteins, such as transmembrane proteins, that extends into the interior space defined by the cell surface membrane. In mammalian cells, the endodomain is the cytoplasmic region of the membrane protein. In cells, the endodomain interacts with intracellular constituents and can be play a role in signal transduction and thus, in some cases, can be an intracellular signaling domain. The endodomain of a cellular transmembrane protein is alternately referred to as a cytoplasmic domain, which, in some cases, can be a cytoplasmic signaling domain.

[0088] The terms "enhanced" or "increased" as used herein in the context of increasing immunological activity of a mammalian lymphocyte means to increase one or more activities the lymphocyte. An increased activity can be one or more of increase cell survival, cell proliferation, cytokine production, or T-cell cytotoxicity, such as by a statistically significant amount. In some embodiments, reference to increased immunological activity means to increase interferon gamma (IFN-gamma) production, such as by a statistically significant amount. In some embodiments, the immunological activity can be assessed in a mixed lymphocyte reaction (MLR) assay. Methods of conducting MLR assays are known in the art. Wang et al., Cancer Immunol Res. 2014 September: 2(9):846-56. Other methods of assessing activities of lymphocytes are known in the art, including any assay as described herein. In some embodiments an enhancement can be an increase of at least 10%, 20%, 30%, 40%, 50%, 75%, 100%, 200%, 300%, 400%, or 500% greater than a non-zero control value.

[0089] The term "engineered cell" as used herein refers to a mammalian cell that has been genetically modified by human intervention such as by recombinant DNA methods or viral transduction. In some embodiments, the cell is an immune cell, such as a lymphocyte (e.g. T cell, B cell, NK cell) or an antigen presenting cell (e.g. dendritic cell). The cell can be a primary cell from a patient or can be a cell line. In some embodiments, an engineered cell of the invention comprises a variant CD155 of the invention engineered to modulate immunological activity of a T-cell expressing TIGIT, CD226, and/or CD96 to which the variant CD155 specifically binds. In some embodiments, the variant CD155 is a transmembrane immunomodulatory protein (hereinafter referred to as "TIP") containing the extracellular domain or a portion thereof containing the IgV domain linked to a transmembrane domain (e.g. a CD155 transmembrane domain) and, optionally, an intracellular signaling domain. In some cases, the TIP is formatted as a chimeric receptor containing a heterologous cytoplasmic signaling domain or endodomain. In some embodiments, an engineered cell is capable of expressing and secreting an immunomodulatory protein as described herein. Among provided engineered cells also are cells further containing an engineered T-cell receptor (TCR) or chimeric antigen receptor (CAR).

[0090] The term "engineered T-cell" as used herein refers to a T-cell such as a T helper cell, cytotoxic T-cell (alternatively, cytotoxic T lymphocyte or CTL), natural killer T-cell, regulatory T-cell, memory T-cell, or gamma delta T-cell, that has been genetically modified by human intervention such as by recombinant DNA methods or viral transduction methods. An engineered T-cell comprises a variant CD155 transmembrane immunomodulatory protein (TIP) or secreted immunodulatory protein (SIP) of the present invention that is expressed on the T-cell and is engineered to modulate immunological activity of the engineered T-cell itself, or a mammalian cell to which the variant CD155 expressed on the T-cell specifically binds.

[0091] The term "engineered T-cell receptor" or "engineered TCR" refers to a T-cell receptor (TCR) engineered to specifically bind with a desired affinity to a major histocompatibility complex (MHC)/peptide target antigen that is selected, cloned, and/or subsequently introduced into a population of T-cells, often used for adoptive immunotherapy. In contrast to engineered TCRs, CARs are engineered to bind target antigens in a MHC independent manner.

[0092] The term "expressed on" as used herein is used in reference to a protein expressed on the surface of a cell, such as a mammalian cell. Thus, the protein is expressed as a membrane protein. In some embodiments, the expressed protein is a transmembrane protein. In some embodiments, the protein is conjugated to a small molecule moiety such as a drug or detectable label. Proteins expressed on the surface of a cell can include cell-surface proteins such as cell surface receptors that are expressed on mammalian cells.

[0093] The term "half-life extending moiety" refers to a moiety of a polypeptide fusion or chemical conjugate that extends the half-life of a protein circulating in mammalian blood serum compared to the half-life of the protein that is not so conjugated to the moiety. In some embodiments, half-life is extended by greater than or greater than about 1.2-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold, or 6.0-fold. In some embodiments, half-life is extended by more than 6 hours, more than 12 hours, more than 24 hours, more than 48 hours, more than 72 hours, more than 96 hours or more than 1 week after in vivo administration compared to the protein without the half-life extending moiety. The half-life refers to the amount of time it takes for the protein to lose half of its concentration, amount, or activity. Half-life can be determined for example, by using an ELISA assay or an activity assay. Exemplary half-life extending moieties include an Fc domain, a multimerization domain, polyethylene glycol (PEG), hydroxyethyl starch (HES), XTEN (extended recombinant peptides; see, WO2013130683), human serum albumin (HSA), bovine serum albumin

(BSA), lipids (acylation), and poly-Pro-Ala-Ser (PAS), and polyglutamic acid (glutamylation).

[0094] The term "immunological synapse" or "immune synapse" as used herein means the interface between a mammalian cell that expresses MHC I (major histocompatibility complex) or MHC II, such as an antigen-presenting cell or tumor cell, and a mammalian lymphocyte such as an effector T cell or Natural Killer (NK) cell.

[0095] An Fc (fragment crystallizable) region or domain of an immunoglobulin molecule (also termed an Fc polypeptide) corresponds largely to the constant region of the immunoglobulin heavy chain, and is responsible for various functions, including the antibody's effector function(s). The Fc domain contains part or all of a hinge domain of an immunoglobulin molecule plus a CH2 and a CH3 domain. The Fc domain can form a dimer of two polypeptide chains joined by one or more disulfide bonds. In some embodiments, the Fc is a variant Fc that exhibits reduced (e.g. reduced greater than 30%, 40%, 50%, 60%, 70%, 80%, 90% or more) activity to facilitate an effector function. In some embodiments, reference to amino acid substitutions in an Fc region is by EU numbering system unless described with reference to a specific SEQ ID NO. EU numbering is known and is according to the most recently updated IMGT Scientific Chart (IMGT®, the international ImMunoGeneTics information System®, http://www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html (created: 17 May 2001, last updated: 10 Jan. 2013) and the EU index as reported in Kabat, E. A. et al. Sequences of Proteins of Immunological interest. 5th ed. US Department of Health and Human Services, NIH publication No. 91-3242 (1991).

[0096] An immunoglobulin Fc fusion ("Fc-fusion"), such as an immunomodulatory Fc fusion protein, is a molecule comprising one or more polypeptides (or one or more small molecules) operably linked to an Fc region of an immunoglobulin. An Fc-fusion may comprise, for example, the Fc region of an antibody (which facilitates effector functions and pharmacokinetics) and a variant CD155. An immunoglobulin Fc region may be linked indirectly or directly to one or more variant CD155 or small molecules (fusion partners). Various linkers are known in the art and can optionally be used to link an Fc to a fusion partner to generate an Fc-fusion. Fc-fusions of identical species can be dimerized to form Fc-fusion homodimers, or using non-identical species to form Fc-fusion heterodimers. In some embodiments, the Fc is a mammalian Fc such as a murine or human Fc.

[0097] The term "host cell" refers to a cell that can be used to express a protein encoded by a recombinant expression vector. A host cell can be a prokaryote, for example, *E. coli*, or it can be a eukaryote, for example, a single-celled eukaryote (e.g., a yeast or other fungus), a plant cell (e.g., a tobacco or tomato plant cell), an animal cell (e.g., a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Examples of host cells include Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media or CHO strain DX-B11, which is deficient in DHFR. Another example is Human Endothelial Kidney 293 cells or their derivatives. In some embodiments, a host cell is a mammalian cell (e.g., a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell).

[0098] The term "immunoglobulin" (abbreviated "Ig") as used herein refers to a mammalian immunoglobulin protein including any of the five human classes of antibody: IgA (which includes subclasses IgA1 and IgA2), IgD, IgE, IgG (which includes subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. The term is also inclusive of immunoglobulins that are less than full-length, whether wholly or partially synthetic (e.g., recombinant or chemical synthesis) or naturally produced, such as antigen binding fragment (Fab), variable fragment (Fv) containing V_H and V_L, the single chain variable fragment (scFv) containing V_H and V_L linked together in one chain, as well as other antibody V region fragments, such as Fab', $F(ab)_2$, F(ab')2, dsFv diabody, Fe, and Fd polypeptide fragments. Bispecific antibodies, homobispecific and heterobispecific, are included within the meaning of the term.

[0099] The term "immunoglobulin superfamily" or "IgSF" as used herein means the group of cell surface and soluble proteins that are involved in the recognition, binding, or adhesion processes of cells. Molecules are categorized as members of this superfamily based on shared structural features with immunoglobulins (i.e., antibodies); they all possess a domain known as an immunoglobulin domain or fold. Members of the IgSF include cell surface antigen receptors, co-receptors and co-stimulatory molecules of the immune system, molecules involved in antigen presentation to lymphocytes, cell adhesion molecules, certain cytokine receptors and intracellular muscle proteins. They are commonly associated with roles in the immune system. Proteins in the immunological synapse are often members of the IgSF. IgSF can also be classified into "subfamilies" based on shared properties such as function. Such subfamilies typically consist of from 4 to 30 IgSF members.

[0100] The terms "IgSF domain" or "immunoglobulin domain" or "Ig domain" as used herein refers to a structural domain of IgSF proteins. Ig domains are named after the immunoglobulin molecules. They contain about 70-110 amino acids and are categorized according to their size and function. Ig-domains possess a characteristic Ig-fold, which has a sandwich-like structure formed by two sheets of antiparallel beta strands. Interactions between hydrophobic amino acids on the inner side of the sandwich and highly conserved disulfide bonds formed between cysteine residues in the B and F strands, stabilize the Ig-fold. One end of the Ig domain has a section called the complementarity determining region that is important for the specificity of antibodies for their ligands. The Ig like domains can be classified (into classes) as: IgV, IgC (which either can be an IgC1 or IgC2), or IgI. Most Ig domains are either variable (IgV) or constant (IgC). IgV domains with 9 beta strands are generally longer than IgC domains with 7 beta strands. Ig domains of some members of the IgSF resemble IgV domains in the amino acid sequence, yet are similar in size to IgC domains. These are called IgC2 domains, while standard IgC domains are called IgC1 domains. T-cell receptor (TCR) chains contain two Ig domains in the extracellular portion; one IgV domain at the N-terminus and one IgC1 domain adjacent to the cell membrane. CD155 contains three Ig domains: IgV and two IgC domains that are IgC2 domains.

[0101] The term "IgSF species" as used herein means an ensemble of IgSF member proteins with identical or substantially identical primary amino acid sequence. Each mammalian immunoglobulin superfamily (IgSF) member defines a unique identity of all IgSF species that belong to that IgSF member. Thus, each IgSF family member is unique

from other IgSF family members and, accordingly, each species of a particular IgSF family member is unique from the species of another IgSF family member. Nevertheless, variation between molecules that are of the same IgSF species may occur owing to differences in post-translational modification such as glycosylation, phosphorylation, ubiquitination, nitrosylation, methylation, acetylation, and lipidation. Additionally, minor sequence differences within a single IgSF species owing to gene polymorphisms constitute another form of variation within a single IgSF species as do wild type truncated forms of IgSF species owing to, for example, proteolytic cleavage. A "cell surface IgSF species" is an IgSF species expressed on the surface of a cell, generally a mammalian cell.

[0102] The term "immunological activity" as used herein in the context of mammalian lymphocytes such as T-cells refers to one or more cell survival, cell proliferation, cytokine production (e.g. interferon-gamma), or T-cell cytotoxicity activities. In some cases, an immunological activity can mean the cell expression of cytokines, such as chemokines or interleukins. Assays for determining enhancement or suppression of immunological activity include the MLR (mixed lymphocyte reaction) assays measuring interferon-gamma cytokine levels in culture supernatants (Wang et al., Cancer Immunol Res. 2014 September: 2(9):846-56), SEB (staphylococcal enterotoxin B) T cell stimulation assay (Wang et al., Cancer Immunol Res. 2014 September: 2(9): 846-56), and anti-CD3 T cell stimulation assays (Li and Kurlander, J Transl Med. 2010: 8: 104). Since T cell activation is associated with secretion of IFN-gamma cytokine, detecting IFN-gamma levels in culture supernatants from these in vitro human T cell assays can be assayed using commercial ELISA kits (Wu et al, Immunol Lett 2008 Apr. 15; 117(1): 57-62). Induction of an immune response results in an increase in immunological activity relative to quiescent lymphocytes. An immunomodulatory protein, such as a variant CD155 polypeptide containing an affinity modified IgSF domain, as provided herein can in some embodiments increase or, in alternative embodiments, decrease IFN-gamma (interferon-gamma) expression in a primary T-cell assay relative to a wild-type IgSF member or IgSF domain control. Those of skill will recognize that the format of the primary T-cell assay used to determine an increase in IFN-gamma expression can differ from that employed to assay for a decrease in IFN-gamma expression. In assaying for the ability of an immunomodulatory protein or affinity modified IgSF domain of the invention to alter IFN-gamma expression in a primary T-cell assay, a Mixed Lymphocyte Reaction (MLR) assay can be used. Conveniently, in some cases, a soluble form of an affinity modified IgSF domain of the invention can be employed to determine its ability to increase or decrease the IFN-gamma expression in a MLR. Alternatively, a co-immobilization assay can be used. In a co-immobilization assay, a T-cell receptor signal, provided in some embodiments by anti-CD3 antibody, is used in conjunction with a co-immobilized affinity modified IgSF domain, such as variant CD155, to determine the ability to increase or decrease IFN-gamma expression relative to a wild-type IgSF domain control. Methods to assay the immunological activity of engineered cells, including to evaluate the activity of a variant CD155 transmembrane immunomodulatory protein, are known in the art and include, but are not limited to, the ability to expand T cells following antigen stimulation, sustain T cell expansion in the absence of re-stimulation, and anti-cancer activities in appropriate animal models. Assays also include assays to assess cytotoxicity, including a standard ^{51}Cr-release assay (see e.g. Milone et al., (2009) Molecular Therapy 17: 1453-1464) or flow based cytotoxicity assays, or an impedance based cytotoxicity assay (Peper et al. (2014) Journal of Immunological Methods, 405:192-198).

[0103] An "immunomodulatory polypeptide" or "immunomodulatory protein" is a polypeptide or protein molecule that modulates immunological activity. By "modulation" or "modulating" an immune response is meant that immunological activity is either increased or decreased. An immunomodulatory protein can be a single polypeptide chain or a multimer (dimers or higher order multimers) of at least two polypeptide chains covalently bonded to each other by, for example, interchain disulfide bonds. Thus, monomeric, dimeric, and higher order multimeric polypeptides are within the scope of the defined term. Multimeric polypeptides can be homomultimeric (of identical polypeptide chains) or heteromultimeric (of non-identical polypeptide chains). An immunomodulatory protein of the invention comprises a variant CD155.

[0104] The term "increase" as used herein means to increase by a statistically significant amount. An increase can be at least 5%, 10%, 20%, 30%, 40%, 50%, 75%, 100%, or greater than a non-zero control value.

[0105] An "isoform" of CD155 is one of a plurality naturally occurring CD155 polypeptides that differ in amino acid sequence. Isoforms can be the product of splice variants of an RNA transcript expressed by a single gene, or the expression product of highly similar but different genes yielding a functionally similar protein such as may occur from gene duplication. As used herein, the term "isoform" of CD155 also refers to the product of different alleles of a CD155 gene.

[0106] The term "lymphocyte" as used herein means any of three subtypes of white blood cell in a mammalian immune system. They include natural killer cells (NK cells) (which function in cell-mediated, cytotoxic innate immunity), T cells (for cell-mediated, cytotoxic adaptive immunity), and B cells (for humoral, antibody-driven adaptive immunity). T cells include: T helper cells, cytotoxic T-cells, natural killer T-cells, memory T-cells, regulatory T-cells, or gamma delta T-cells. Innate lymphoid cells (ILC) are also included within the definition of lymphocyte.

[0107] The terms "mammal," or "patient" specifically includes reference to at least one of a: human, chimpanzee, rhesus monkey, cynomolgus monkey, dog, cat, mouse, or rat.

[0108] The term "membrane protein" as used herein means a protein that, under physiological conditions, is attached directly or indirectly to a lipid bilayer. A lipid bilayer that forms a membrane can be a biological membrane such as a eukaryotic (e.g., mammalian) cell membrane or an artificial (i.e., man-made) membrane such as that found on a liposome. Attachment of a membrane protein to the lipid bilayer can be by way of covalent attachment, or by way of non-covalent interactions such as hydrophobic or electrostatic interactions. A membrane protein can be an integral membrane protein or a peripheral membrane protein. Membrane proteins that are peripheral membrane proteins are non-covalently attached to the lipid bilayer or non-covalently attached to an integral membrane protein. A peripheral membrane protein forms a temporary attachment

to the lipid bilayer such that under the range of conditions that are physiological in a mammal, peripheral membrane protein can associate and/or disassociate from the lipid bilayer. In contrast to peripheral membrane proteins, integral membrane proteins form a substantially permanent attachment to the membrane's lipid bilayer such that under the range of conditions that are physiological in a mammal, integral membrane proteins do not disassociate from their attachment to the lipid bilayer. A membrane protein can form an attachment to the membrane by way of one layer of the lipid bilayer (monotopic), or attached by way of both layers of the membrane (polytopic). An integral membrane protein that interacts with only one lipid bilayer is an "integral monotopic protein". An integral membrane protein that interacts with both lipid bilayers is an "integral polytopic protein" alternatively referred to herein as a "transmembrane protein".

[0109] The terms "modulating" or "modulate" as used herein in the context of an immune response, such as a mammalian immune response, refer to any alteration, such as an increase or a decrease, of existing or potential immune responses that occurs as a result of administration of an immunomodulatory polypeptide comprising a variant CD155 of the present invention or as a result of administration of engineered cells expresses an immunomodulatory protein, such as a variant CD155 transmembrane immunomodulatory protein of the present invention. Thus, it refers to an alteration, such as an increase or decrease, of an immune response as compared to the immune response that occurs or is present in the absence of the administration of the immunomodulatory protein comprising the variant CD155 or cells expressing such an immunomodulatory polypeptide. Such modulation includes any induction, activation, suppression or alteration in degree or extent of immunological activity of an immune cell. Immune cells include B cells, T cells, NK (natural killer) cells, NK T cells, professional antigen-presenting cells (APCs), and non-professional antigen-presenting cells, and inflammatory cells (neutrophils, macrophages, monocytes, eosinophils, and basophils). Modulation includes any change imparted on an existing immune response, a developing immune response, a potential immune response, or the capacity to induce, regulate, influence, or respond to an immune response. Modulation includes any alteration in the expression and/or function of genes, proteins and/or other molecules in immune cells as part of an immune response. Modulation of an immune response or modulation of immunological activity includes, for example, the following: elimination, deletion, or sequestration of immune cells; induction or generation of immune cells that can modulate the functional capacity of other cells such as autoreactive lymphocytes, antigen presenting cells, or inflammatory cells; induction of an unresponsive state in immune cells (i.e., anergy); enhancing or suppressing the activity or function of immune cells, including but not limited to altering the pattern of proteins expressed by these cells. Examples include altered production and/or secretion of certain classes of molecules such as cytokines, chemokines, growth factors, transcription factors, kinases, costimulatory molecules, or other cell surface receptors or any combination of these modulatory events. Modulation can be assessed, for example, by an alteration in IFN-gamma (interferon gamma) expression relative to the wild-type CD155 control in a primary T cell assay (see, Zhao and Ji, Exp Cell Res. 2016 Jan. 1; 340(1): 132-138). Modulation can be assessed, for example, by an alteration of an immunological activity of engineered cells, such as an alteration in in cytotoxic activity of engineered cells or an alteration in cytokine secretion of engineered cells relative to cells engineered with a wild-type CD155 transmembrane protein

[0110] The term "molecular species" as used herein means an ensemble of proteins with identical or substantially identical primary amino acid sequence. Each mammalian immunoglobulin superfamily (IgSF) member defines a collection of identical or substantially identical molecular species. Thus, for example, human CD155 is an IgSF member and each human CD155 molecule is a molecular species of CD155. Variation between molecules that are of the same molecular species may occur owing to differences in post-translational modification such as glycosylation, phosphorylation, ubiquitination, nitrosylation, methylation, acetylation, and lipidation. Additionally, minor sequence differences within a single molecular species owing to gene polymorphisms constitute another form of variation within a single molecular species as do wild type truncated forms of a single molecular species owing to, for example, proteolytic cleavage. A "cell surface molecular species" is a molecular species expressed on the surface of a mammalian cell. Two or more different species of protein, each of which is present exclusively on one or exclusively the other (but not both) of the two mammalian cells forming the IS, are said to be in "cis" or "cis configuration" with each other. Two different species of protein, the first of which is exclusively present on one of the two mammalian cells forming the IS and the second of which is present exclusively on the second of the two mammalian cells forming the IS, are said to be in "trans" or "trans configuration." Two different species of protein each of which is present on both of the two mammalian cells forming the IS are in both cis and trans configurations on these cells.

[0111] The term, a "multimerization domain" refers to a sequence of amino acids that promotes stable interaction of a polypeptide molecule with one or more additional polypeptide molecules, each containing a complementary multimerization domain (e.g. a first multimerization domain and a second multimerization domain), which can be the same or a different multimerization domain. The interactions between complementary multimerization domains, e.g. interaction between a first multimerication domain and a second multimerization domain, form a stable protein-protein interaction to produce a multimer of the polypeptide molecule with the additional polypeptide molecule. In some cases, the multimerization domain is the same and interacts with itself to form a stable proten-protein interaction between two polypeptide chains. Generally, a polypeptide is joined directly or indirectly to the multimerization domain. Exemplary multimerization domains include the immunoglobulin sequences or portions thereof, leucine zippers, hydrophobic regions, hydrophilic regions, and compatible protein-protein interaction domains. The multimerization domain, for example, can be an immunoglobulin constant region or domain, such as, for example, the Fc domain or portions thereof from IgG, including IgG1, IgG2, IgG3 or IgG4 subtypes, IgA, IgE, IgD and IgM and modified forms thereof.

[0112] The terms "nucleic acid" and "polynucleotide" are used interchangeably to refer to a polymer of nucleic acid residues (e.g., deoxyribonucleotides or ribonucleotides) in

either single- or double-stranded form. Unless specifically limited, the terms encompass nucleic acids containing known analogues of natural nucleotides and that have similar binding properties to it and are metabolized in a manner similar to naturally-occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary nucleotide sequences as well as the sequence explicitly indicated (a "reference sequence"). Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues. The term nucleic acid or polynucleotide encompasses cDNA or mRNA encoded by a gene.

[0113] The term "non-competitive binding" as used herein means the ability of a protein to specifically bind simultaneously to at least two cognate binding partners. Thus, the protein is able to bind to at least two different cognate binding partners at the same time, although the binding interaction need not be for the same duration such that, in some cases, the protein is specifically bound to only one of the cognate binding partners. In some embodiments, the binding occurs under specific binding conditions. In some embodiments, the simultaneous binding is such that binding of one cognate binding partner does not substantially inhibit simultaneous binding to a second cognate binding partner. In some embodiments, non-competitive binding means that binding a second cognate binding partner to its binding site on the protein does not displace the binding of a first cognate binding partner to its binding site on the protein. Methods of assessing non-competitive binding are well known in the art such as the method described in Perez de La Lastra et al., Immunology, 1999 April: 96(4): 663-670. In some cases, in non-competitive interactions, the first cognate binding partner specifically binds at an interaction site that does not overlap with the interaction site of the second cognate binding partner such that binding of the second cognate binding partner does not directly interfere with the binding of the first cognate binding partner. Thus, any effect on binding of the cognate binding partner by the binding of the second cognate binding partner is through a mechanism other than direct interference with the binding of the first cognate binding partner. For example, in the context of enzyme-substrate interactions, a non-competitive inhibitor binds to a site other than the active site of the enzyme. Non-competitive binding encompasses uncompetitive binding interactions in which a second cognate binding partner specifically binds at an interaction site that does not overlap with the binding of the first cognate binding partner but binds to the second interaction site only when the first interaction site is occupied by the first cognate binding partner.

[0114] The term "pharmaceutical composition" refers to a composition suitable for pharmaceutical use in a mammalian subject, often a human. A pharmaceutical composition typically comprises an effective amount of an active agent (e.g., an immunomodulatory polypeptide comprising a variant CD155 or engineered cells expressing a variant CD155 transmembrane immunomodulatory protein) and a carrier, excipient, or diluent. The carrier, excipient, or diluent is typically a pharmaceutically acceptable carrier, excipient or diluent, respectively.

[0115] The terms "polypeptide" and "protein" are used interchangeably herein and refer to a molecular chain of two or more amino acids linked through peptide bonds. The terms do not refer to a specific length of the product. Thus, "peptides," and "oligopeptides," are included within the definition of polypeptide. The terms include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. The terms also include molecules in which one or more amino acid analogs or non-canonical or unnatural amino acids are included as can be synthesized, or expressed recombinantly using known protein engineering techniques. In addition, proteins can be derivatized.

[0116] The term "primary T-cell assay" as used herein refers to an in vitro assay to measure interferon-gamma ("IFN-gamma") expression. A variety of such primary T-cell assays are known in the art. In a preferred embodiment, the assay used is an anti-CD3 coimmobilizaton assay. In this assay, primary T cells are stimulated by anti-CD3 immobilized with or without additional recombinant proteins. Culture supernatants are harvested at timepoints, usually 24-72 hours. In another embodiment, the assay used is the MLR. In this assay, primary T cells are stimulated with allogeneic APC. Culture supernatants are harvested at timepoints, usually 24-72 hours. Human IFN-gamma levels are measured in culture supernatants by standard ELISA techniques. Commercial kits are available from vendors and the assay is performed according to manufacturer's recommendation.

[0117] The term "purified" as applied to nucleic acids, such as encoding immunomodulatory proteins of the invention, generally denotes a nucleic acid or polypeptide that is substantially free from other components as determined by analytical techniques well known in the art (e.g., a purified polypeptide or polynucleotide forms a discrete band in an electrophoretic gel, chromatographic eluate, and/or a media subjected to density gradient centrifugation). For example, a nucleic acid or polypeptide that gives rise to essentially one band in an electrophoretic gel is "purified." A purified nucleic acid or protein of the invention is at least about 50% pure, usually at least about 75%, 80%, 85%, 90%, 95%, 96%, 99% or more pure (e.g., percent by weight or on a molar basis).

[0118] The term "recombinant" indicates that the material (e.g., a nucleic acid or a polypeptide) has been artificially (i.e., non-naturally) altered by human intervention. The alteration can be performed on the material within, or removed from, its natural environment or state. For example, a "recombinant nucleic acid" is one that is made by recombining nucleic acids, e.g., during cloning, affinity modification, DNA shuffling or other well-known molecular biological procedures. A "recombinant DNA molecule," is comprised of segments of DNA joined together by means of such molecular biological techniques. The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule which is expressed using a recombinant DNA molecule. A "recombinant host cell" is a cell that contains and/or expresses a recombinant nucleic acid or that is otherwise altered by genetic engineering, such as by introducing into the cell a nucleic acid molecule encoding a recombinant protein, such as a transmembrane immunomodulatory protein provided herein. Transcriptional control signals in eukaryotes comprise "promoter" and "enhancer" elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with

cellular proteins involved in transcription. Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in yeast, insect and mammalian cells and viruses (analogous control elements, i.e., promoters, are also found in prokaryotes). The selection of a particular promoter and enhancer depends on what cell type is to be used to express the protein of interest. The terms "in operable combination," "in operable order" and "operably linked" as used herein refer to the linkage of nucleic acid sequences in such a manner or orientation that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced.

[0119] The term "recombinant expression vector" as used herein refers to a DNA molecule containing a desired coding sequence and appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host cell. Nucleic acid sequences necessary for expression in prokaryotes include a promoter, optionally an operator sequence, a ribosome binding site and possibly other sequences. Eukaryotic cells are known to utilize promoters, enhancers, and termination and polyadenylation signals. A secretory signal peptide sequence can also, optionally, be encoded by the recombinant expression vector, operably linked to the coding sequence for the recombinant protein, such as a recombinant fusion protein, so that the expressed fusion protein can be secreted by the recombinant host cell, for easier isolation of the fusion protein from the cell, if desired. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Among the vectors are viral vectors, such as lentiviral vectors.

[0120] The term "selectivity" refers to the preference of a subject protein, or polypeptide, for specific binding of one substrate, such as one cognate binding partner, compared to specific binding for another substrate, such as a different cognate binding partner of the subject protein. Selectivity can be reflected as a ratio of the binding activity (e.g. binding affinity) of a subject protein and a first substrate, such as a first cognate binding partner, (e.g., Kai) and the binding activity (e.g. binding affinity) of the same subject protein with a second cognate binding partner (e.g., K_{d2}).

[0121] The term "sequence identity" as used herein refers to the sequence identity between genes or proteins at the nucleotide or amino acid level, respectively. "Sequence identity" is a measure of identity between proteins at the amino acid level and a measure of identity between nucleic acids at nucleotide level. The protein sequence identity may be determined by comparing the amino acid sequence in a given position in each sequence when the sequences are aligned. Similarly, the nucleic acid sequence identity may be determined by comparing the nucleotide sequence in a given position in each sequence when the sequences are aligned. Methods for the alignment of sequences for comparison are well known in the art, such methods include GAP, BESTFIT, BLAST, FASTA and TFASTA. The BLAST algorithm calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Center for Biotechnology Information (NCBI) website.

[0122] The term "soluble" as used herein in reference to proteins, means that the protein is not a membrane protein. In general, a soluble protein contains only the extracellular domain of an IgSF family member receptor, or a portion thereof containing an IgSF domain or domains or specific-binding fragments thereof, but does not contain the transmembrane domain. In some cases, solubility of a protein can be improved by linkage or attachment, directly or indirectly via a linker, to an Fc domain, which, in some cases, also can improve the stability and/or half-life of the protein. In some aspects, a soluble protein is an Fc fusion protein.

[0123] The term "species" as used herein with respect to polypeptides or nucleic acids means an ensemble of molecules with identical or substantially identical sequences. Variation between polypeptides that are of the same species may occur owing to differences in post-translational modification such as glycosylation, phosphorylation, ubiquitination, nitrosylation, methylation, acetylation, and lipidation. Slightly truncated sequences of polypeptides that differ (or encode a difference) from the full length species at the amino-terminus or carboxy-terminus by no more than 1, 2, or 3 amino acid residues are considered to be of a single species. Such microbeterogeneities are a common feature of manufactured proteins.

[0124] The term "specific binding fragment" as used herein in reference to a full-length wild-type mammalian CD155 polypeptide or an IgV or an IgC (e.g. IgC2) domain thereof, means a polypeptide having a subsequence of the full-length polypeptide or an IgV and/or IgC domain and that specifically binds in vitro and/or in vivo to a mammalian TIGIT, mammalian CD226, and/or mammalian CD96 such as a human or murine TIGIT, CD226, or CD96. In some embodiments, the specific binding fragment comprises an CD155 IgV, a CD155 IgC2 subsequence that is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% the sequence length of the full-length wild-type sequence or an IgV or an IgC (e.g. IgC2) sequence thereof. The specific binding fragment can be altered in sequence to form a variant CD155 of the invention.

[0125] The term "specifically binds" as used herein means the ability of a protein, under specific binding conditions, to bind to a target protein such that its affinity or avidity is at least 5 times as great, but optionally at least 10, 20, 30, 40, 50, 100, 250 or 500 times as great, or even at least 1000 times as great as the average affinity or avidity of the same protein to a collection of random peptides or polypeptides of sufficient statistical size. A specifically binding protein need not bind exclusively to a single target molecule but may specifically bind to a non-target molecule due to similarity in structural conformation between the target and non-target (e.g., paralogs or orthologs). Those of skill will recognize that specific binding to a molecule having the same function in a different species of animal (i.e., ortholog) or to a non-target molecule having a substantially similar epitope as the target molecule (e.g., paralog) is possible and does not detract from the specificity of binding which is determined relative to a statistically valid collection of unique non-targets (e.g., random polypeptides). Thus, a polypeptide of the invention may specifically bind to more than one distinct species of target molecule due to cross-reactivity. Solid-phase ELISA immunoassays, ForteBio Octet, or Biacore measurements can be used to determine specific binding between two proteins. Generally, interactions between two binding proteins have dissociation constants (K_d) less than 1×10^{-5} M, and often as low as 1×10^{-12} M. In certain embodiments of the present disclosure, interactions between

two binding proteins have dissociation constants of less than or less than about 1×10^{-6} M, 1×10^{-7} M, 1×10^{-8} M, 1×10^{-9} M, 1×10^{-10} M or 1×10^{-11} M or less.

[0126] The terms "surface expresses" or "surface expression" in reference to a mammalian cell expressing a polypeptide means that the polypeptide is expressed as a membrane protein. In some embodiments, the membrane protein is a transmembrane protein.

[0127] As used herein, "synthetic," with reference to, for example, a synthetic nucleic acid molecule or a synthetic gene or a synthetic peptide refers to a nucleic acid molecule or polypeptide molecule that is produced by recombinant methods and/or by chemical synthesis methods.

[0128] The term "targeting moiety" as used herein refers to a composition that is covalently or non-covalently attached to, or physically encapsulates, a polypeptide comprising a variant CD155 of the present invention. The targeting moiety has specific binding affinity for a desired counter-structure such as a cell surface receptor (e.g., the B7 family member PD-L1), or a tumor antigen such as tumor specific antigen (TSA) or a tumor associated antigen (TAA) such as B7-H6. Typically, the desired counter-structure is localized on a specific tissue or cell-type. Targeting moieties include: antibodies, antigen binding fragment (Fab), variable fragment (Fv) containing V_H and V_L, the single chain variable fragment (scFv) containing V_H and V_L linked together in one chain, as well as other antibody V region fragments, such as Fab', $F(ab)_2$, F(ab')2, dsFv diabody, nanobodies, soluble receptors, receptor ligands, affinity matured receptors or ligands, as well as small molecule (<500 dalton) compositions (e.g., specific binding receptor compositions). Targeting moieties can also be attached covalently or non-covalently to the lipid membrane of liposomes that encapsulate a polypeptide of the present invention.

[0129] The term "transmembrane protein" as used herein means a membrane protein that substantially or completely spans a lipid bilayer such as those lipid bilayers found in a biological membrane such as a mammalian cell, or in an artificial construct such as a liposome. The transmembrane protein comprises a transmembrane domain ("transmembrane domain") by which it is integrated into the lipid bilayer and by which the integration is thermodynamically stable under physiological conditions. Transmembrane domains are generally predictable from their amino acid sequence via any number of commercially available bioinformatics software applications on the basis of their elevated hydrophobicity relative to regions of the protein that interact with aqueous environments (e.g., cytosol, extracellular fluid). A transmembrane domain is often a hydrophobic alpha helix that spans the membrane. A transmembrane protein can pass through the both layers of the lipid bilayer once or multiple times. A transmembrane protein includes the provided transmembrane immunomodulatory proteins described herein. In addition to the transmembrane domain, a transmembrane immunomodulatory protein of the invention further comprises an ectodomain and, in some embodiments, an endodomain.

[0130] The terms "treating," "treatment," or "therapy" of a disease or disorder as used herein mean slowing, stopping or reversing the disease or disorders progression, as evidenced by decreasing, cessation or elimination of either clinical or diagnostic symptoms, by administration of a therapeutic composition (e.g. containing an immunomodulatory protein or engineered cells) of the invention either alone or in combination with another compound as described herein. "Treating," "treatment," or "therapy" also means a decrease in the severity of symptoms in an acute or chronic disease or disorder or a decrease in the relapse rate as for example in the case of a relapsing or remitting autoimmune disease course or a decrease in inflammation in the case of an inflammatory aspect of an autoimmune disease. As used herein in the context of cancer, the terms "treatment" or, "inhibit," "inhibiting" or "inhibition" of cancer refers to at least one of: a statistically significant decrease in the rate of tumor growth, a cessation of tumor growth, or a reduction in the size, mass, metabolic activity, or volume of the tumor, as measured by standard criteria such as, but not limited to, the Response Evaluation Criteria for Solid Tumors (RECIST), or a statistically significant increase in progression free survival (PFS) or overall survival (OS). "Preventing," "prophylaxis," or "prevention" of a disease or disorder as used in the context of this invention refers to the administration of an immunomodulatory polypeptide or engineered cells of the invention, either alone or in combination with another compound, to prevent the occurrence or onset of a disease or disorder or some or all of the symptoms of a disease or disorder or to lessen the likelihood of the onset of a disease or disorder.

[0131] The term "tumor specific antigen" or "TSA" as used herein refers to a counter-structure that is present primarily on tumor cells of a mammalian subject but generally not found on normal cells of the mammalian subject. A tumor specific antigen need not be exclusive to tumor cells but the percentage of cells of a particular mammal that have the tumor specific antigen is sufficiently high or the levels of the tumor specific antigen on the surface of the tumor are sufficiently high such that it can be targeted by anti-tumor therapeutics, such as immunomodulatory polypeptides of the invention, and provide prevention or treatment of the mammal from the effects of the tumor. In some embodiments, in a random statistical sample of cells from a mammal with a tumor, at least 50% of the cells displaying a TSA are cancerous. In other embodiments, at least 60%, 70%, 80%, 85%, 90%, 95%, or 99% of the cells displaying a TSA are cancerous.

[0132] The term "variant" (also "modified" or mutant") as used in reference to a variant CD155 means a CD155, such as a mammalian (e.g., human or murine) CD155 created by human intervention. The variant CD155 is a polypeptide having an altered amino acid sequence, relative to an unmodified or wild-type CD155. The variant CD155 is a polypeptide which differs from a wild-type CD155 isoform sequence by one or more amino acid substitutions, deletions, additions, or combinations thereof. For purposes herein, the variant CD155 contains at least one affinity modified domain, whereby one or more of the amino acid differences occurs in an IgSF domain (e.g. IgV domain). A variant CD155 can contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more amino acid differences, such as amino acid substitutions. A variant CD155 polypeptide generally exhibits at least 50%, 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to a corresponding wild-type or unmodified CD155, such as to the sequence of SEQ ID NO:20, a mature sequence thereof (lacking the signal sequence) or a portion thereof containing the extracellular domain or an IgSF domain thereof. In some embodiments,

a variant CD155 polypeptide exhibits at least 50%, 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to a corresponding wild-type or unmodified CD155 comprising the sequence set forth in SEQ ID NO:47 or SEQ ID NO: 58 or 155. Non-naturally occurring amino acids as well as naturally occurring amino acids are included within the scope of permissible substitutions or additions. A variant CD155 is not limited to any particular method of making and includes, for example, de novo chemical synthesis, de novo recombinant DNA techniques, or combinations thereof. A variant CD155 of the invention specifically binds to at least one or more of: TIGIT, CD226, or CD96 of a mammalian species. In some embodiments, the altered amino acid sequence results in an altered (i.e., increased or decreased) binding affinity or avidity to TIGIT, CD226, and/or CD96 compared to the wild-type or unmodified CD155 protein. An increase or decrease in binding affinity or avidity can be determined using well known binding assays such as flow cytometry. Larsen et al., American Journal of Transplantation, Vol 5: 443-453 (2005). See also, Linsley et al., Immunity, Vol 1 (9): 793-801 (1994). An increase in variant CD155 binding affinity or avidity to TIGIT, CD226, and/or CD96 is to a value at least 5% greater than that of the wild-type or unmodified CD155 and in some embodiments, at least 10%, 15%, 20%, 30%, 40%, 50%, 100% greater than that of the wild-type or unmodified CD155 control value. A decrease in CD155 binding affinity or avidity to TIGIT, CD226, and/or CD96 is to a value no greater than 95% of the wild-type or unmodified control values, and in some embodiments no greater than 80%, 70% 60%, 50%, 40%, 30%, 20%, 10%, 5%, or no detectable binding affinity or avidity of the wild-type or unmodified control values. A variant CD155 is altered in primary amino acid sequence by substitution, addition, or deletion of amino acid residues. The term "variant" in the context of variant CD155 is not to be construed as imposing any condition for any particular starting composition or method by which the variant CD155 is created. A variant CD155 can, for example, be generated starting from wild type mammalian CD155 sequence information, then modeled in silico for binding to TIGIT, CD226, and/or CD96, and finally recombinantly or chemically synthesized to yield a variant CD155 of the present invention. In but one alternative example, a variant CD155 can be created by site-directed mutagenesis of a wild-type CD155. Thus, variant CD155 denotes a composition and not necessarily a product produced by any given process. A variety of techniques including recombinant methods, chemical synthesis, or combinations thereof, may be employed.

[0133] The term "wild-type" or "natural" or "native" as used herein is used in connection with biological materials such as nucleic acid molecules, proteins (e.g., CD155), IgSF members, host cells, and the like, refers to those which are found in nature and not modified by human intervention.

II. Variant CD155 Polypeptides

[0134] Provided herein are variant CD155 polypeptides that exhibit altered (increased or decreased) binding activity or affinity for one or more of a CD155 cognate binding partner. In some embodiments, the CD155 cognate binding partner is TIGIT, CD226, or CD96. In some embodiments, the CD155 cognate binding partner is TIGIT or CD226. In some embodiments, the variant CD155 polypeptide contains one or more amino acids modifications, such as one or more substitutions (alternatively, "mutations" or "replacements"), deletions or addition, in an immunoglobulin superfamily (IgSF) domain (IgD) relative to a wild-type or unmodified CD155 polypeptide or a portion of a wild-type or unmodified CD155 containing the IgD or a specific binding fragment thereof. Thus, a provided variant CD155 polypeptide is or comprises a variant IgD (hereinafter called "vIgD") in which the one or more amino acid modifications (e.g. substitutions) is in an IgD.

[0135] In some embodiments, the IgD comprises an IgV domain or an IgC (e.g. IgC2) domain or specific binding fragment of the IgV domain or the IgC (e.g. IgC2) domain, or combinations thereof. In some embodiments, the IgD can be an IgV only, the combination of the IgV and IgC, including the entire extracellular domain (ECD), or any combination of Ig domains of CD155. Table 2 provides exemplary residues that correspond to IgV or IgC regions of CD155. In some embodiments, the variant CD155 polypeptide contains an IgV domain or an IgC domain or specific binding fragments thereof in which the at least one of the amino acid modifications (e.g. substitutions) is in the IgV domain or IgC domain or a specific binding fragment thereof. In some embodiments, the variant CD155 polypeptide contains an IgV domain or specific binding fragments thereof in which the at least one of the amino acid modifications (e.g. substitutions) is in the IgV domain or a specific binding fragment thereof. In some embodiments, by virtue of the altered binding activity or affinity, the altered IgV domain or IgC (e.g. IgC2) domain is an affinity-modified IgSF domain.

[0136] In some embodiments, the variant is modified in one more IgSF domains relative to the sequence of an unmodified CD155 sequence. In some embodiments, the unmodified CD155 sequence is a wild-type CD155. In some embodiments, the unmodified or wild-type CD155 has the sequence of a native CD155 or an ortholog thereof. In some embodiments, the unmodified CD155 is or comprises the extracellular domain (ECD) of CD155 or a portion thereof containing one or more IgSF domain (see Table 2). In some embodiments, the extracellular domain of an unmodified or wild-type CD155 polypeptide comprises an IgV domain and an IgC (e.g. IgC2) domain or domains. However, the variant CD155 polypeptide need not comprise both the IgV domain and the IgC (e.g. IgC2) domain or domains. In some embodiments, the variant CD155 polypeptide comprises or consists essentially of the IgV domain or a specific binding fragment thereof. In some embodiments, the variant CD155 polypeptide comprises or consists essentially of one or both of the IgC (e.g. IgC2) domain or specific binding fragments thereof. In some embodiments, the variant CD155 polypeptide comprises or consists essentially of only one of the IgC (e.g. IgC2) domain or a specific binding fragment thereof. In some embodiments, the variant CD155 polypeptide comprises the IgV domain or a specific binding fragment thereof, and the first and second IgC (e.g. IgC2) domains or specific binding fragment thereof. In some embodiments, the variant CD155 is soluble and lacks a transmembrane domain. In some embodiments, the variant CD155 further comprises a transmembrane domain and, in some cases, also a cytoplasmic domain.

[0137] In some embodiments, the wild-type or unmodified CD155 sequence is a mammalian CD155 sequence. In some embodiments, the wild-type or unmodified CD155 sequence can be a mammalian CD155 that includes, but is not limited

to, human, mouse, cynomolgus monkey, or rat. In some embodiments, the wild-type or unmodified CD155 sequence is human.

[0138] In some embodiments, the wild-type or unmodified CD155 sequence has (i) the sequence of amino acids set forth in SEQ ID NO:20 or a mature form thereof lacking the signal sequence, (ii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:20 or the mature form thereof, or (iii) is a portion of (i) or (ii) containing an IgV domain or IgC (e.g. IgC2) domain or specific binding fragments thereof.

[0139] In some embodiments, the wild-type or unmodified CD155 sequence is or comprises an extracellular domain of the CD155 or a portion thereof. In some embodiments, the unmodified or wild-type CD155 polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 47, or an ortholog thereof. In some cases, the unmodified or wild-type CD155 polypeptide can comprise (i) the sequence of amino acids set forth in SEQ ID NO: 47, (ii) a sequence of amino acids that has at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 47, or (iii) is a specific binding fragment of the sequence of (i) or (ii) comprising an IgV domain or an IgC (e.g. IgC2) domain.

[0140] In some embodiments, the wild-type or unmodified CD155 polypeptide comprises an IgV domain or an IgC (e.g. IgC2) domain or domains, or a specific binding fragment thereof. In some embodiments, the IgV domain of the wild-type or unmodified CD155 polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 58 or 155 (corresponding to amino acid residues 24-139 or 21-142, respectively, of SEQ ID NO: 20), or an ortholog thereof. For example, the IgV domain of the unmodified or wild-type CD155 polypeptide can contain (i) the sequence of amino acids set forth in SEQ ID NO: 58 or 155, (ii) a sequence of amino acids that has at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 58 or 155, or (iii) a specific binding fragment of the sequence of (i) or (ii). In some embodiments, the wild-type or unmodified IgV domain is capable of binding one or more CD155 cognate binding proteins, such as one or more of TIGIT, CD226, or CD96.

[0141] In some embodiments, a first IgC2 domain of the wild-type or unmodified CD155 polypeptide comprises the amino acid sequence set forth as residues 145-237 of SEQ ID NO: 20, or an ortholog thereof. For example, an IgC2 domain of the unmodified or wild-type CD155 polypeptide can contain (i) the sequence of amino acids set forth as residues 145-237 of SEQ ID NO: 20, (ii) a sequence of amino acids that has at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to residues 145-237 of SEQ ID NO: 20, or (iii) a specific binding fragment of (i) or (ii). In some embodiments, the second IgC2 domain of the wild-type or unmodified CD155 polypeptide comprises the amino acid sequence set forth as residues 244-328 of SEQ ID NO: 20, or an ortholog thereof. For example, an IgC2 domain of the unmodified or wild-type CD155 polypeptide can contain (i) the sequence of amino acids set forth as residues 244-328 of SEQ ID NO: 20, (ii) a sequence of amino acids that has at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to residues 244-328 of SEQ ID NO: 20, or (iii) a specific binding fragment of (i) or (ii). In some embodiments, one or both of the wild-type or unmodified IgC domain is capable of binding one or more CD155 cognate binding proteins.

[0142] In some embodiments, the wild-type or unmodified CD155 polypeptide contains a specific binding fragment of CD155, such as a specific binding fragment of the IgV domain or the IgC (e.g. IgC2) domain. In some embodiments the specific binding fragment can bind TIGIT, CD226, and/or CD96. The specific binding fragment can have an amino acid length of at least 50 amino acids, such as at least 60, 70, 80, 90, 100, or 110 amino acids. In some embodiments, a specific binding fragment of the IgV domain contains an amino acid sequence that is at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% of the length of the IgV domain set forth as amino acids 24-139 of SEQ ID NO: 20. In some embodiments, a specific binding fragment of an IgC (e.g. IgC2) domain comprises an amino acid sequence that is at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% of the length of the IgC domain set forth as amino acids 145-237 of SEQ ID NO: 20. In some embodiments, a specific binding fragment of an IgC (e.g. IgC2) domain comprises an amino acid sequence that is at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% of the length of the IgC domain set forth as amino acids 244-328 of SEQ ID NO: 20.

[0143] In some embodiments, the variant CD155 polypeptide comprises the ECD domain or a portion thereof comprising one or more affinity modified IgSF domains. In some embodiments, the variant CD155 polypeptides can comprise an IgV domain or an IgC (e.g. IgC2) domain or domains, or a specific binding fragment of the IgV domain or a specific binding fragment of the IgC (e.g. IgC2) domain or domains in which one or more of the IgSF domains (IgV or IgC) contains the one or more amino acid modifications (e.g. substitutions). In some embodiments, the variant CD155 polypeptides can comprise an IgV domain and an IgC (e.g. IgC2) domain or domains, or a specific binding fragment of the IgV domain and a specific binding fragment of the IgC (e.g. IgC2) domain or domains, in which at least one of the IgV or IgC domain contains the amino acid modification(s) (e.g. substitutions). In some embodiments, the variant CD155 polypeptide comprises a full-length IgV domain. In some embodiments, the variant CD155 polypeptide comprises a full-length IgC (e.g. IgC2) domain or domains. In some embodiments, the variant CD155 polypeptide comprises a specific binding fragment of the IgV domain. In some embodiments, the variant CD155 polypeptide comprises a specific binding fragment of the IgC (e.g. IgC2) domain or domains. In some embodiments, the variant CD155 polypeptide comprises a full-length IgV domain and a full-length IgC (e.g. IgC2) domain or domains. In some embodiments, the variant CD155 polypeptide comprises a full-length IgV domain and a specific binding fragment of an IgC (e.g. IgC2) domain or domains. In some embodiments, the variant CD155 polypeptide comprises a specific binding fragment of an IgV domain and a full-length IgC (e.g. IgC2) domain or domains. In some embodiments, the variant CD155 polypeptide comprises a specific binding fragment of an IgV domain and a specific binding fragment of an IgC (e.g. IgC2) domain or domains.

[0144] In any of such embodiments, the one or more amino acid modifications (e.g. substitutions) of the variant CD155 polypeptides can be located in any one or more of the CD155 polypeptide IgSF domains. For example, in some embodiments, one or more amino acid modifications (e.g. substitutions) are located in the extracellular domain of the variant CD155 polypeptide. In some embodiments, one or more amino acid modifications (e.g. substitutions) are located in the IgV domain or specific binding fragment of the IgV domain. In some embodiments, one or more amino acid modifications (e.g. substitutions) are located in an IgC (e.g. IgC2) domain or specific binding fragment of an IgC (e.g. IgC2) domain.

[0145] Generally, each of the various attributes of polypeptides are separately disclosed below (e.g., soluble and membrane bound polypeptides, affinity of CD155 for TIGIT, CD226, and CD96, number of variations per polypeptide chain, number of linked polypeptide chains, the number and nature of amino acid alterations per variant CD155, etc.). However, as will be clear to the skilled artisan, any particular polypeptide can comprise a combination of these independent attributes. It is understood that reference to amino acids, including to a specific sequence set forth as a SEQ ID NO used to describe domain organization of an IgSF domain are for illustrative purposes and are not meant to limit the scope of the embodiments provided. It is understood that polypeptides and the description of domains thereof are theoretically derived based on homology analysis and alignments with similar molecules. Thus, the exact locus can vary, and is not necessarily the same for each protein. Hence, the specific IgSF domain, such as specific IgV domain or IgC domain, can be several amino acids (such as one, two, three or four) longer or shorter.

[0146] Further, various embodiments of the invention as discussed below are frequently provided within the meaning of a defined term as disclosed above. The embodiments described in a particular definition are therefore to be interpreted as being incorporated by reference when the defined term is utilized in discussing the various aspects and attributes described herein. Thus, the headings, the order of presentation of the various aspects and embodiments, and the separate disclosure of each independent attribute is not meant to be a limitation to the scope of the present disclosure.

[0147] Exemplary Modifications

[0148] Provided herein are variant CD155 polypeptides containing at least one affinity-modified IgSF domain (e.g. IgV or IgC) or a specific binding fragment thereof relative to an IgSF domain contained in a wild-type or unmodified CD155 polypeptide such that the variant CD155 polypeptide exhibits altered (increased or decreased) binding activity or affinity for one or more ligands TIGIT, CD226, or CD96 compared to a wild-type or unmodified CD155 polypeptide. In some embodiments, a variant CD155 polypeptide has a binding affinity for TIGIT, CD226, and/or CD96 that differs from that of a wild-type or unmodified CD155 polypeptide control sequence as determined by, for example, solid-phase ELISA immunoassays, flow cytometry, ForteBio Octet or Biacore assays. In some embodiments, the variant CD155 polypeptide has an increased binding affinity for TIGIT, CD226, and/or CD96. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for TIGIT, CD226, and/or CD96, relative to a wild-type or unmodified CD155 polypeptide. The TIGIT, CD226, and/or the CD96 can be a mammalian protein, such as a human protein or a murine protein.

[0149] Binding affinities for each of the cognate binding partners are independent; that is, in some embodiments, a variant CD155 polypeptide has an increased binding affinity for one, two or three of TIGIT, CD226, and/or CD96, and a decreased binding affinity for one, two or three of TIGIT, CD226, and CD96, relative to a wild-type or unmodified CD155 polypeptide.

[0150] In some embodiments, the variant CD155 polypeptide has an increased binding affinity for TIGIT, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has an increased binding affinity for CD226, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has an increased binding affinity for CD96, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for TIGIT, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for CD226, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for CD96, relative to a wild-type or unmodified CD155 polypeptide.

[0151] In some embodiments, the variant CD155 polypeptide has an increased binding affinity for TIGIT and CD226, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has an increased binding affinity for TIGIT and a decreased binding affinity for CD226, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for TIGIT and CD226, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for TIGIT and an increased binding affinity for CD226, relative to a wild-type or unmodified CD155 polypeptide.

[0152] In some embodiments, the variant CD155 polypeptide has an increased binding affinity for TIGIT and CD96, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has an increased binding affinity for TIGIT and a decreased binding affinity for CD96, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for TIGIT and CD96, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for TIGIT and an increased binding affinity for CD96, relative to a wild-type or unmodified CD155 polypeptide.

[0153] In some embodiments, the variant CD155 polypeptide has an increased binding affinity for CD226 and CD96, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has an increased binding affinity for CD226 and a decreased binding affinity for CD96, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for CD226 and CD96, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for

CD226 and an increased binding affinity for CD96, relative to a wild-type or unmodified CD155 polypeptide.

[0154] In some embodiments, the variant CD155 polypeptide has an increased binding affinity for TIGIT, CD226, and CD96, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has an increased binding affinity for TIGIT and CD226, and a decreased binding affinity for CD96, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has an increased binding affinity for TIGIT and CD96, and a decreased binding affinity for CD226, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for TIGIT and CD226, and an increased binding affinity for CD96, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for TIGIT and an increased binding affinity for CD226 and CD96, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has an increased binding affinity for TIGIT, and a decreased binding affinity for CD226 and CD96, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for TIGIT, CD96, and CD226, relative to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide has a decreased binding affinity for TIGIT, and an increased binding affinity for CD226 and CD96, relative to a wild-type or unmodified CD155 polypeptide.

[0155] In some embodiments, a variant CD155 polypeptide with increased or greater binding affinity to TIGIT, CD226, and/or CD96 will have an increase in binding affinity relative to the wild-type or unmodified CD155 polypeptide control of at least about 5%, such as at least about 10%, 15%, 20%, 25%, 35%, or 50% for the TIGIT, CD226, and/or CD96. In some embodiments, the increase in binding affinity relative to the wild-type or unmodified CD155 polypeptide is more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold 40-fold or 50-fold. In such examples, the wild-type or unmodified CD155 polypeptide has the same sequence as the variant CD155 polypeptide except that it does not contain the one or more amino acid modifications (e.g. substitutions).

[0156] In some embodiments, a variant CD155 polypeptide with reduced or decreased binding affinity to TIGIT, CD226, and/or CD96 will have decrease in binding affinity relative to the wild-type or unmodified CD155 polypeptide control of at least 5%, such as at least about 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more for the TIGIT, CD226, and/or CD96. In some embodiments, the decrease in binding affinity relative to the wild-type or unmodified CD155 polypeptide is more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold 40-fold or 50-fold. In such examples, the wild-type or unmodified CD155 polypeptide has the same sequence as the variant CD155 polypeptide except that it does not contain the one or more amino acid modifications (e.g. substitutions).

[0157] In some embodiments, the equilibrium dissociation constant (K_d) of any of the foregoing embodiments to TIGIT, CD226, and/or CD96 can be less than 1×10^{-5} M, 1×10^{-6} M, 1×10^{-7} M, 1×10^{-8} M, 1×10^{-9} M, 1×10^{-10} M or 1×10^{-11} M, or 1×10^{-12} M or less.

[0158] The wild-type or unmodified CD155 sequence does not necessarily have to be used as a starting composition to generate variant CD155 polypeptides described herein. Therefore, use of the term "modification", such as "substitution" does not imply that the present embodiments are limited to a particular method of making variant CD155 polypeptides. Variant CD155 polypeptides can be made, for example, by de novo peptide synthesis and thus does not necessarily require a modification, such as a "substitution" in the sense of altering a codon to encode for the modification, e.g. substitution. This principle also extends to the terms "addition" and "deletion" of an amino acid residue which likewise do not imply a particular method of making. The means by which the variant CD155 polypeptides are designed or created is not limited to any particular method. In some embodiments, however, a wild-type or unmodified CD155 encoding nucleic acid is mutagenized from wild-type or unmodified CD155 genetic material and screened for desired specific binding affinity and/or induction of IFN-gamma expression or other functional activity. In some embodiments, a variant CD155 polypeptide is synthesized de novo utilizing protein or nucleic acid sequences available at any number of publicly available databases and then subsequently screened. The National Center for Biotechnology Information provides such information and its website is publicly accessible via the internet as is the UniProtKB database as discussed previously.

[0159] Unless stated otherwise, as indicated throughout the present disclosure, the amino acid modification(s) are designated by amino acid position number corresponding to the numbering of positions of the unmodified ECD sequence set forth in SEQ ID NO:47 or, where applicable, the unmodified IgV sequence set forth in SEQ ID NO:58 or SEQ ID NO: 155 as follows:

(SEQ ID NO: 47)

WPPPGTGDVVVQAPTQVPGFLGDSVTLPCYLQVPNMEVTHVSQLTWARHG

ESGSMAVFHQTQGPSYSESKRLEFVAARLGAELRNASLRMFGLRVEDEGN

YTCLFVTFPQGSRSVDIWLRVLAKPQNTAEVQKVQLTGEPVPMARCVSTG

GRPPAQITWHSDLGGMPNTSQVPGFLSGTVTVTSLWILVPSSQVDGKNVT

CKVEHESFEKPQLLTVNLTVYYPPEVSISGYDNNWYLGQNEATLTCDARS

NPEPTGYNWSTTMGPLPPFAVAQGAQLLIRPVDKPINTTLICNVTNALGA

RQAELTVQVKEGPPSEHSGISRN

(SEQ ID NO: 58)

PGTGDVVVQAPTQVPGFLGDSVTLPCYLQVPNMEVTHVSQLTWARHGESG

SMAVFHQTQGPSYSESKRLEFVAARLGAELRNASLRMFGLRVEDEGNYTC

LFVTFPQGSRSVDIWL

(SEQ ID NO: 155)

WPPPGTGDVVVQAPTQVPGFLGDSVTLPCYLQVPNMEVTHVSQLTWARHG

ESGSMAVFHQTQGPSYSESKRLEFVAARLGAELRNASLRMFGLRVEDEGN

YTCLFVTFPQGSRSVDIWLRVL

[0160] It is within the level of a skilled artisan to identify the corresponding position of a modification, e.g. amino acid substitution, in a CD155 polypeptide, including portion

thereof containing an IgSF domain (e.g. IgV) thereof, such as by alignment of a reference sequence with SEQ ID NO:47 or SEQ ID NO:58 or SEQ ID NO:155. In the listing of modifications throughout this disclosure, the amino acid position is indicated in the middle, with the corresponding unmodified (e.g. wild-type) amino acid listed before the number and the identified variant amino acid substitution listed after the number. If the modification is a deletion of the position a "del" is indicated and if the modification is an insertion at the position an "ins" is indicated. In some cases, an insertion is listed with the amino acid position indicated in the middle, with the corresponding unmodified (e.g. wild-type) amino acid listed before and after the number and the identified variant amino acid insertion listed after the unmodified (e.g. wild-type) amino acid.

[0161] In some embodiments, the variant CD155 polypeptide has one or more amino acid modification, e.g. substitutions in a wild-type or unmodified CD155 sequence. The one or more amino acid modification, e.g. substitutions can be in the ectodomain (extracellular domain) of the wild-type or unmodified CD155 sequence. In some embodiments, the one or more amino acid modification, e.g. substitutions are in the IgV domain or specific binding fragment thereof. In some embodiments, the one or more amino acid modification, e.g. substitutions are in an IgC (e.g. IgC2) domain or specific binding fragment thereof. In some embodiments of the variant CD155 polypeptide, some of the one or more amino acid modification, e.g. substitutions are in the IgV domain or a specific binding fragment thereof, and some of the one or more amino acid modification, e.g. substitutions are in an IgC domain or domains (e.g. IgC2) or a specific binding fragment thereof.

[0162] In some embodiments, the variant CD155 polypeptide has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid modification, e.g. substitutions. The substitutions can be in the IgV domain or the IgC (e.g. IgC2) domain or domains. In some embodiments, the variant CD155 polypeptide has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid modification, e.g. substitutions in the IgV domain or specific binding fragment thereof. In some embodiments, the variant CD155 polypeptide has up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid modification, e.g. substitutions in the IgC (e.g. IgC2) domain or domains or specific binding fragment thereof. In some embodiments, the variant CD155 polypeptide has at least about 85%, 86%, 86%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the wild-type or unmodified CD155 polypeptide or specific binding fragment thereof, such as with the amino acid sequence of SEQ ID NO: 47, 58 or 155.

[0163] In some embodiments, the variant CD155 polypeptide has one or more amino acid modification, e.g. substitutions in an unmodified CD155 or specific binding fragment thereof corresponding to position(s) 7, 8, 9, 10, 11, 12, 13, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 67, 68, 69, 70, 72, 73, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 87, 88, 89, 90, 91, 92, 94, 95, 96, 97, 98, 99, 100, 102, 104, 106, 107, 108, 110, 111, 112, 113, 114, 115, or 116 with reference to positions set forth in SEQ ID NO: 47. In some embodiments, such variant CD155 polypeptides exhibit altered binding affinity to one or more of TIGIT, CD226, and/or CD96 compared to the wild-type or unmodified CD155 polypeptide. For example, in some embodiments, the variant CD155 polypeptide exhibits increased binding affinity to TIGIT, CD226, and/or CD96 compared to a wild-type or unmodified CD155 polypeptide. In some embodiments, the variant CD155 polypeptide exhibits decreased binding affinity to TIGIT, CD226, or CD96 compared to a wild-type or unmodified CD155 polypeptide.

[0164] In some embodiments, the variant CD155 polypeptide has one or more amino acid modifications selected from G7E, D8G, V9A, V9D, V9I, V9L, V10F, V10G, V10I, V11A, V11E, V11M, Q12H, Q12K, Q12L, A13E, A13R, T15I, T15S, Q16H, P18C, P18F, P18H, P18L, P18S, P18T, P18Y, G19D, F20I, F20S, F20Y, L21S, L21M, G22S, D23A, D23G, D23N, D23Y, S24A, S24P, V25A, V25E, T26M, C29R, Y30C, Y30F, Y30H, Q32L, Q32R, V33M, P34S, N35D, N35F, N35S, M36I, M36R, M36T, E37G, E37P, E37S, E37V, V38A, V38G, T39A, T39S, H40Q, H40R, H40T, V41A, V41M, S42A, S42C, S42G, S42L, S42N, S42P, S42Q, S42T, S42V, S42W, L44P, L44V, T45A, T45G, T45I, T45S, T45Q, T45V, W46C, W46R, A47E, A47G, A47V, R48Q, H49L, H49Q, H49R, G50S, E51G, E51K, E51V, S52A, S52E, S52G, S52K, S52L, S52M, S52P, S52Q, S52R, S52T, S52W, G53R, S54C, S54G, S54H, S54N, S54R, M55I, M55L, M55V, A56V, V57A, V57L, V57T, F58L, F58Y, H59E, H59N, N59R, Q60H, Q60K, Q60P, Q60R, T61A, T61G, T61K, T61M, T61R, T61S, Q62F, Q62H, Q62K, Q62L, Q62M, Q62R, Q62Y, P64S, S65A, S65C, S65G, S65D, S65T, S65Y, S65H, S65N, S65T, S65W, S67A, S67E, S67G, S67H, S67L, S67T, S67V, S67W, E68G, S69L, S69P, K70E, K70R, K70Q, L72Q, E73D, E73G, E73R, V75A, V75L, A76E, A76G, A76T, A77T, A77V, R78G, R78K, R78S, L79P, L79Q, L79V, G80D, G80S, A81E, A81P, A81T, A81V, E82D, E82G, L83P, L83Q, R84W, N85D, N85Y, N87T, L88P, R89K, M90I, M90L, M90V, F91S, F91P, F91T, G92A, G92E, G92W, R94H, V95A, E96D, D97G, E98D, E98S, G99D, G99Y, N100Y, T102S, L104E, L104M, L104N, L104P, L104Q, L104T, L104Y, V106A, V1061, V106L, T107A, T107L, T107M, T107S, T107V, F108H, F108L, F108Y, Q110R, G111D, G111R, S112I, S112N, S112V, R113G, R113W, S114N, S114T, V115A, V115M, D116G, or D116N, or a conservative amino acid substitution thereof. A conservative amino acid substitution is any amino acid that falls in the same class of amino acids as the substituted amino acids, other than the wild-type or unmodified amino acid. The classes of amino acids are aliphatic (glycine, alanine, valine, leucine, and isoleucine), hydroxyl or sulfur-containing (serine, cysteine, threonine, and methionine), cyclic (proline), aromatic (phenylalanine, tyrosine, tryptophan), basic (histidine, lysine, and arginine), and acidic/amide (aspartate, glutamate, asparagine, and glutamine).

[0165] In some embodiments, the variant CD155 polypeptide has two or more amino acid modifications selected from G7E, D8G, V9A, V9D, V9I, V9L, V10F, V10G, V10I, V11A, V11E, V11M, Q12H, Q12K, Q12L, A13E, A13R, T15I, T15S, Q16H, P18C, P18F, P18H, P18L, P18S, P18T, P18Y, G19D, F20I, F20S, F20Y, L21S, L21M, G22S, D23A, D23G, D23N, D23Y, S24A, S24P, V25A, V25E, T26M, C29R, Y30C, Y30F, Y30H, Q32L, Q32R, V33M, P34S, N35D, N35F, N35S, M36I, M36R, M36T, E37G, E37P, E37S, E37V, V38A, V38G, T39A, T39S, H40Q, H40R, H40T, V41A, V41M, S42A, S42C, S42G, S42L, S42N, S42P, S42Q, S42T, S42V, S42W, L44P, L44V, T45A, T45G,

T45I, T45S, T45Q, T45V, W46C, W46R, A47E, A47G, A47V, R48Q, H49L, H49Q, H49R, G50S, E51G, E51K, E51V, S52A, S52E, S52G, S52K, S52L, S52M, S52P, S52Q, S52R, S52T, S52W, G53R, S54C, S54G, S54H, S54N, S54R, M55I, M55L, M55V, A56V, V57A, V57L, V57T, F58L, F58Y, H59E, H59N, N59R, Q60H, Q60K, Q60P, Q60R, T61A, T61G, T61K, T61M, T61R, T61S, Q62F, Q62H, Q62K, Q62L, Q62M, Q62R, Q62Y, P64S, S65A, S65C, S65G, S65D, S65T, S65Y, S65H, S65N, S65T, S65W, S67A, S67E, S67G, S67H, S67L, S67T, S67V, S67W, E68D, E68G, S69L, S69P, K70E, K70R, K70Q, L72Q, E73D, E73G, E73R, V75A, V75L, A76E, A76G, A76T, A77T, A77V, R78G, R78K, R78S, L79P, L79Q, L79V, G80D, G80S, A81E, A81P, A81T, A81V, E82D, E82G, L83P, L83Q, R84W, N85D, N85Y, N87T, L88P, R89K, M90I, M90L, M90V, F91S, F91T, F91P, G92A, G92E, G92W, R94H, V95A, E96D, D97G, E98D, E98S, G99D, G99Y, N100Y, T102S, L104E, L104M, L104N, L104P, L104Q, L104T, L104Y, V106A, V1061, V106L, T107A, T107L, T107M, T107S, T107V, F108H, F108L, F108Y, Q110R, G111D, G111R, S112I, S112N, S112V, R113G, R113W, S114N, S114T, V115A, V115M, D116G, or D116N. In some embodiments, the two or more amino acid modifications is P18S/P64S/F91S, P18S/F91S/L104P, P18L/L79V/F91S, P18S/F91S, P18T/F91S, P18T/S42P/F91S, G7E/P18T/Y30C/F91S, P18T/F91S/G111D, P18S/F91P, P18T/F91S/F108L, P18S/F91S, P18T/T45A/F91S, P18T/F91S/R94H, P18S/Y30C/F91S, A81V/L83P, A13E/P18S/A56V/F91S, P18T/F91S/V115A, P18T/Q60K, S52M, T45Q/S52L/L104E/G111R, S42G, Q62F, S52Q, S42A/L104Q/G111R, S42A/S52Q/L104Q/G111R, S52W/L104E, S42C, S52W, S52M/L104Q, S42L/S52L/Q62F/L104Q, S42W, S42Q, S52L, S52R, L104E, G111R, S52E, Q62Y, T45Q/S52M/L104E, S42N/L104Q/G111R, S52M/V57L, S42N/S52Q/Q62F, S42A/S52L/L104E/G111R, S42W/S52Q/V57L/Q62Y, L104Q, S42L/S52Q/L104E, S42C/S52L, S42W/S52R/Q62Y/L104Q, T45Q/S52R/L104E, S52R/Q62F/L104Q/G111R, T45Q/S52L/V57L/L104E, S52M/Q62Y, Q62F/L104E/G111R, T45Q/S52Q, S52L/L104E, S42V/S52E, T45Q/S52R/G111R, S42G/S52Q/L104E/G111R, S42N/S52E/V57L/L104E, S42C/S52M/Q62F, S42L, S42A, S42G/S52L/Q62F/L104Q, S42N, P18T/S65A/S67V/F91S, P18F/T39A/T45Q/T61R/S65N/S67L/E73G/R78G, P18T/T45Q/T61R/S65N/S67L, P18F/S65A/S67V/F91S, P18F/T45Q/T61R/S65N/S67L/F91S/L104P, P18S/L79P/L104M, P18S/L104M, L79P/L104M, P18T/T45Q/L79P, P18T/T45Q/T61R/S65H/S67H, P18T/A81E, P18S/D23Y/E37P/S52G/Q62M/G80S/A81P/G99Y/S 112N, A13R/D23Y/E37P/S42P/Q62Y/A81E, A13R/D23Y/E37P/G99Y/S112N, A13R/D23Y/E37P/Q62M/A77V/G80S/A81P/G99Y, P18L/E37S/Q62M/G80S/A81P/G99Y/S112N, P18S/L104T, P18S/Q62H/L79Q/F91S, T45Q/S52K/Q62F/L104Q/G111R, T45Q/S52Q/Q62Y/L104Q/G111R, T45Q/S52Q/Q62Y/L104E/G111R, V57A/T61M/S65W/S67A/E96D/L104T, P18L/V57T/T61S/S65Y/S67A/L104T, P18T/T45Q, P18L/V57A/T61M/S65W/S67A/L104T, T61M/S65W/S67A/L104T, P18S/V41A/S42G/T45G/L104N, P18H/S42G/T45I/S52T/G53R/S54H/V57L/H59E/T61S/S65D/E68G/L104N, P18S/S42G/T45V/F58L/S67W/L104N, P18S/T45I/L104N, P18S/S42G/T45G/L104N/V106A, P18H/H40R/S42G/T45I/S52T/G53R/S54H/V57L/H59E/T61S/S65D/E68G/L104Y/V106L/F108H, E37V/S42G/T45G/L104N, P18S/T45Q/L79P/L104T, P18L/Q62R, A13R/D23Y/E37P/S42L/S52G/Q62Y/A81E, P18L/H49R/L104T/D116N, A13R/D23Y/E37P/Q62M/G80S/A81P/L104T, S65T/L104T, A13R/D23Y/E37P/S52G/V57A/Q62M/K70E/L104T, P18L/A47V/Q62Y/E73D/L104T, H40T/V411M/A47V/S52Q/Q62L/S65T/E73R/D97G/E98S/L104T/D116N, P18L/S42P/T45Q/T61G/S65H/S67E/L104T/D116N, P18S/H40T/V41M/A47V/S52Q/Q62L/S65T/E73R/L104M/V106A, H40T/V41M/A47V/S52Q/Q62L/S65T/E68G/E73R/D97G/E98S/L104T, T45Q/S52E/L104E, T45Q/S52E/Q62F/L104E, P18F/T26M/L44V/Q62K/L79P/F91S/L104M/G111D, P18S/T45S/T61K/S65W/S67A/F91S/G111R, P18S/L79P/L104M/T107M, P18S/S65W/S67A/M90V/V95A/L104Q/G111R, P18S/A47G/L79P/F91S/L104M/T107A/R113W, P18T/D23G/S24A/N35D/H49L/L79P/F91S/L104M/G111R, V9L/P18S/Q60R/V75L/L79P/R89K/F91S/L104E/G111R, P18S/H49R/E73D/L79P/N85D/F91S/V95A/L104M/G111R, V11A/P18S/L79P/F91S/L104M/G111R, V11A/P18S/S54R/Q60P/Q62K/L79P/N85D/F91S/T107M, P18T/S52P/S65A/S67V/L79P/F91S/L104M/G111R, P18T/M36T/L79P/F91S/G111R, D8G/P18S/M36I/V38A/H49Q/A76E/F91S/L104M/T107A/R113W, P18S/S52P/S65A/S67V/L79P/F91S/L104M/T107S/R113W, T15I/P18T/L79P/F91S/L104M/G111R, P18F/T26M/L44V/Q62K/L79P/E82D/F91S/L104M/G111D, P18T/E37G/G53R/Q62K/L79P/F91S/E98D/L104M/T107M, P18L/K70E/L79P/F91S/V95A/G111R, V9I/Q12K/P18F/S65A/S67V/L79P/L104T/G111R/S112I, P18F/S65A/S67V/F91S/L104M/G111R, V9I/V10I/P18S/F20S/T45A/L79P/F91S/L104M/F108Y/G111R/S112V, V9L/P18L/L79P/M90I/F91S/T102S/L104M/G111R, P18C/T26M/L44V/M55I/Q62K/L79P/F91S/L104M/T107M, V9I/P18T/D23G/L79P/F91S/G111R, P18F/L79P/M90L/F91S/V95A/L104M/G111R, P18T/M36T/S65A/S67E/L79Q/A81T/F91S/G111R, V9L/P18T/Q62R/L79P/F91S/L104M/G111R, P18S/S65W/S67A/L104Q/G111R, P18T/G19D/M36T/S54N/L79P/L83Q/F91S/T107M/F108Y, V9L/P18L/M55V/S69L/L79P/A81E/F91S/T107M, P18F/H40Q/T61K/Q62K/L79P/F91S/L104M/T107V, P18S/Q32R/Q62K/R78G/L79P/F91S/T107A/R113W, Q12H/P18T/L21S/G22S/V57A/Q62R/L79P/F91S/T107M, V9I/P18S/S24P/H49Q/F58Y/Q60R/Q62K/L79P/F91S/T107M, P18T/W46C/H49R/S65A/S67V/A76T/L79P/S87T/L104M, P18S/S42T/E51G/L79P/F91S/G92W/T107M, V10F/T15S/P18L/R48Q/L79P/F91S/T107MIV 115M, P18S/L21M/Y30F/N35D/R84W/F91S/T107M/D 116G, P18F/E51V/S54G/Q60R/L79Q/E82G/S87T/M901/F91S/G92R/T107M, Q16H/P18F/F91S/T107M, P18T/D23G/Q60R/S67L/L79P/F91S/T107M/V115A, D8G/V9I/V11A/P18T/T26M/S52P/L79P/F91S/G92A/T107L/V115A, V9I/P18F/A47E/G50S/E68G/L79P/F91S/T107M, P18S/M55I/Q62K/S69P/L79P/F91S/T107M, P18T/T39S/S52P/S54R/L79P/F91S/T107M, P18S/D23N/L79P/F91S/T107M/S114N, P18S/P34S/E51V/L79P/F91S/G111R, P18S/H59N/V75A/L79P/A81T/F91S/L104M/T107M, P18S/W46R/E68D/L79P/F91S/T107M/R113G, V9L/P18F/T45A/S65A/S67V/R78K/L79V/F91S/T107M/S114T, P18T/M55L/T61R/L79P/F91S/V106I/T107M, T15I/P18S/V33M/N35F/T39S/M55L/R78S/L79P/F91S/T107M, P18S/Q62K/K70E/L79P/F91S/G92E/R113W, P18F/F20I/T26M/A47V/E51K/L79P/F91S, P18T/D23A/Q60H/L79P/M90V/F91S/T107M, P18S/D23G/C29R/N35D/E37G/M55I/Q62K/S65A/S67G/R78G/L79P/F91S/L104M/T107M/Q110R, A13E/P18S/M36R/Q62K/S67T/L79P/N85D/F91S/T107M, V9I/P18T/H49R/L79P/N85D/F91S/L104T/T107M, V9A/P18F/T61S/Q62L/L79P/F91S/

G111R, D8E/P18T/T61A/L79P/F91S/T107M, P18S/V41A/H49R/S54C/L79S/N85Y/L88P/F91S/L104M/T107M, V11E/P18H/F20Y/V25E/N35S/H49R/L79P/F91S/T107M/G111R, V11A/P18F/D23A/L79P/G80D/V95A/T107M, P18S/K70R/L79P/F91S/G111R, V9L/V11M/P18S/N35S/S54G/Q62K/L79P/L104M/T107M/V115M, V9L/P18Y/V25A/V38G/M55V/A77T/L79P/M90I/F91S/L104M, V10G/P18T/L72Q/L79P/F91S/T107M, P18S/H59R/A76G/R78S/L79P, V9A/P18S/M36T/S65G/L79P/F91S/L104T/G111R/S112I, P18T/S52A/V57A/Q60R/Q62K/S65C/L79P/F91T/N100Y/T107M, V11A/P18F/N35D/A47E/Q62K/L79P/F91S/G99D/T107M/S114N, V11A/P18T/N35S/L79P/S87T/F91S, V9D/V11M/Q12L/P18S/E37V/M55I/Q60R/K70Q/L79P/F91S/L104M/T107M, or T15S/P18S/Y30H/Q32L/Q62R/L79P/F91S/T107M.

[0166] In some embodiments, the variant CD155 polypeptide comprises any of the substitutions (mutations) listed in Table 1. Table 1 also provides exemplary sequences by reference to SEQ ID NO for the extracellular domain (ECD) or IgV domain of wild-type CD155 or exemplary variant CD155 polypeptides. As indicated, the exact locus or residues corresponding to a given domain can vary, such as depending on the methods used to identify or classify the domain. Also, in some cases, adjacent N- and/or C-terminal amino acids of a given domain (e.g. IgV) also can be included in a sequence of a variant IgSF polypeptide, such as to ensure proper folding of the domain when expressed. Thus, it is understood that the exemplification of the SEQ ID NOSs in Table 1 is not to be construed as limiting. For example, the particular domain, such as the IgV domain, of a variant CD155 polypeptide can be several amino acids longer or shorter, such as 1-10, e.g. 1, 2, 3, 4, 5, 6 or 7 amino acids longer or shorter, than the sequence of amino acids set forth in the respective SEQ ID NO. In some embodiments, the variant CD155 polypeptide comprises any of the mutations listed in Table 1. In some embodiments, the variant CD155 polypeptide comprises any of the extracellular domain (ECD) sequences listed in Table 1 (i.e., any one of SEQ ID NOS: 59-80, 178-274, 1230-1252, 1269, and 1610-1655). In some embodiments, the variant CD155 polypeptide comprises a polypeptide sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any of the extracellular domain (ECD) sequences listed in Table 1 (i.e., any one of SEQ ID NOS: 59-80, 178-274, 1230-1252, 1269, and 1610-1655) and contains the amino acid modification(s), e.g. substitution(s) not present in the wild-type or unmodified CD155. In some embodiments, the variant CD155 polypeptide comprises a specific binding fragment of any of the extracellular domain (ECD) sequences listed in Table 1 (i.e., any one of SEQ ID NOS: 59-80, 178-274, 1230-1252, 1269, and 1610-1655) and contains the amino acid modification(s), e.g. substitution(s) not present in the wild-type or unmodified CD155. In some embodiments, the variant CD155 polypeptide comprises any of the IgV sequences listed in Table 1 (i.e., any one of SEQ ID NOS: 81-102, 156-177, 275-468, 1184-1229, 1270-1271, 1656-1747). In some embodiments, the variant CD155 polypeptide comprises a polypeptide sequence that exhibits at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, such as at least 96% identity, 97% identity, 98% identity, or 99% identity to any of the IgV sequences listed in Table 1 (i.e., any one of SEQ ID NOS: 81-102, 156-177, 275-468, 1184-1229, 1270-1271, 1656-1747) and contains the amino acid modification(s), e.g. substitution(s) not present in the wild-type or unmodified CD155. In some embodiments, the variant CD155 polypeptide comprises a specific binding fragment of any of the IgV sequences listed in Table 1 (i.e., any one of SEQ ID NOS: 81-102, 156-177, 275-468, 1184-1229, 1270-1271, 1656-1747) and contains the amino acid modification(s), e.g. substitution(s) not present in the wild-type or unmodified CD155.

TABLE 1

Exemplary variant CD155 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
Wild-type	47	58, 155
P18S, P64S, F91S	59	81, 156
P18S, F91S, L104P	60	82, 157
L44P	61	83, 158
A56V	62	84, 159
P18L, L79V, F91S	63	85, 160
P18S, F91S	64	86, 161
P18T, F91S	65	87, 162
P18T, S42P, F91S	66	88, 163
G7E, P18T, Y30C, F91S	67	89, 164
P18T, F91S, G111D	68	90,165
P18S, F91P	69	91, 166
P18T, F91S, F108L	70	92, 167
P18T, T45A, F91S	72	94, 169
P18T, F91S, R94H	73	95, 170
P18S, Y30C, F91S	74	96, 171
A81V, L83P	75	97, 172
L88P	76	98, 173
R94H	77	99, 174
A13E, P18S, A56V, F91S	78	100, 175
P18T, F91S, V115A	79	101, 176
P18T, Q60K	80	102, 177
S52M	178	275, 372
T45Q, S52L, L104E, G111R	179	276, 373
S42G	180	277, 374
Q62F	181	278, 375
S52Q	182	279, 376
S42A, L104Q, G111R	183	280, 377
S42A, S52Q, L104Q, G111R	184	281, 378
S52W, L104E	185	282, 379
S42C	186	283, 380
S52W	187	284, 381
S52M, L104Q	188	285, 382
S42L, S52L, Q62F, L104Q	189	286, 383
S42W	190	287, 384
S42Q	191	288, 385
S52L	192	289, 386
S52R	193	290, 387
L104E	194	291, 388
G111R	195	292, 389
S52E	196	293, 390
Q62Y	197	294, 391
T45Q, S52M, L104E	198	295, 392
S42N, L104Q, G111R	199	296, 393
S52M, V57L	200	297, 394
S42N, S52Q, Q62F	201	298, 395
S42A, S52L, L104E, G111R	202	299, 396
S42W, S52Q, V57L, Q62Y	203	300, 397
L104Q	204	301, 398
S42L, S52Q, L104E	205	302, 399
S42C, S52L	206	303, 400
S42W, S52R, Q62Y, L104Q	207	304, 401
T45Q, S52R, L104E	208	305, 402
S52R, Q62F, L104Q, G111R	209	306, 403
T45Q, S52L, V57L, L104E	210	307, 404

TABLE 1-continued

Exemplary variant CD155 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
S52M, Q62Y	211	308, 405
Q62F, L104E, G111R	212	309, 406
T45Q, S52Q	213	310, 407
S52L, L104E	214	311, 408
S42V, S52E	215	312, 409
T45Q, S52R, G111R	216	313, 410
S42G, S52Q, L104E, G111R	217	314, 411
S42N, S52E, V57L, L104E	218	315, 412
S42C, S52M, Q62F	219	316, 413
S42L	220	317, 414
S42A	221	318, 415
S42G, S52L, Q62F, L104Q	222	319, 416
S42N	223	320, 417
P18T, S65A, S67V, F91S	224	321, 418
P18F, T39A, T45Q, T61R, S65N, S67L, E73G, R78G	225	322, 419
P18T, T45Q, T61R, S65N, S67L	226	323, 420
P18F, S65A, S67V, F91S	227	324, 421
P18F, T45Q, T61R, S65N, S67L, F91S, L104P	228	325, 422
P18S, L79P, L104M	229	326, 423
P18S, L104M	230	327, 424
L79P, L104M	231	328, 425
P18T, T45Q, L79P	232	329, 426
P18T, T45Q, T61R, S65H, S67H	233	330, 427
P18T, A81E	234	331, 428
P18S, D23Y, E37P, S52G, Q62M, G80S, A81P, G99Y, S112N	235	332, 429
A13R, D23Y, E37P, S42P, Q62Y, A81E	236	333, 430
A13R, D23Y, E37P, G99Y, S112N	237	334, 431
A13R, D23Y, E37P, Q62M, A77V, G80S, A81P, G99Y	238	335, 432
P18L, E37S, Q62M, G80S, A81P, G99Y, S112N	239	336, 433
P18S, L104T	240	337, 434
P18S, Q62H, L79Q, F91S	241	338, 435
T45Q, S52K, Q62F, L104Q, G111R	242	339, 436
T45Q, S52Q, Q62Y, L104Q, G111R	243	340, 437
T45Q, S52Q, Q62Y, L104E, G111R	244	341, 438
V57A, T61M, S65W, S67A, E96D, L104T	245	342, 439
P18L, V57T, T61S, S65Y, S67A, L104T	246	343, 440
P18T, T45Q	247	344, 441
P18L, V57A, T61M, S65W, S67A, L104T	248	345, 442
T61M, S65W, S67A, L104T	249	346, 443
P18S, V41A, S42G, T45G, L104N	250	347, 444
P18H, S42G, T45I, S52T, G53R, S54H, V57L, H59E, T61S, S65D, E68G, L104N	251	348, 445
P18S, S42G, T45V, F58L, S67W, L104N	252	349, 446
P18S, T45I, L104N	253	350, 447
P18S, S42G, T45G, L104N, V106A	254	351, 448
P18H, H40R, S42G, T45I, S52T, G53R, S54H, V57L, H59E, T61S, S65D, E68G, L104Y, V106L, F108H	255	352, 449
E37V, S42G, T45G, L104N	256	353, 450
P18S, T45Q, L79P, L104T	257	354, 451
P18L, Q62R	258	355, 452
A13R, D23Y, E37P, S42L, S52G, Q62Y, A81E	259	356, 453
P18L, H49R, L104T, D116N	260	357, 454
A13R, D23Y, E37P, Q62M, G80S, A81P, L104T S65T, L104T	261	358, 455
	262	359, 456
A13R, D23Y, E37P, S52G, V57A, Q62M, K70E, L104T	263	360, 457
P18L, A47V, Q62Y, E73D, L104T	264	361, 458
H40T, V41M, A47V, S52Q, Q62L, S65T, E73R, D97G, E98S, L104T, D116N	265	362, 459
P18L, S42P, T45Q, T61G, S65H, S67E, L104T, D116N	266	363, 460
P18S, H40T, V41M, A47V, S52Q, Q62L, S65T, E73R, L104M, V106A	267	364, 461

TABLE 1-continued

Exemplary variant CD155 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
H40T, V41M, A47V, S52Q, Q62L, S65T, E68G, E73R, D97G, E98S, L104T	268	365, 462
T45Q, S52E, L104E	269	366, 463
T45Q, S52E, Q62F, L104E	270	367, 464
P18F, T26M, L44V, Q62K, L79P, F91S, L104M, G111D	271	368, 465
P18S, T45S, T61K, S65W, S67A, F91S, G111R	272	369, 466
P18S, L79P, L104M, T107M	273	370, 467
P18S, S65W, S67A, M90V, V95A, L104Q, G111R	274	371, 468
P18S, A47G, L79P, F91S, L104M, T107A, R113W	1230	1184, 1207
P18T, D23G, S24A, N35D, H49L, L79P, F91S, L104M, G111R	1231	1185, 1208
V9L, P18S, Q60R, V75L, L79P, R89K, F91S, L104E, G111R	1232	1186, 1209
P18S, H49R, E73D, L79P, N85D, F91S, V95A, L104M, G111R	1233	1187, 1210
V11A, P18S, L79P, F91S, L104M, G111R	1234	1188, 1211
V11A, P18S, S54R, Q60P, Q62K, L79P, N85D, F91S, T107M	1235	1189, 1212
P18T, S52P, S65A, S67V, L79P, F91S, L104M, G111R	1236	1190, 1213
P18T, M36T, L79P, F91S, G111R	1237	1191, 1214
D8G, P18S, M36I, V38A, H49Q, A76E, F91S, L104M, T107A, R113W	1238	1192, 1215
P18S, S52P, S65A, S67V, L79P, F91S, L104M, T107S, R113W	1239	1193, 1216
T15I, P18T, L79P, F91S, L104M, G111R	1240	1194, 1217
P18F, T26M, L44V, Q62K, L79P, E82D, F91S, L104M, G111D	1241	1195, 1218
P18T, E37G, G53R, Q62K, L79P, F91S, E98D, L104M, T107M	1242	1196, 1219
P18L, K70E, L79P, F91S, V95A, G111R	1243	1197, 1220
V9I, Q12K, P18F, S65A, S67V, L79P, L104T, G111R, S112I	1244	1198, 1221
P18F, S65A, S67V, F91S, L104M, G111R	1245	1199, 1222
V9I, V10I, P18S, F20S, T45A, L79P, F91S, L104M, F108Y, G111R, S112V	1246	1200, 1223
V9L, P18L, L79P, M90I, F91S, T102S, L104M, G111R	1247	1201, 1224
P18C, T26M, L44V, M55I, Q62K, L79P, F91S, L104M, T107M	1248	1202, 1225
V9I, P18T, D23G, L79P, F91S, G111R	1249	1203, 1226
P18F, L79P, M90L, F91S, V95A, L104M, G111R	1250	1204, 1227
P18T, M36T, S65A, S67E, L79Q, A81T, F91S, G111R	1251	1205, 1228
V9L, P18T, Q62R, L79P, F91S, L104M, G111R	1252	1206, 1229
P18S, S65W, S67A, L104Q, G111R	1269	1270, 1271
P18T, G19D, M36T, S54N, L79P, L83Q, F91S, T107M, F108Y	1610	1656, 1702
V9L, P18L, M55V, S69L, L79P, A81E, F91S, T107M	1611	1657, 1703
P18F, H40Q, T61K, Q62K, L79P, F91S, L104M, T107V	1612	1658, 1704
P18S, Q32R, Q62K, R78G, L79P, F91S, T107A, R113W	1613	1659, 1705
Q12H, P18T, L21S, G22S, V57A, Q62R, L79P, F91S, T107M	1614	1660, 1706
V9I, P18S, S24P, H49Q, F58Y, Q60R, Q62K, L79P, F91S, T107M	1615	1661, 1707
P18T, W46C, H49R, S65A, S67V, A76T, L79P, S87T, L104M	1616	1662, 1708
P18S, S42T, E51G, L79P, F91S, G92W, T107M	1617	1663, 1709
V10F, T15S, P18L, R48Q, L79P, F91S, T107M, V115M	1618	1664, 1710
P18S, L21M, Y30F, N35D, R84W, F91S, T107M, D116G	1619	1665, 1711
P18F, E51V, S54G, Q60R, L79Q, E82G, S87T, M90I, F91S, G92R, T107M	1620	1666, 1712

TABLE 1-continued

Exemplary variant CD155 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
Q16H, P18F, F91S, T107M	1621	1667, 1713
P18T, D23G, Q60R, S67L, L79P, F91S, T107M, V115A	1622	1668, 1714
D8G, V9I, V11A, P18T, T26M, S52P, L79P, F91S, G92A, T107L, V115A	1623	1669, 1715
V9I, P18F, A47E, G50S, E68G, L79P, F91S, T107M	1624	1670, 1716
P18S, M55I, Q62K, S69P, L79P, F91S, T107M	1625	1671, 1717
P18T, T39S, S52P, S54R, L79P, F91S, T107M	1626	1672, 1718
P18S, D23N, L79P, F91S, T107M, S114N	1627	1673, 1719
P18S, P34S, E51V, L79P, F91S, G111R	1628	1674, 1720
P18S, H59N, V75A, L79P, A81T, F91S, L104M, T107M	1629	1675, 1721
P18S, W46R, E68D, L79P, F91S, T107M, R113G	1630	1676, 1722
V9L, P18F, T45A, S65A, S67V, R78K, L79V, F91S, T107M, S114T	1631	1677, 1723
P18T, M55L, T61R, L79P, F91S, V106I, T107M	1632	1678, 1724
T15I, P18S, V33M, N35F, T39S, M55L, R78S, L79P, F91S, T107M	1633	1679, 1725
P18S, Q62K K70E, L79P, F91S, G92E, R113W	1634	1680, 1726
P18F, F20I, T26M, A47V, E51K, L79P, F91S	1635	1681, 1727
P18T, D23A, Q60H, L79P, M90V, F91S, T107M	1636	1682, 1728
P18S, D23G, C29R, N35D, E37G, M55I, Q62K, S65A, S67G, R78G, L79P, F91S, L104M, T107M, Q110R	1637	1683, 1729
A13E, P18S, M36R, Q62K, S67T, L79P, N85D, F91S, T107M	1638	1684, 1730
V9I, P18T, H49R, L79P, N85D, F91S, L104T, T107M	1639	1685, 1731
V9A, P18F, T61S, Q62L, L79P, F91S, G111R	1640	1686, 1732
D8E, P18T, T61A, L79P, F91S, T107M	1641	1687, 1733
P18S, V41A, H49R, S54C, L79S, N85Y, L88P, F91S, L104M, T107M	1642	1688, 1734
V11E, P18H, F20Y, V25E, N35S, H49R, L79P, F91S, T107M, G111R	1643	1689, 1735
V11A, P18F, D23A, L79P, G80D, V95A, T107M	1644	1690, 1736
P18S, K70R, L79P, F91S, G111R	1645	1691, 1737
V9L, V11M, P18S, N35S, S54G, Q62K, L79P, L104M, T107M, V115M	1646	1692, 1738
V9L, P18Y, V25A, V38G, M55V, A77T, L79P, M90I, F91S, L104M	1647	1693, 1739
V10G, P18T, L72Q, L79P, F91S, T107M	1648	1694, 1740
P18S, H59R, A76G, R78S, L79P	1649	1695, 1741
V9A, P18S, M36T, S65G, L79P, F91S, L104T, G111R, S112I	1650	1696, 1742
P18T, S52A, V57A, Q60R, Q62K, S65C, L79P, F91T, N100Y, T107M	1651	1697, 1743
V11A, P18F, N35D, A47E, Q62K, L79P, F91S, G99D, T107M, S114N	1652	1698, 1744
V11A, P18T, N35S, L79P, S87T, F91S	1653	1699, 1745
V9D, V11M, Q12L, P18S, E37V, M55I, Q60R, K70Q, L79P, F91S, L104M, T107M	1654	1700, 1746
T15S, P18S, Y30H, Q32L, Q62R, L79P, F91S, T107M	1655	1701, 1747

[0167] In some embodiments, the variant CD155 polypeptide exhibits increased affinity for the ectodomain of TIGIT compared to the wild-type or unmodified CD155 polypeptide, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155. In some embodiments, the CD155 polypeptide exhibits increased affinity for the ectodomain of CD226 compared to the wild-type or unmodified CD155, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155. In some embodiments, the CD155 polypeptide exhibits increased affinity for the ectodomain of CD96 compared to the wild-type or unmodified CD155, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155. In some embodiments, the CD155 polypeptide exhibits increased affinity for the ectodomain of TIGIT, the ectodomain of CD226, and the ectodomain of CD96 compared to the wild-type or unmodified CD155, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155. In some embodiments, the CD155 polypeptide exhibits increased affinity for the ectodomain of TIGIT and the ectodomain of CD226 compared to the wild-type or unmodified CD155, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155. In some embodiments, the CD155 polypeptide exhibits increased affinity for the ectodomain of TIGIT and the ectodomain of CD96 compared to the wild-type or unmodified CD155, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155. In some embodiments, the CD155 polypeptide exhibits increased affinity for the ectodomain of CD226 and the ectodomain of CD96 compared to the wild-type or unmodified CD155, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155.

[0168] In some embodiments, the variant CD155 polypeptide exhibits increased binding affinity for binding one of the ectodomains of TIGIT, CD226, or CD96 and exhibits decreased binding affinity for binding to the other of the ectodomains of TIGIT, CD226, or CD96 compared to the wild-type or unmodified CD155 polypeptide, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155. In some embodiments, the variant CD155 polypeptide exhibits increased affinity for the ectodomain of TIGIT or the ectodomain of CD226, and decreased affinity for the ectodomain of CD96, compared to wild-type or unmodified CD155 polypeptide, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155. In some embodiments, the variant CD155 polypeptide exhibits increased affinity for the ectodomain of TIGIT or the ectodomain of CD96, and decreased affinity for the ectodomain of CD226, compared to wild-type or unmodified CD155 polypeptide, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155. In some embodiments, the variant CD155 polypeptide exhibits increased affinity for the ectodomain of CD226 or the ectodomain of CD96, and decreased affinity for the ectodomain of TIGIT, compared to wild-type or unmodified CD155 polypeptide, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155.

[0169] In some embodiments, the variant CD155 polypeptide exhibits increased affinity for the ectodomain of TIGIT and the ectodomain of CD226, and decreased affinity for the ectodomain of CD96, compared to wild-type or unmodified CD155 polypeptide, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155. In some embodiments, the variant CD155 polypeptide exhibits increased affinity for the ectodomain of TIGIT and the ectodomain of CD96, and decreased affinity for the ectodomain of CD226, compared to wild-type or unmodified CD155 polypeptide, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155. In some embodiments, the variant CD155 polypeptide exhibits increased affinity for the ectodomain of CD226 and the ectodomain of CD96, and decreased affinity for the ectodomain of TIGIT, compared to wild-type or unmodified CD155 polypeptide, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155.

[0170] In some embodiments, the variant CD155 polypeptide exhibits increased affinity for the ectodomain of TIGIT, and decreased affinity for the ectodomain of CD226 and the ectodomain of CD96, compared to wild-type or unmodified

CD155 polypeptide, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155. In some embodiments, the variant CD155 polypeptide exhibits increased affinity for the ectodomain of CD96, and decreased affinity for the ectodomain of TIGIT and the ectodomain of CD226, compared to wild-type or unmodified CD155 polypeptide, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155. In some embodiments, the variant CD155 polypeptide exhibits increased affinity for the ectodomain of CD226, and decreased affinity for the ectodomain of TIGIT and the ectodomain of CD96, compared to wild-type or unmodified CD155 polypeptide, such as comprising the sequence set forth in SEQ ID NO: 47, 58 or 155.

[0171] In some embodiments, a variant CD155 polypeptide exhibits increased selectivity for TIGIT versus CD226 compared to the ratio of binding of the unmodified CD155 polypeptide (e.g. set forth in SEQ ID NO:47, 58 or 155) for TIGIT versus CD226, such as indicated by a ratio of TIGIT binding to CD226 binding (TIGIT:CD226 binding ratio) that is greater than 1. In some embodiments, the variant CD155 polypeptide exhibits a ratio of binding to TIGIT versus CD226 that is greater than or greater than about or 1.1, 1.2, 1.3, 1.4, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, or more.

III. Format of Variant Polypeptides

[0172] The immunomodulatory polypeptide comprising a variant CD155 provided herein in which is contained a vIgD can be formatted in a variety of ways, including as a soluble protein, membrane bound protein or secreted protein. In some embodiments, the particular format can be chosen for the desired therapeutic application. In some cases, an immunomodulatory polypeptide comprising a variant CD155 polypeptide is provided in a format to antagonize or block activity of its cognate binding partner, e.g. TIGIT. In some embodiments, antagonism of TIGIT may be useful to promote immunity in oncology. In some cases, an immunomodulatory polypeptide comprising a variant CD155 polypeptide is provided in a format to agonize or stimulate activity of its cognate binding partner, e.g. TIGIT. In some embodiments, agonism of TIGIT may be useful for treating inflammation or autoimmunity. A skilled artisan can readily determine the activity of a particular format, such as for antagonizing or agonizing one or more specific cognate binding partner. Exemplary methods for assessing such activities are provided herein, including in the examples.

[0173] In some aspects, provided are immunomodulatory proteins comprising a vIgD of CD155 in which such proteins are soluble, e.g. fused to an Fc chain. In some aspects, one or more additional IgSF domain, such as one or more additional vIgD, may be linked to a vIgD of CD155 as provided herein (hereinafter called a "stack" or "stacked" immunomodulatory protein). In some embodiments, the modular format of the provided immunomodulatory proteins provides flexibility for engineering or generating immunomodulatory proteins for modulating activity of multiple counterstructures (multiple cognate binding partners). In some embodiments, such "stack" molecules can be provided in a soluble format or, in some cases, may be provided as membrane bound or secreted proteins. In some embodiments, a variant CD155 immunomodulatory protein is provided as a conjugate in which is contained a vIgD of CD155 linked, directly or indirectly, to a targeting agent or moiety, e.g. to an antibody or other binding molecules that specifically binds to a ligand, e.g. an antigen, for example, for targeting or localizing the vIgD to a specific environment or cell, such as when administered to a subject. In some embodiments, the targeting agent, e.g. antibody or other binding molecule, binds to a tumor antigen, thereby localizing the variant CD155 containing the vIgD to the tumor microenvironment, for example, to modulate activity of tumor infiltrating lymphocytes (TILs) specific to the tumor microenvironment.

[0174] In some embodiments, provided immunomodulatory proteins are expressed in cells and provided as part of an engineered cellular therapy (ECT). In some embodiments, the variant CD155 polypeptide is expressed in a cell, such as an immune cell (e.g. T cell or antigen presenting cell), in membrane-bound form, thereby providing a transmembrane immunomodulatory protein (hereinafter also called a "TIP"). In some embodiments, depending on the cognate binding partner recognized by the TIP, engineered cells expressing a TIP can agonize a cognate binding partner by providing a costimulatory signal, either positive to negative, to other engineered cells and/or to endogenous T cells. In some aspects, the variant CD155 polypeptide is expressed in a cell, such as an immune cell (e.g. T cell or antigen presenting cell), in secretable form to thereby produce a secreted or soluble form of the variant CD155 polypeptide (hereinafter also called a "SIP"), such as when the cells are administered to a subject. In some aspects, a SIP can antagonize a cognate binding partner in the environment (e.g. tumor microenvironment) in which it is secreted. In some embodiments, a variant CD155 polypeptide is expressed in an infectious agent (e.g. viral or bacterial agent) which, upon administration to a subject, is able to infect a cell in vivo, such as an immune cell (e.g. T cell or antigen presenting cell), for delivery or expression of the variant polypeptide as a TIP or a SIP in the cell.

[0175] In some embodiments, a soluble immunomodulatory polypeptide, such as a variant CD155 containing a vIgD, can be encapsulated within a liposome which itself can be conjugated to any one of or any combination of the provided conjugates (e.g., a targeting moiety). In some embodiments, the soluble or membrane bound immunomodulatory polypeptides of the invention are deglycosylated. In more specific embodiments, the variant CD155 sequence is deglycosylated. In even more specific embodiments, the IgV and/or IgC (e.g. IgC2) domain or domains of the variant CD155 is deglycosylated.

[0176] Non-limiting examples of provided formats are described in FIGS. **1**A-**1**C and further described below.

[0177] A. Soluble Protein

[0178] In some embodiments, the immunomodulatory protein containing a variant CD155 polypeptide is a soluble protein. Those of skill will appreciate that cell surface proteins typically have an intracellular, transmembrane, and extracellular domain (ECD) and that a soluble form of such proteins can be made using the extracellular domain or an immunologically active subsequence thereof. Thus, in some embodiments, the immunomodulatory protein containing a variant CD155 polypeptide lacks a transmembrane domain or a portion of the transmembrane domain. In some embodiments, the immunomodulatory protein containing a variant CD155 lacks the intracellular (cytoplasmic) domain or a portion of the intracellular domain. In some embodiments, the immunomodulatory protein containing the variant

CD155 polypeptide only contains the vIgD portion containing the ECD domain or a portion thereof containing an IgV domain and/or IgC (e.g. IgC2) domain or domains or specific binding fragments thereof containing the amino acid modification(s).

[0179] In some embodiments, an immunomodulatory polypeptide comprising a variant CD155 can include one or more variant CD155 polypeptides of the invention. In some embodiments a polypeptide of the invention will comprise exactly 1, 2, 3, 4, 5 variant CD155 sequences. In some embodiments, at least two of the variant CD155 sequences are identical variant CD155 sequences.

[0180] In some embodiments, the provided immunomodulatory polypeptide comprises two or more vIgD sequences of CD155. Multiple variant CD155 polypeptides within the polypeptide chain can be identical (i.e., the same species) to each other or be non-identical (i.e., different species) variant CD155 sequences. In addition to single polypeptide chain embodiments, in some embodiments two, three, four, or more of the polypeptides of the invention can be covalently or non-covalently attached to each other. Thus, monomeric, dimeric, and higher order (e.g., 3, 4, 5, or more) multimeric proteins are provided herein. For example, in some embodiments exactly two polypeptides of the invention can be covalently or non-covalently attached to each other to form a dimer. In some embodiments, attachment is made via interchain cysteine disulfide bonds. Compositions comprising two or more polypeptides of the invention can be of an identical species or substantially identical species of polypeptide (e.g., a homodimer) or of non-identical species of polypeptides (e.g., a heterodimer). A composition having a plurality of linked polypeptides of the invention can, as noted above, have one or more identical or non-identical variant CD155 polypeptides of the invention in each polypeptide chain.

[0181] In some embodiments, the immunomodulatory protein comprises a variant CD155 polypeptide attached to an immunoglobulin Fc (yielding an "immunomodulatory Fc fusion," such as a "CD155-Fc variant fusion," also termed a CD155 vIgD-Fc fusion). In some embodiments, the attachment of the variant CD155 polypeptide is at the N-terminus of the Fc. In some embodiments, the attachment of the variant CD155 polypeptide is at the C-terminus of the Fc. In some embodiments, two or more CD155 variant polypeptides (the same or different) are independently attached at the N-terminus and at the C-terminus.

[0182] In some embodiments, the Fc is murine or human Fc. In some embodiments, the Fc is a mammalian or human IgG1, lgG2, lgG3, or lgG4 Fc regions. In some embodiments, the Fc is derived from IgG1, such as human IgG1. In some embodiments, the Fc comprises the amino acid sequence set forth in SEQ ID NO: 56 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 56.

[0183] In some embodiments, the Fc region contains one more modifications to alter (e.g. reduce) one or more of its normal functions. In general, the Fc region is responsible for effector functions, such as complement-dependent cytotoxicity (CDC) and antibody-dependent cell cytotoxicity (ADCC), in addition to the antigen-binding capacity, which is the main function of immunoglobulins. Additionally, the FcRn sequence present in the Fc region plays the role of regulating the IgG level in serum by increasing the in vivo half-life by conjugation to an in vivo FcRn receptor. In some embodiments, such functions can be reduced or altered in an Fc for use with the provided Fc fusion proteins.

[0184] In some embodiments, one or more amino acid modifications may be introduced into the Fc region of a CD155-Fc variant fusion provided herein, thereby generating an Fc region variant. In some embodiments, the Fc region variant has decreased effector function. There are many examples of changes or mutations to Fc sequences that can alter effector function. For example, WO 00/42072, WO2006019447, WO2012125850, WO2015/107026, US2016/0017041 and Shields et al. J Biol. Chem. 9(2): 6591-6604 (2001) describe exemplary Fc variants with improved or diminished binding to FcRs. The contents of those publications are specifically incorporated herein by reference.

[0185] In some embodiments, the provided variant CD155-Fc fusions comprise an Fc region that exhibits reduced effector functions, which makes it a desirable candidate for applications in which the half-life of the CD155-Fc variant fusion in vivo is important yet certain effector functions (such as CDC and ADCC) are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the CD155-Fc variant fusion lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 (see, e.g. Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); U.S. Pat. No. 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assay methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (Cell Technology, Inc. Mountain View, Calif; and CytoTox 96™ non-radioactive cytotoxicity assay (Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the CD155-Fc variant fusion is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996); Cragg, M. S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M. S. and M. J. Glennie, Blood 103:2738-2743 (2004)). FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S. B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0186] CD155-Fc variant fusions with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 by EU

numbering (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327 by EU numbering, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

[0187] In some embodiments, the Fc region of CD155-Fc variant fusions has an Fc region in which any one or more of amino acids at positions 234, 235, 236, 237, 238, 239, 270, 297, 298, 325, and 329 (indicated by EU numbering) are substituted with different amino acids compared to the native Fc region. Such alterations of Fc region are not limited to the above-described alterations, and include, for example, alterations such as deglycosylated chains (N297A and N297Q), IgG1-N297G, IgG1-L234A/L235A, IgG1-L234A/L235E/G237A, IgG1-A325A/A330S/P331S, IgG1-C226S/C229S, IgG1-C226S/C229S/E233P/L234V/L235A, IgG1-E233P/L234V/L235A/G236del/S267K, IgG1-L234F/L235E/P331S, IgG1-S267E/L328F, IgG2-V234A/G237A, IgG2-H268Q/V309L/A330S/A331S, IgG4-L235A/G237A/E318A, and IgG4-L236E described in Current Opinion in Biotechnology (2009) 20 (6), 685-691; alterations such as G236R/L328R, L235G/G236R, N325A/L328R, and N325LL328R described in WO 2008/092117; amino acid insertions at positions 233, 234, 235, and 237 (indicated by EU numbering); and alterations at the sites described in WO 2000/042072.

[0188] Certain Fc variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Pat. No. 6,737,056; WO 2004/056312, WO2006019447 and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[0189] In some embodiments, there is provided a CD155-Fc variant fusion comprising a variant Fc region comprising one or more amino acid substitutions which increase half-life and/or improve binding to the neonatal Fc receptor (FcRn). Antibodies with increased half-lives and improved binding to FcRn are described in US2005/0014934A1 (Hinton et al.) or WO2015107026. Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434 by EU numbering, e.g., substitution of Fc region residue 434 (U.S. Pat. No. 7,371,826).

[0190] In some embodiments, the Fc region of a CD155-Fc variant fusion comprises one or more amino acid substitution E356D and M358L by EU numbering. In some embodiments, the Fc region of a CD155-Fc variant fusion comprises one or more amino acid substitutions C220S, C226S and/or C229S by EU numbering. In some embodiments, the Fc region of a CD155 variant fusion comprises one or more amino acid substitutions R292C and V302C. See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Pat. Nos. 5,648,260; 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

[0191] In some embodiments, alterations are made in the Fc region that result in diminished C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in U.S. Pat. No. 6,194,551, WO 99/51642, and Idusogie et al., *J. Immunol.* 164: 4178-4184 (2000).

[0192] In some embodiments, there is provided a CD155-Fc variant fusion comprising a variant Fc region comprising one or more amino acid modifications, wherein the variant Fc region is derived from IgG1, such as human IgG1. In some embodiments, the variant Fc region is derived from the amino acid sequence set forth in SEQ ID NO: 56. In some embodiments, the Fc contains at least one amino acid substitution that is N82G by numbering of SEQ ID NO: 56 (corresponding to N297G by EU numbering). In some embodiments, the Fc further contains at least one amino acid substitution that is R77C or V87C by numbering of SEQ ID NO: 56 (corresponding to R292C or V302C by EU numbering). In some embodiments, the variant Fc region further comprises a C5S amino acid modification by numbering of SEQ ID NO: 56 (corresponding to C220S by EU numbering). For example, in some embodiments, the variant Fc region comprises the following amino acid modifications: V297G and one or more of the following amino acid modifications C220S, R292C or V302C by EU numbering (corresponding to N82G and one or more of the following amino acid modifications C5S, R77C or V87C with reference to SEQ ID NO:56), e.g. the Fc region comprises the sequence set forth in SEQ ID NO:1135. In some embodiments, the variant Fc region comprises one or more of the amino acid modifications C220S, L234A, L235E or G237A, e.g. the Fc region comprises the sequence set forth in SEQ ID NO:1136. In some embodiments, the variant Fc region comprises one or more of the amino acid modifications C220S, L235P, L234V, L235A, G236del or S267K, e.g. the Fc region comprises the sequence set forth in SEQ ID NO:1137. In some embodiments, the variant Fc comprises one or more of the amino acid modifications C220S, L234A, L235E, G237A, E356D or M358L, e.g. the Fc region comprises the sequence set forth in SEQ ID NO:1119.

[0193] In some embodiments, the Fc region lacks the C-terminal lysine corresponding to position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 56 (corresponding to K447del by EU numbering). In some aspects, such an Fc region can additionally include one or more additional modifications, e.g. amino acid substitutions, such as any as described. Exemplary of such an Fc region is set forth in SEQ ID NO:1253, 1748, 1749 or 1750.

[0194] In some embodiments, there is provided a CD155-Fc variant fusion comprising a variant Fc region in which the variant Fc comprises the sequence of amino acids set forth in any of SEQ ID NOS:1119, 1135, 1136, 1137, 1253, 1748, 1749, or 1750 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 1119, 1135, 1136, 1137, 1253, 1748, 1749, or 1750.

[0195] In some embodiments, the Fe is derived from IgG2, such as human IgG2. In some embodiments, the Fc comprises the amino acid sequence set forth in SEQ ID NO: 57 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 57.

[0196] In some embodiments, the Fc comprises the amino acid sequence of human IgG4 set forth in SEQ ID NO: 1178 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:1178. In some embodiments, the IgG4 Fc is a stabilized Fc in which the CH3 domain of human IgG4 is substituted with the CH3 domain of human IgG1 and which exhibits inhibited aggregate formation, an antibody in which the

CH3 and CH2 domains of human IgG4 are substituted with the CH3 and CH2 domains of human IgG1, respectively, or an antibody in which arginine at position 409 indicated in the EU index proposed by Kabat et al. of human IgG4 is substituted with lysine and which exhibits inhibited aggregate formation (see e.g. U.S. Pat. No. 8,911,726. In some embodiments, the Fc is an IgG4 containing the S228P mutation, which has been shown to prevent recombination between a therapeutic antibody and an endogenous IgG4 by Fab-arm exchange (see e.g. Labrijin et al. (2009) Nat. Biotechnol., 27(8):767-71.) In some embodiments, the Fc comprises the amino acid sequence set forth in human IgG4 with S228P set forth in SEQ ID NO: 1179 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:1179.

[0197] In some embodiments, the variant CD155 polypeptide is directly linked to the Fc sequence. In some embodiments, the variant CD155 polypeptide is indirectly linked to the Fc sequence, such as via a linker. In some embodiments, one or more "peptide linkers" link the variant CD155 polypeptide and the Fc domain. In some embodiments, a peptide linker can be a single amino acid residue or greater in length. In some embodiments, the peptide linker has at least one amino acid residue but is no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues in length. In some embodiments, the linker is three alanines (AAA). In some embodiments, the linker is (in one-letter amino acid code): GGGGS ("4GS"; SEQ ID NO:1752) or multimers of the 4GS linker, such as repeats of 2, 3, 4, or 5 4GS linkers, such as set forth in SEQ ID NO: 1182 (2×GGGGS) or SEQ ID NO: 1181 (3×GGGGS). In some embodiments, the linker (in one-letter amino acid code) is GSGGGGS (SEQ ID NO:1751).

[0198] In some embodiments, the variant CD155-Fc fusion protein is a dimer formed by two variant CD155 Fc polypeptides linked to an Fe domain. In some embodiments, the dimer is a homodimer in which the two variant CD155 Fc polypeptides are the same. In some embodiments, the dimer is a heterodimer in which the two variant CD155 Fc polypeptides are different.

[0199] Also provided are nucleic acid molecules encoding the variant CD155-Fc fusion protein. In some embodiments, for production of an Fc fusion protein, a nucleic acid molecule encoding a variant CD155-Fc fusion protein is inserted into an appropriate expression vector. The resulting variant CD155-Fc fusion protein can be expressed in host cells transformed with the expression where assembly between Fe domains occurs by interchain disulfide bonds formed between the Fc moieties to yield dimeric, such as divalent, variant CD155-Fc fusion proteins.

[0200] The resulting Fc fusion proteins can be easily purified by affinity chromatography over Protein A or Protein G columns. For the generation of heterodimers, additional steps for purification can be necessary. For example, where two nucleic acids encoding different variant CD155 polypeptides are transformed into cells, the formation of heterodimers must be biochemically achieved since variant CD155 molecules carrying the Fc-domain will be expressed as disulfide-linked homodimers as well. Thus, homodimers can be reduced under conditions that favor the disruption of interchain disulfides, but do no effect intra-chain disulfides. In some cases, different variant-CD155 Fc monomers are mixed in equimolar amounts and oxidized to form a mixture of homo- and heterodimers. The components of this mixture are separated by chromatographic techniques. Alternatively, the formation of this type of heterodimer can be biased by genetically engineering and expressing Fc fusion molecules that contain a variant CD155 polypeptide using knob-into-hole methods described below.

[0201] B. Stack Molecules with Additional IgSF Domains

[0202] In some embodiments, the immunomodulatory proteins can contain any of the variant CD155 polypeptides provided herein linked, directly or indirectly, to one or more other immunoglobulin superfamily (IgSF) domain ("stacked" immunomodulatory protein construct and also called a "Type II" immunomodulatory protein). In some aspects, this can create unique multi-domain immunomodulatory proteins that bind two or more, such as three or more, cognate binding partners, thereby providing a multi-targeting modulation of the immune synapse.

[0203] In some embodiments, an immunomodulatory protein comprises a combination (a "non-wild-type combination") and/or arrangement (a "non-wild type arrangement" or "non-wild-type permutation") of a variant CD155 domain with one or more other affinity modified and/or non-affinity modified IgSF domain sequences of another IgSF family member (e.g. a mammalian IgSF family member) that are not found in wild-type IgSF family members. In some embodiments, the immunomodulatory protein contains 2, 3, 4, 5 or 6 immunoglobulin superfamily (IgSF) domains, where at least one of the IgSF domain is a variant CD155 IgSF domain (vIgD of CD155) according to the provided description.

[0204] In some embodiments, the sequences of the additional IgSF domains can be a modified IgSF domain that contains one or more amino acid modifications, e.g. substitutions, compared to a wildtype or unmodified IgSF domain. In some embodiments, the IgSF domain can be non-affinity modified (e.g., wild-type) or have been affinity modified. In some embodiments, the unmodified or wild-type IgSF domain can be from mouse, rat, cynomolgus monkey, or human origin, or combinations thereof. In some embodiments, the additional IgSF domains can be an IgSF domain of an IgSF family member set forth in Table 2. In some embodiments, the additional IgSF domain can be an affinity-modified IgSF domain containing one or more amino acid modifications, e.g. substitutions, compared to an IgSF domain contained in an IgSF family member set forth in Table 2.

[0205] In some embodiments, the additional IgSF domain is an affinity or non-affinity modified IgSF domain contained in an IgSF family member of a family selected from Signal-Regulatory Protein (SIRP) Family, Triggering Receptor Expressed On Myeloid Cells Like (TREML) Family, Carcinoembryonic Antigen-related Cell Adhesion Molecule (CEACAM) Family, Sialic Acid Binding Ig-Like Lectin (SIGLEC) Family, Butyrophilin Family, B7 family, CD28 family, V-set and Immunoglobulin Domain Containing (VSIG) family, V-set transmembrane Domain (VSTM) family, Major Histocompatibility Complex (MHC) family, Signaling lymphocytic activation molecule (SLAM) family, Leukocyte immunoglobulin-like receptor (LIR), Nectin (Nec) family, Nectin-like (NECL) family, Poliovirus receptor related (PVR) family, Natural cytotoxicity triggering receptor (NCR) family, T cell immunoglobulin and mucin (TIM) family or Killer-cell immunoglobulin-like receptors (KIR) family. In some embodiments, the additional IgSF

domains are independently derived from an IgSF protein selected from the group consisting of CD80(B7-1), CD86 (B7-2), CD274 (CD155, B7-H1), PDCD1LG2(CD155, CD273), ICOSLG(B7RP1, CD275, ICOSL, B7-H2), CD276(B7-H3), VTCN1(B7-H4), CD28, CTLA4, PDCD1 (TIGIT), ICOS, BTLA(CD272), CD4, CD8A(CD8-alpha), CD8B(CD8-beta), LAG3, HAVCR2(TIM-3), CEACAM1, TIGIT, PVR(CD155), PVRL2(CD112), CD226, CD2, CD160, CD200, CD200R1(CD200R), and NCR3 (NKp30).

[0206] The first column of Table 2 provides the name and, optionally, the name of some possible synonyms for that particular IgSF member. The second column provides the protein identifier of the UniProtKB database, a publicly available database accessible via the internet at uniprot.org or, in some cases, the GenBank Number. The Universal Protein Resource (UniProt) is a comprehensive resource for protein sequence and annotation data. The UniProt databases include the UniProt Knowledgebase (UniProtKB). UniProt is a collaboration between the European Bioinformatics Institute (EMBL-EBI), the SIB Swiss Institute of Bioinformatics and the Protein Information Resource (PIR) and supported mainly by a grant from the U.S. National Institutes of Health (NIH). GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (Nucleic Acids Research, 2013 January; 41(D1):D36-42). The third column provides the region where the indicated IgSF domain is located. The region is specified as a range where the domain is inclusive of the residues defining the range. Column 3 also indicates the IgSF domain class for the specified IgSF region. Column 4 provides the region where the indicated additional domains are located (signal peptide, S; extracellular domain, E; transmembrane domain, T; cytoplasmic domain, C). It is understood that description of domains can vary depending on the methods used to identify or classify the domain, and may be identified differently from different sources. The description of residues corresponding to a domain in Table 2 is for exemplification only and can be several amino acids (such as one, two, three or four) longer or shorter. Column 5 indicates for some of the listed IgSF members, some of its cognate cell surface binding partners.

TABLE 2

IgSF members according to the present disclosure.

IgSF Member (Synonyms)	NCBI Protein Accession Number/ UniProtKB Protein Identifier	IgSF Region & Domain Class	Other Domains	Cognate Cell Surface Binding Partners	IgSF Member Amino Acid Sequence (SEQ ID NO) Precursor (mature residues)	Mature	ECD
CD80 (B7-1)	NP_005182.1 P33681	35-135, 35-138, or 35-14137-138 IgV, 145-230 or 154-232 IgC	S: 1-34, E: 35-242, T: 243-263, C: 264-288	CD28, CTLA4, PD-L1	1 (35-288)	103	28
CD86 (B7-2)	P42081.2	33-131 IgV, 150-225 IgC2	S: 1-23, E: 24-247, T: 248-268, C: 269-329	CD28, CTLA4	2 (24-329)	104	29
CD274 (PD-L1, B7-H1)	Q9NZQ7.1	19-127, 24-130 IgV, 133-225 IgC2	S: 1-18, E: 19-238, T: 239-259, C: 260-290	PD-1, B7-1	3 (19-290)	105	30
PDCD1LG2 (PD-L2, CD273)	Q9BQ51.2	21-118 IgV, 122-203 IgC2	S: 1-19, E: 20-220, T: 221-241, C: 242-273	PD-1, RGMb	4 (20-273)	106	31
ICOSLG (B7RP1, CD275, ICOSL, B7-H2)	O75144.2	19-129 IgV, 141-227 IgC2	S: 1-18, E: 19-256, T: 257-277, C: 278-302	ICOS, CD28, CTLA4	5 (19-302)	107	32
CD276 (B7-H3)	Q5ZPR3.1	29-139 IgV, 145-238 IgC2, 243-357 IgV2, 367-453, 363-456 IgC2	S: 1-28, E: 29-466, T: 467-487, C: 488-534		6 (29-534)	108	33
VTCN1 (B7-H4)	Q7Z7D3.1	35-146 IgV, 153-241 IgV	S: 1-24, E: 25-259, T: 260-280, C: 281-282		7 (25-282)	109	34
CD28	P10747.1	28-137 IgV	S: 1-18, E: 19-152, T: 153-179, C: 180-220	B7-1, B7-2, B7RP1	8 (19-220)	110	35
CTLA4	P16410.3	39-140 IgV	S: 1-35, E: 36-161, T: 162-182, C: 183-223	B7-1, B7-2, B7RP1	9 (36-223)	111	36

TABLE 2-continued

IgSF members according to the present disclosure.

IgSF Member (Synonyms)	NCBI Protein Accession Number/ UniProtKB Protein Identifier	IgSF Region & Domain Class	Other Domains	Cognate Cell Surface Binding Partners	IgSF Member Amino Acid Sequence (SEQ ID NO)		
					Precursor (mature residues)	Mature	ECD
PDCD1 (PD-1)	Q15116.3	35-145 IgV	S: 1-20, E: 21-170, T: 171-191, C: 192-288	PD-L1, PD-L2	10 (21-288)	112	37
ICOS	Q9Y6W8.1	30-132 IgV	S: 1-20, E: 21-140, T: 141-161, C: 162-199	B7RP1	11 (21-199)	113	38
BTLA (CD272)	Q7Z6A9.3	31-132 IgV	S: 1-30, E: 31-157, T: 158-178, C: 179-289	HVEM	12 (31-289)	114	39
CD4	P01730.1	26-125 IgV, 126-203 IgC2, 204-317 IgC2, 317-389, 318-374 IgC2	S: 1-25, E: 26-396, T: 397-418, C: 419-458	MHC class II	13 (26-458)	115	40
CD8A (CD8-alpha)	P01732.1	22-135 IgV	S: 1-21, E: 22-182, T: 183-203, C: 204-235	MHC class I	14 (22-235)	116	41
CD8B (CD8-beta)	P10966.1	22-132 IgV	S: 1-21, E: 22-170, T: 171-191, C: 192-210	MHC class I	15 (22-210)	117	42
LAG3	P18627.5	37-167 IgV, 168-252 IgC2, 265-343 IgC2, 349-419 IgC2	S: 1-28, E: 29-450, T: 451-471, C: 472-525	MHC class II	16 (29-525)	118	43
HAVCR2 (TIM-3)	Q8TDQ0.3	22-124 IgV	S: 1-21, E: 22-202, T: 203-223, C: 224-301	CEACAM-1, phosphatidylserine, Galectin-9, HMGB1	17 (22-301)	119	44
CEACAM1	P13688.2	35-142 IgV, 145-232 IgC2, 237-317 IgC2, 323-413 IgCs	S: 1-34, E: 35-428, T: 429-452, C: 453-526	TIM-3	18 (35-526)	120	45
TIGIT	Q495A1.1	22-124 IgV	S: 1-21, E: 22-141, T: 142-162, C: 163-244	CD155, CD112	19 (22-244)	121	46
PVR (CD155)	P15151.2	24-139 IgV, 145-237 IgC2, 244-328 IgC2	S: 1-20, E: 21-343, T: 344-367, C: 368-417	TIGIT, CD226, CD96, poliovirus	20 (21-417)	122	47
PVRL2 (CD112)	Q92692.1	32-156 IgV, 162-256 IgC2, 261-345 IgC2	S: 1-31, E: 32-360, T: 361-381, C: 382-538	TIGIT, CD226, CD112R	21 (32-538)	123	48
CD226	Q15762.2	19-126 IgC2, 135-239 IgC2	S: 1-18, E: 19-254, T: 255-275, C: 276-336	CD155, CD112	22 (19-336)	124	49
CD2	P06729.2	25-128 IgV, 129-209 IgC2	S: 1-24, E: 25-209, T: 210-235, C: 236-351	CD58	23 (25-351)	125	50
CD160	O95971.1	27-122 IgV	N/A	HVEM, MHC family of proteins	24 (27-159)	126	51
CD200	P41217.4	31-141 IgV, 142-232 IgC2	S: 1-30, E: 31-232, T: 233-259, C: 260-278	CD200R	25 (31-278)	127	52

TABLE 2-continued

IgSF members according to the present disclosure.

IgSF Member (Synonyms)	NCBI Protein Accession Number/ UniProtKB Protein Identifier	IgSF Region & Domain Class	Other Domains	Cognate Cell Surface Binding Partners	IgSF Member Amino Acid Sequence (SEQ ID NO) Precursor (mature residues)	Mature	ECD
CD200R1 (CD200R)	Q8TD46.2	53-139 IgV, 140-228 IgC2	S: 1-28, E: 29-243, T: 244-264, C: 265-325	CD200	26 (29-325)	128	53
NCR3 (NKp30)	O14931.1	19-126 IgC-like	S: 1-18, E: 19-135, T: 136-156, C: 157-201	B7-H6	27 (19-201)	129	54
VSIG8	Q5VU13	22-141 IgV1, 146-257 IgV2	S: 1-21 E: 22-263 T: 264-284 C: 285-414	VISTA	132 (22-414)	133	134

[0207] The number of such non-affinity modified or affinity modified IgSF domains present in a "stacked" immunomodulatory protein construct (whether non-wild type combinations or non-wild type arrangements) is at least 2, 3, 4, or 5 and in some embodiments exactly 2, 3, 4, or 5 IgSF domains (whereby determination of the number of affinity modified IgSF domains disregards any non-specific binding fractional sequences thereof and/or substantially immunologically inactive fractional sequences thereof).

[0208] In some embodiments of a stacked immunomodulatory protein provided herein, the number of IgSF domains is at least 2 wherein the number of affinity modified and the number of non-affinity modified IgSF domains is each independently at least: 0, 1, 2, 3, 4, 5, or 6. Thus, the number of affinity modified IgSF domains and the number of non-affinity modified IgSF domains, respectively, (affinity modified IgSF domain: non-affinity modified IgSF domain), can be exactly or at least: 2:0 (affinity modified: wild-type), 0:2, 2:1, 1:2, 2:2, 2:3, 3:2, 2:4, 4:2, 1:1, 1:3, 3:1, 1:4, 4:1, 1:5, or 5:1.

[0209] In some embodiments of a stacked immunomodulatory protein, at least two of the non-affinity modified and/or affinity modified IgSF domains are identical IgSF domains.

[0210] In some embodiments, a stacked immunomodulatory protein provided herein comprises at least two affinity modified and/or non-affinity modified IgSF domains from a single IgSF member but in a non-wild-type arrangement (alternatively, "permutation"). One illustrative example of a non-wild type arrangement or permutation is an immunomodulatory protein comprising a non-wild-type order of affinity modified and/or non-affinity modified IgSF domain sequences relative to those found in the wild-type CD155 whose IgSF domain sequences served as the source of the variant IgSF domains as provided herein. Thus, in one example, the immunomodulatory protein can comprise an IgV proximal and an IgC distal to the transmembrane domain albeit in a non-affinity modified and/or affinity modified form. The presence, in an immunomodulatory protein provided herein, of both non-wild-type combinations and non-wild-type arrangements of non-affinity modified and/or affinity modified IgSF domains, is also within the scope of the provided subject matter.

[0211] In some embodiments of a stacked immunomodulatory protein, the non-affinity modified and/or affinity modified IgSF domains are non-identical (i.e., different) IgSF domains. Non-identical affinity modified IgSF domains specifically bind, under specific binding conditions, different cognate binding partners and are "non-identical" irrespective of whether or not the wild-type or unmodified IgSF domains from which they are engineered was the same. Thus, for example, a non-wild-type combination of at least two non-identical IgSF domains in an immunomodulatory protein can comprise at least one IgSF domain sequence whose origin is from and unique to one CD155, and at least one of a second IgSF domain sequence whose origin is from and unique to another IgSF family member that is not CD155, wherein the IgSF domains of the immunomodulatory protein are in non-affinity modified and/or affinity modified form. However, in alternative embodiments, the two non-identical IgSF domains originate from the same IgSF domain sequence but at least one is affinity modified such that they specifically bind to different cognate binding partners.

[0212] In some embodiments, the provided immunomodulatory proteins, in addition to containing a variant CD155 polypeptide, also contains at least 1, 2, 3, 4, 5 or 6 additional immunoglobulin superfamily (IgSF) domains, such as an IgD domain of an IgSF family member set forth in Table 2. In some embodiments, the provided immunomodulatory protein contains at least one additional IgSF domain (e.g. second IgSF domain). In some embodiments, the provided immunomodulatory protein contains at least two additional IgSF domains (e.g. second and third IgSF domain). In some embodiments, the provided immunomodulatory protein contains at least three additional IgSF domains (e.g. second, third and fourth). In some embodiments, the provided immunomodulatory protein contains at least four additional IgSF domains (e.g. second, third, fourth and fifth). In some embodiments, the provided immunomodulatory protein contains at least five additional IgSF domains (e.g. second, third, fourth, fifth and sixth). In some embodiments, the provided immunomodulatory protein contains at least six

additional IgSF domains (e.g. second, third, fourth, fifth, sixth and seventh). In some embodiments, each of the IgSF domains in the immunomodulatory protein are different. In some embodiments, at least one of the additional IgSF domain is the same as at least one other IgSF domain in the immunomodulatory protein. In some embodiments, each of the IgSF domains is from or derived from a different IgSF family member. In some embodiments, at least two of the IgSF domains is from or derived from the same IgSF family member.

[0213] In some embodiments, the additional IgSF domain comprises an IgV domain or an IgC (e.g., IgC2) domain or domains, or a specific binding fragment of the IgV domain or a specific binding fragment of the IgC (e.g., IgC2) domain or domains. In some embodiments, the additional IgSF domain is or comprises a full-length IgV domain. In some embodiments, the additional IgSF domain is or comprises a full-length IgC (e.g., IgC2) domain or domains. In some embodiments, the additional IgSF domain is or comprises a specific binding fragment of the IgV domain. In some embodiments, the additional IgSF domain is or comprises a specific binding fragment of the IgC (e.g., IgC2) domain or domains. In some embodiments, the immunomodulatory protein contains at least two additional IgSF domains from a single (same) IgSF member. For example, in some aspects, the immunomodulatory protein contains an ECD or portion thereof of an IgSF member containing a full-length IgV domain and a full-length IgC (e.g., IgC2) domain or domains or specific binding fragments thereof.

[0214] In some embodiments, the provided immunomodulatory proteins contain at least one additional IgSF domain (e.g. a second or, in some cases, also a third IgSF domain) in which at least one additional, e.g., second or third IgSF domain, is an IgSF domain set forth in a wild-type or unmodified IgSF domain or a specific binding fragment thereof contained in the sequence of amino acids set forth in any of SEQ ID NOS: 1-27 and 132. In some embodiments, the wild-type or unmodified IgSF domain is an IgV domain or an IgC domain, such as an IgC1 or IgC2 domain.

[0215] In some embodiments, the provided immunomodulatory proteins, in addition to containing a variant CD155 polypeptide, also contains at least one additional affinity-modified IgSF domain (e.g. a second or, in some cases, also a third affinity-modified IgSF domain and so on) in which at least one additional IgSF domain is a vIgD that contains one or more amino acid modifications (e.g. substitution, deletion or mutation) compared to an IgSF domain in a wild-type or unmodified IgSF domain, such as an IgSF domain in an IgSF family member set forth in Table 2. In some embodiments, the additional, e.g., second or third affinity-modified IgSF domain comprises at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to a wild-type or unmodified IgSF domain or a specific binding fragment thereof contained in the sequence of amino acids set forth in any of SEQ ID NOS: 1-27 and 132. In some embodiments, the wild-type or unmodified IgSF domain is an IgV domain or an IgC domain, such as an IgC1 or IgC2 domain. In some embodiments, the additional, e.g., second or third IgSF domain is an affinity-modified IgV domain and/or IgC domain. In some embodiments, the one or more additional IgSF domain is an affinity-modified IgSF domain that contains an IgV domain and/or an IgC (e.g., IgC2) domain or domains, or a specific binding fragment of the IgV domain and/or a specific binding fragment of the IgC (e.g., IgC2) domain or domains, in which the IgV and/or IgC domain contains the amino acid modification(s) (e.g., substitution(s)). In some embodiments, the one or more additional affinity-modified IgSF domain contains an IgV domain containing the amino acid modification(s) (e.g. substitution(s)). In some embodiments, the one or more additional affinity-modified IgSF domain include IgSF domains present in the ECD or a portion of the ECD of the corresponding unmodified IgSF family member, such as a full-length IgV domain and a full-length IgC (e.g., IgC2) domain or domains, or specific binding fragments thereof, in which one or both of the IgV and IgC contain the amino acid modification(s) (e.g. substitution(s)).

[0216] In some embodiments, the provided immunomodulatory protein contains at least one additional, (e.g., second or, in some cases, also a third IgSF domain and so on) IgSF domain that is a vIgD that contains one or more amino acid substitutions compared to an IgSF domain (e.g. IgV) of a wild-type or unmodified IgSF domain other than CD155.

[0217] In some embodiments, the one or more additional IgSF domain (e.g. second or third IgSF) domain is an IgSF domain (e.g. IgV) of another IgSF family member that itself also binds to an inhibitory receptor In some aspects, the one or more additional IgSF domain (e.g. second or third IgSF) domain is an affinity-modified IgSF domain that is a variant IgSF domain (vIgD) of an IgSF family member that bind to an inhibitory receptor and that contains one or more amino acid substitutions in such an IgSF domain (e.g. IgV), in which, in some cases, the one or more amino acid modifications result in increased binding to the inhibitory receptor. In some embodiments, the vIgD contains one or more amino acid modifications (e.g. substitutions, deletions or additions) in a wild-type or unmodified IgSF domain (e.g. IgV) of an IgSF family member that binds to an inhibitory receptor. In addition to TIGIT, exemplary of such inhibitory receptors are CD112R CTLA-4, LAG3, PD-1, TIM-3, or BTLA. In some embodiments, the one or more additional IgSF domain is from an IgSF family member selected from CD112, PD-L1, PD-L2, CD80 or CEACAM1. Thus, in some aspects, provided are multi-target checkpoint antagonists that target or block activity of more than one inhibitory receptor.

[0218] In some embodiments, there is provided an immunomodulatory protein containing any one of the variant CD155 polypeptides and one or more IgSF domain of an inhibitory receptor, such as a wild-type or unmodified inhibitory receptor. In some embodiments, there is provided an immunomodulatory protein containing any one of the variant CD155 polypeptides and one or more IgSF domain of CD80, e.g. wild-type or unmodified CD80, such as an IgV domain set forth in SEQ ID NO: 969 or 1043 or an ECD or a portion thereof (containing the IgV and IgC domain or specific binding fragments thereof) set forth in SEQ ID NO:28 or a portion thereof. In some embodiments, there is provided an immunomodulatory protein containing any one of the variant CD155 polypeptides and one or more IgSF domain of PD-L1, e.g. wild-type or unmodified PD-L1, such as an IgV domain set forth in SEQ ID NO:469 or 665 or an ECD or a portion thereof (containing the IgV and IgC domain or specific binding fragments thereof) set forth in SEQ ID NO:30 or 1756 or a portion thereof. In some embodiments, there is provided an immunomodulatory protein containing any one of the variant CD155 polypeptides and one or more IgSF domain of PD-L2, e.g. wild-type or

unmodified PD-L2, such as an IgV domain set forth in SEQ ID NO: 666 or 726 or an ECD or a portion thereof (containing the IgV and IgC domain or specific binding fragments thereof) set forth in SEQ ID NO:31 or a portion thereof. In some embodiments, there is provided an immunomodulatory protein containing any one of the variant CD155 polypeptides and one or more IgSF domain of CD112, e.g. wild-type or unmodified CD112, such as an IgV domain set forth in SEQ ID NO:1272 or 1367 or an ECD or a portion thereof (containing the IgV and IgC domain or specific binding fragments thereof) set forth in SEQ ID NO:48 or a portion thereof.

[0219] In some embodiments, there is provided an immunomodulatory protein containing one or more additional IgSF domain (e.g., second or third IgSF) that is a vIgD of an IgSF family member that binds to an inhibitory receptor in which the one or more amino acid modifications in an IgSF domain (e.g. IgV) results in increased binding affinity of the vIgD, or a fusion or immunomodulatory protein containing the vIgD, for its inhibitory receptor cognate binding partner compared to the unmodified IgSF domain, such as binding affinity that is increased more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold 40-fold or 50-fold. In some embodiments, the one or more amino acid modifications in an IgSF domain (e.g. IgV) results in increased selectivity of the vIgD, or a fusion or immunomodulatory protein containing the vIgD for its inhibitory receptor compared to the unmodified IgSF domain. In some embodiments, the increased selectivity is a greater ratio of binding of the vIgD for the inhibitory receptor versus another cognate binding partner, such as a cognate binding partner that is not an inhibitory receptor, compared to the ratio of binding of the unmodified IgSF for the inhibitory receptor versus the another cognate binding partner. In some embodiments, the ratio is greater by at least or at least about 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold 40-fold or 50-fold.

[0220] In some embodiments, the at least one additional (e.g., second or third) vIgD is an IgSF domain (e.g. IgV) of a variant CD80 polypeptide that contains one or more amino acid modifications (e.g., substitutions, deletions or additions) in the IgSF domain (e.g., IgV) compared to unmodified or wild-type CD80, which, in some aspects, result in increased binding to the inhibitory receptor CTLA-4. Exemplary amino acid modifications, such as substitutions, deletions or additions, in an IgSF domain (e.g. IgV or ECD containing IgV and IgC) of a variant CD80 polypeptide are set forth in Table 3. In some embodiments, there is provided an immunomodulatory protein containing any of the provided variant CD155 polypeptides and a variant CD80 polypeptide containing an IgV domain including any of the amino acid modifications set forth in Table 3, such as the IgV domain set forth in any of SEQ ID NOS: 969-1002, 1004-1042, 1044-1076, 1078-1116 or an IgV domain that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any of SEQ ID NOS: 969-1002, 1004-1042, 1044-1076, 1078-1116 and contains the one or more amino acid modifications. In some embodiments, there is provided an immunomodulatory protein containing any of the provided variant CD155 polypeptides and a variant CD80 polypeptide containing an ECD or a portion thereof containing the IgV and/or IgC domains, in which is contained any of the amino acid modifications set forth in Table 3, such as the ECD set forth in any of SEQ ID NOS: 897-928, 930-968 or an ECD that contains at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any of SEQ ID NOS: 897-928, 930-968 and contains the one or more amino acid modifications.

[0221] In some embodiments, the at least one additional (e.g. second or third) vIgD is an IgSF domain (e.g. IgV) of a variant PD-L1 polypeptide that contains one or more amino acid modifications (e.g. substitutions, deletions or additions) in an IgSF domain (e.g. IgV) of PD-L1 or PD-L2, which are IgSF family members that bind to the inhibitory receptor PD-1. In some embodiments, the at least one additional (e.g. second or third) vIgD contains one or more amino acid modifications (e.g. substitutions, deletions or additions) in an IgSF domain (e.g. IgV) of CD112, which is IgSF family members that bind to the inhibitory receptor CD112R. In some embodiments, the at least one additional (e.g. second or third) vIgD contains one or more amino acid modifications (e.g. substitutions, deletions or additions) in the IgSF domain (e.g. IgVor ECD) compared to unmodified or wild-type of PD-L1, which, in some aspects, result in increased binding to the inhibitory receptor PD-1. Exemplary amino acid modifications, such as substitutions, deletions or additions, in an IgSF domain (e.g. IgV or ECD containing IgV and IgC) of a variant PD-L1 polypeptide are set forth in Table 4. In some embodiments, there is provided an immunomodulatory protein containing any of the provided variant CD155 polypeptides and a variant PD-L1 polypeptide containing an IgV domain including any of the amino acid modifications set forth in Table 4, such as the IgV domain set forth in any of SEQ ID NOS: 535-664, 1754, 1755, 1936-1965, or an IgV domain that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any of SEQ ID NOS: 535-664, 1754, 1755, 1936-1965 and contains the one more more amino acid modifications. In some embodiments, there is provided an immunomodulatory protein containing any of the provided variant CD155 polypeptides and a variant PD-L1 polypeptide containing an ECD or a portion thereof containing the IgV and/or IgC domains, in which is contained any of the amino acid modifications set forth in Table 4, such as the ECD set forth in any of SEQ ID NOS: 470-534, 1753, 1757-1935, 1966-2031, or an ECD that contains at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any of SEQ ID NOS: 470-534, 1753, 1757-1935, 1966-2031 and contains the one or more amino acid modifications.

[0222] In some embodiments, the at least one additional (e.g., second or third) vIgD is an IgSF domain (e.g. IgV) of a variant PD-L2 polypeptide that contains one or more amino acid modifications (e.g., substitutions, deletions or additions) in the IgSF domain (e.g., IgV) compared to unmodified or wild-type PD-L2, which, in some aspects, result in increased binding to the inhibitory receptor PD-1. Exemplary amino acid modifications, such as substitutions, deletions or additions, in an IgSF domain (e.g. IgV or ECD containing IgV and IgC) of a variant PD-L2 polypeptide are set forth in Table 5. In some embodiments, there is provided an immunomodulatory protein containing any of the provided variant CD155 polypeptides and a variant PD-L2 polypeptide containing an IgV domain including any of the amino acid modifications set forth in Table 5, such as the IgV domain set forth in any of SEQ ID NOS:744-794,

796-870, 872-895 or an IgV domain that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any of SEQ ID NOS: 744-794, 796-870, 872-895 and contains the one more more amino acid modifications. In some embodiments, there is provided an immunomodulatory protein containing any of the provided variant CD155 polypeptides and a variant PD-L2 polypeptide containing an ECD or a portion thereof containing the IgV and/or IgC domains, in which is contained any of the amino acid modifications set forth in Table 5, such as the ECD set forth in any of SEQ ID NOS: 667-717, 719-743 or an ECD that contains at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any of SEQ ID NOS: 667-717, 719-743 and contains the one or more amino acid modifications.

[0223] In some embodiments, the at least one additional (e.g., second or third) vIgD is an IgSF domain (e.g. IgV) of a variant CD112 polypeptide that contains one or more amino acid modifications (e.g., substitutions, deletions or additions) in the IgSF domain (e.g., IgV) compared to unmodified or wild-type CD112, which, in some aspects, result in increased binding to the inhibitory receptor TIGIT. Exemplary amino acid modifications, such as substitutions, deletions or additions, in an IgSF domain (e.g. IgV or ECD containing IgV and IgC) of a variant CD112 polypeptide are set forth in Table 8. In some embodiments, there is provided an immunomodulatory protein containing any of the provided variant CD155 polypeptides and a variant CD112 polypeptide containing an IgV domain including any of the amino acid modifications set forth in Table 8, such as the IgV domain set forth in any of SEQ ID NOS: 1320-1366, 1368-1414, 1497-1537, 1586-1609, or an IgV domain that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any of SEQ ID NOS: 1320-1366, 1368-1414, 1497-1537, 1586-1609 and contains the one more more amino acid modifications. In some embodiments, there is provided an immunomodulatory protein containing any of the provided variant CD155 polypeptides and a variant CD112 polypeptide containing an ECD or a portion thereof containing the IgV and/or IgC domains, in which is contained any of the amino acid modifications set forth in Table 8, such as the ECD set forth in any of SEQ ID NOS: 1273-1319, 1415-1455, 1538-1561, or an ECD that contains at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any of SEQ ID NOS: 1273-1319, 1415-1455, 1538-1561 and contains the one or more amino acid modifications.

[0224] In some embodiments, the one or more additional IgSF domain (e.g. second or third IgSF) domain is an IgSF domain (e.g. IgV) of another IgSF family member that binds or recognizes a tumor antigen. In such embodiments, the IgSF family member serves as a tumor-localizing moiety, thereby bringing the vIgD of CD155 in close proximity to immune cells in the tumor microenvironment. In some embodiments, the additional IgSF domain (e.g. second or third IgSF) domain is an IgSF domain of NKp30, which binds or recognizes B7-H6 expressed on a tumor cell. In some embodiments, the at least one additional (e.g. second or third) IgSF domain, e.g. NKp30, is an affinity-modified IgSF domain or vIgD that contains one or more amino acid modifications (e.g. substitutions, deletions or additions). In some embodiments, the one or more amino acid modifications increase binding affinity and/or selectivity to B7-H6 compared to unmodified IgSF domain, e.g. NKp30, such as by at least or at least about 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold 40-fold or 50-fold. Exemplary amino acid modifications, such as substitutions, deletions or additions, in an IgSF domain (e.g. IgC-like or full ECD) of a variant NKp30 polypeptide are set forth in Table 6. Among the exemplary polypeptides is an NKp30 variant that contains the mutations L30V/A60V/S64P/S86G with reference to positions in the NKp30 extracellular domain corresponding to positions set forth in SEQ ID NO: 54. In some embodiments, there is provided an immunomodulatory protein containing any of the provided variant CD155 polypeptides and a variant NKp30 polypeptide containing an IgC-like domain including any of the amino acid modifications set forth in Table 6, such as the IgC-like domain set forth in any of SEQ ID NOS: 1162-1166 or an IgC-like domain that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any of SEQ ID NOS: 1162-1166 and contains the one more amino acid modifications. In some embodiments, there is provided an immunomodulatory protein containing any of the provided variant CD155 polypeptides and a variant NKp30 polypeptide containing an ECD or a portion thereof containing an IgSF domain or domains, in which is contained any of the amino acid modifications set forth in Table 6, such as the ECD set forth in any of SEQ ID NOS: 1156-1160 or an ECD that contains at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any of SEQ ID NOS: 1156-1160 and contains the one or more amino acid modifications.

[0225] In some embodiments, the at least one additional (e.g., second or third) vIgD is an IgSF domain (e.g. IgV) of a variant CD86 polypeptide that contains one or more amino acid modifications (e.g., substitutions, deletions or additions) in the IgSF domain (e.g., IgV) compared to unmodified or wild-type CD86, which, in some aspects, result in increased binding to its cognate binding partner. Exemplary amino acid modifications, such as substitutions, deletions or additions, in an IgSF domain (e.g. IgV or ECD containing IgV and IgC) of a variant CD86 polypeptide are set forth in Table 7. Among exemplary polypeptides include CD86 variants that contain the mutations Q35H/H90L/Q102H with reference to positions in the CD86 extracellular domain corresponding to positions set forth in SEQ ID NO: 29. In some embodiments, there is provided an immunomodulatory protein containing any of the provided variant CD155 polypeptides and a variant CD86 polypeptide containing an IgV domain including any of the amino acid modifications set forth in Table 7, such as the IgV domain set forth in any of SEQ ID NOS: 1174-1177 or an IgV domain that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any of SEQ ID NOS: 1174-1177 and contains the one more more amino acid modifications. In some embodiments, there is provided an immunomodulatory protein containing any of the provided variant CD155 polypeptides and a variant CD86 polypeptide containing an ECD or a portion thereof containing the IgV and/or IgC domains, in which is contained any of the amino acid modifications set forth in Table 7, such as the ECD set forth in any of SEQ ID NOS: 1169-1172 or an ECD that contains at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% to any of SEQ ID NOS: 1169-1172 and contains the one or more amino acid modifications.

[0226] Tables 3-8 provide exemplary polypeptides containing one or more affinity-modified IgSF domains that can be used in stack constructs provided herein.

TABLE 3

Exemplary variant CD80 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
Wild-type	28	969, 1043
L70P	896	970, 1044
I30F/L70P	897	971, 1045
Q27H/T41S/A71D	898	972, 1046
I30T/L70R	899	973, 1047
T13R/C16R/L70Q/A71D	900	974, 1048
T57I	901	975, 1049
M43I/C82R	902	976, 1050
V22L/M38V/M47T/A71D/L85M	903	977, 1051
I30V/T57I/L70P/A71D/A91T	904	978, 1052
V22I/L70M/A71D	905	979, 1053
N55D/L70P/E77G	906	980, 1054
T57A/I69T	907	981, 1055
N55D/K86M	908	982, 1056
L72P/T79I	909	983, 1057
L70P/F92S	910	984, 1058
T79P	911	985, 1059
E35D/M47I/L65P/D90N	912	986, 1060
L25S/E35D/M47I/D90N	913	987, 1061
A71D	915	989, 1063
E81K/A91S	917	991, 1065
A12V/M47V/L70M	918	992, 1066
K34E/T41A/L72V	919	993, 1067
T41S/A71D/V84A	920	994, 1068
E35D/A71D	921	995, 1069
E35D/M47I	922	996, 1070
K36R/G78A	923	997, 1071
Q33E/T41A	924	998, 1072
M47V/N48H	925	999, 1073
M47L/V68A	926	1000, 1074
S44P/A71D	927	1001, 1075
Q27H/M43I/A71D/R73S	928	1002, 1076
E35D/T57I/L70Q/A71D	930	1004, 1078
M47I/E88D	931	1005, 1079
M42I/I61V/A71D	932	1006, 1080
P51A/A71D	933	1007, 1081
H18Y/M47I/T57I/A71G	934	1008, 1082
V20I/M47V/T57I/V84I	935	1009, 1083
V20I/M47V/A71D	936	1010, 1084
A71D/L72V/E95K	937	1011, 1085
V22L/E35G/A71D/L72P	938	1012, 1086
E35D/A71D	939	1013, 1087
E35D/I67L/A71D	940	1014, 1088
Q27H/E35G/A71D/L72P/T79I	941	1015, 1089
T13R/M42V/M47I/A71D	942	1016, 1090
E35D	943	1017, 1091
E35D/M47I/L70M	944	1018, 1092
E35D/A71D/L72V	945	1019, 1093
E35D/M43L/L70M	946	1020, 1094
A26P/E35D/M43I/L85Q/E88D	947	1021, 1095
E35D/D46V/L85Q	948	1022, 1096
Q27L/E35D/M47I/T57I/L70Q/E88D	949	1023, 1097
M47V/I69F/A71D/V83I	950	1024, 1098
E35D/T57A/A71D/L85Q	951	1025, 1099
H18Y/A26T/E35D/A71D/L85Q	952	1026, 1100
E35D/M47L	953	1027, 1101
E23D/M42V/M43I/I58V/L70R	954	1028, 1102
V68M/L70M/A71D/E95K	955	1029, 1103
N55I/T57I/169F	956	1030, 1104
E35D/M43I/A71D	957	1031, 1105
T41S/T57I/L70R	958	1032, 1106
H18Y/A71D/L72P/E88V	959	1033, 1107
V20I/A71D	960	1034, 1108
E23G/A26S/E35D/T62N/A71D/L72V/L85M	961	1035, 1109
A12T/E24D/E35D/D46V/I61V/L72P/E95V	962	1036, 1110
V22L/E35D/M43L/A71G/D76H	963	1037, 1111

TABLE 3-continued

Exemplary variant CD80 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
E35G/K54E/A71D/L72P	964	1038, 1112
L70Q/A71D	965	1039, 1113
A26E/E35D/M47L/L85Q	966	1040, 1114
D46E/A71D	967	1041, 1115
Y31H/E35D/T41S/V68L/K93R/R94W	968	1042, 1116

TABLE 4

Exemplary variant PD-L1 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
Wild-type	30, 1756	469, 665
K28N/M41V/N45T/H51N/K57E	470, 1966	535, 600
I20L/I36T/N45D/I47T	471, 1967	536, 601
I20L/M41K/K44E	472, 1968	537, 602
P6S/N45T/N78I/183T	473, 1969	538, 603
N78I	474, 1970	539, 604
M41K/N78I	475, 1971	540, 605
N45T/N78I	476, 1972	541, 606
I20L/N45T	477, 1973	542, 607
N45T	478, 1974	543, 608
M41K	479, 1975	544, 609
I20L/I36T/N45D	480, 1976	545, 610
N17D/N45T/V50A/D72G	481, 1977	546, 611
I20L/F49S	482, 1978	547, 612
N45T/V50A	483, 1979	548, 613
I20L/N45T/N78I	484, 1980	549, 614
I20L/N45T/V50A	485, 1981	550, 615
M41V/N45T	486, 1982	551, 616
M41K/N45T	487, 1983	552, 617
A33D/S75P/D85E	488, 1984	553, 618
M18I/M41K/D43G/H51R/N78I	489, 1985	554, 619
V11E/I20L/I36T/N45D/H60R/S75P	490, 1986	555, 620
A33D/V50A	491, 1987	556, 621
S16G/A33D/K71E/S75P	492, 1988	557, 622
E27G/N45T/M97I	493, 1989	558, 623
E27G/N45T/K57R	494, 1990	559, 624
A33D/E53V	495, 1991	560, 625
D43G/N45D/V58A	496, 1992	561, 626
E40G/D43V/N45T/V50A	497, 1993	562, 627
Y14S/K28E/N45T	498, 1994	563, 628
A33D/N78S	499, 1995	564, 629
A33D/N78I	500, 1996	565, 630
A33D/N45T	501, 1997	566, 631
A33D/N45T/N78I	502, 1998	567, 632
E27G/N45T/V50A	503, 1999	568, 633
N45T/V50A/N78S	504, 2000	569, 634
I20L/N45T/V110M	505, 2001	570,635
I20L/I36T/N45T/V50A	506, 2002	571, 636
N45T/L74P/S75P	507, 2003	572, 637
N45T/S75P	508, 2004	573, 638
S75P/K106R	509, 2005	574, 639
S75P	510, 2006	575, 640
A33D/S75P	511, 2007	576, 641
A33D/S75P/D104G	512, 2008	577, 642
A33D/S75P	513, 2009	578, 643
I20L/E27G/N45T/V50A	514, 2010	579, 644
I20L/E27G/D43G/N45D/V58A/N78I	515, 2011	580, 645
I20L/D43G/N45D/V58A/N78I	516, 2012	581, 646
I20L/A33D/D43G/N45D/V58A/N78I	517, 2013	582, 647
I20L/D43G/N45D/N78I	518, 2014	583, 648
E27G/N45T/V50A/N78I	519, 2015	584, 649
N45T/V50A/N78I	520, 2016	585, 650
V11A/I20L/E27G/D43G/N45D/H51Y/S99G	521, 2017	586, 651
I20L/E27G/D43G/N45T/V50A	522, 2018	587, 652
I20L/K28E/D43G/N45D/V58A/Q89R	523, 2019	588, 653

TABLE 4-continued

Exemplary variant PD-L1 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
I20L/I36T/N45D	524, 2020	589, 654
I20L/K28E/D43G/N45D/E53G/V58A/N78I	525, 2021	590, 655
A33D/D43G/N45D/V58A/S75P	526, 2022	591, 656
K23R/D43G/N45D	527, 2023	592, 657
I20L/D43G/N45D/V58A/N78I/D90G/G101D	528, 2024	593, 658
D43G/N45D/L56Q/V58A/G101G-ins(G101GG)	529, 2025	594, 659
I20L/K23E/D43G/N45D/V58A/N78I	530, 2026	595, 660
I20L/K23E/D43G/N45D/V50A/N78I	531, 2027	596, 661
T19I/E27G/N45I/V50A/N781/M97K	532, 2028	597, 662
I20L/M41K/D43G/N45D	533, 2029	598, 663
K23R/N45T/N78I	534, 2030	599, 664
I20L/K28E/D43G/N45D/V58A/ Q89R/G101G-ins (G101GG)	1753, 2031	1754, 1755
K57R/S99G	1757, 1847	1936, 1951
K57R/S99G/F189L	1758, 1848	
M18V/M97L/F193S/R195G/E200K/H202Q	1759, 1849	
I36S/M41K/M97L/K144Q/R195G/ E200K/H202Q/L206	1760, 1850	
C22R/Q65L/L124S/K144Q/R195G/ E200N/H202Q/T221	1761	
M18V/I98L/L124S/P198T/L206F	1762, 1851	
S99G/N117S/I148V/K171R/R180S	1763, 1852	
I36T/M97L/A103V/Q155H	1764, 1853	
K28I/S99G	1765, 1854	1937, 1952
R195S	1766, 1855	
A79T/S99G/T185A/R195G/E200K/ H202Q/L206F	1767, 1856	
K57R/S99G/L124S/K144Q	1768, 1857	
K57R/S99G/R195G	1769, 1858	
D55V/M97L/S99G	1770, 1859	1938, 1953
E27G/I36T/D55N/M97L/K111E	1771, 1860	1939, 1954
E54G/M97L/S99G	1772, 1861	1940, 1955
G15A/I36T/M97L/K111E/H202Q	1773, 1862	
G15A/I36T/V129D	1774, 1863	
G15A/I36T/V129D/R195G	1775, 1864	
G15A/V129D	1776, 1865	
I36S/M97L	1777, 1866	1941, 1956
I36T/D55N/M97L/K111E/A204T	1778, 1867	
I36T/D55N/M97L/K111E/V129A/F173L	1779, 1868	
I36T/D55S/M97L/K111E/I148V/R180S	1780, 1869	
I36T/G52R/M97L/V112A/K144E/ V175A/P198T	1781, 1870	
I36T/I46V/D55G/M97L/K106E/ K144E/T185A/R195G	1782, 1871	
I36T/I83T/M97L/K144E/P198T	1783, 1872	
I36T/M97L/K111E	1784, 1873	1942, 1957
I36T/M97L/K144E/P198T	1785, 1874	
I36T/M97L/Q155H/F193S/N201Y	1786, 1875	
I36T/M97L/V129D	1787, 1876	
L35P/I36S/M97L/K111E	1788, 1877	1943, 1958
M18I/I36T/E53G/M97L/K144E/E199G/V207A	1789, 1878	
M18T/I36T/D55N/M97L/K111E	1790, 1879	1944, 1959
M18V/M97L/T176N/R195G	1791, 1880	
M97L/S99G	1792, 1881	1945, 1960
N17D/M97L/S99G	1793, 1882	1946, 1961
S99G/T185A/R195G/P198T	1794, 1883	
V129D/H202Q	1795, 1884	
V129D/P198T	1796, 1885	
V129D/T150A	1797, 1886	
V93E/V129D	1798, 1887	
Y10F/M18V/S99G/Q138R/T203A	1799, 1888	
N45D	1800, 1889	1947, 1962
K160M/R195G	1801, 1890	
N45D/K144E	1802, 1891	
N45D/P198S	1803, 1892	
N45D/P198T	1804, 1893	
N45D/R195G	1805, 1894	
N45D/R195S	1806, 1895	
N45D/S131F	1807, 1896	
N45D/V58D	1808, 1897	1948, 1963
V129D/R195S	1809, 1898	

TABLE 4-continued

Exemplary variant PD-L1 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
I98T/F173Y/L196S	1810, 1899	
N45D/E134G/L213P	1811, 1900	
N45D/F173I/S177C	1812, 1901	
N45D/I148V/R195G	1813, 1902	
N45D/K111T/R195G	1814, 1903	
N45D/N113Y/R195S	1815, 1904	
N45D/N165Y/E170G	1816, 1905	
N45D/Q89R/198V	1817, 1906	1949, 1964
N45D/S131F/P198S	1818, 1907	
N45D/S75P/P198S	1819, 1908	
N45D/V50A/R195T	1820, 1909	
E27D/N45D/T183A/I188V	1821, 1910	
F173Y/T183I/L196S/T203A	1822, 1911	
K23N/N45D/S75P/N120S	1823, 1912	
N45D/G102D/R194W/R195G	1824, 1913	
N45D/G52V/Q121L/P198S	1825, 1914	
N45D/I148V/R195G/N201D	1826, 1915	
N45D/K111T/T183A/I188V	1827, 1916	
N45D/Q89R/F189S/P198S	1828, 1917	
N45D/S99G/C137R/V207A	1829, 1918	
N45D/T163I/K167R/R195G	1830, 1919	
N45D/T183A/T192S/R194G	1831, 1920	
N45D/V50A/I119T/K144E	1832, 1921	
T19A/N45D/K144E/R195G	1833, 1922	
V11E/N45D/T130A/P198T	1834, 1923	
V26A/N45D/T163I/T185A	1835, 1924	
K23N/N45D/L124S/K167T/R195G	1836, 1925	
K23N/N45D/Q73R/T163I	1837, 1926	
K28E/N45D/W149R/S158G/P198T	1838, 1927	
K28R/N45D/K57E/I98V/R195S	1839, 1928	
K28R/N45D/V129D/T163N/R195T	1840, 1929	
M41K/D43G/N45D/R64S/R195G	1841, 1930	
M41K/D43G/N45D/R64S/S99G	1842, 1931	1950, 1965
N45D/R68L/F173L/D197G/P198S	1843, 1932	
N45D/V50A/I148V/R195G/N201D	1844, 1933	
M41K/D43G/K44E/N45D/R195G/N201D	1845, 1934	
N45D/V50A/L124S/K144E/L179P/R195G	1846, 1935	

TABLE 5

Exemplary variant PD-L2 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
Wild-type	31	666, 726
H15Q	667	744, 820
N24D	668	745, 821
E44D	669	746, 822
V89D	670	747, 823
Q82R/V89D	671	748, 824
E59G/Q82R	672	749, 825
S39I/V89D	673	750, 826
S67L/V89D	674	751, 827
S67L/I85F	675	752, 828
S67L/I86T	676	753, 829
H15Q/K65R	677	754, 830
H15Q/Q72H/V89D	678	755, 831
H15Q/S67L/R76G	679	756, 832
H15Q/R76G/I85F	680	757, 833
H15Q/T47A/Q82R	681	758, 834
H15Q/Q82R/V89D	682	759, 835
H15Q/C23S/I86T	683	760, 836
H15Q/S39I/I86T	684	761, 837
H15Q/R76G/I85F	685	762, 838
E44D/V89D/W91R	686	763, 839
I13V/S67L/V89D	687	764, 840
H15Q/S67L/I86T	688	765, 841

TABLE 5-continued

Exemplary variant PD-L2 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
I13V/H15Q/S67L/I86T	689	766, 842
I13V/H15Q/E44D/V89D	690	767, 843
I13V/S39I/E44D/Q82R/V89D	691	768, 844
I13V/E44D/Q82R/V89D	692	769, 845
I13V/Q72H/R76G/I86T	693	770, 846
I13V/H15Q/R76G/I85F	694	771, 847
H15Q/S39I/R76G/V89D	695	772, 848
H15Q/S67L/R76G/I85F	696	773, 849
H15Q/T47A/Q72H/R76G/I86T	697	774, 850
H15Q/T47A/Q72H/R76G	698	775, 851
I13V/H15Q/T47A/Q72H/R76G	699	776, 852
H15Q/E44D/R76G/I85F	700	777, 853
H15Q/S39I/S67L/V89D	701	778, 854
H15Q/N32D/S67L/V89D	702	779, 855
N32D/S67L/V89D	703	780, 856
H15Q/S67L/Q72H/R76G/V89D	704	781, 857
H15Q/Q72H/Q74R/R76G/I86T	705	782, 858
G28V/Q72H/R76G/I86T	706	783, 859
I13V/H15Q/S39I/E44D/S67L	707	784, 860
E44D/S67L/Q72H/Q82R/V89D	708	785, 861
H15Q/V89D	709	786, 862
H15Q/T47A	710	787, 863
I13V/H15Q/Q82R	711	788, 864
I13V/H15Q/V89D	712	789, 865
I13V/S67L/Q82R/V89D	713	790, 866
I13V/H15Q/Q82R/V89D	714	791, 867
H15Q/V31M/S67L/Q82R/V89D	715	792, 868
I13V/H15Q/T47A/Q82R	716	793, 869
I13V/H15Q/V31A/N45S/Q82R/V89D	717	794, 870
H15Q/T47A/H69L/Q82R/V89D	719	796, 872
I13V/H15Q/T47A/H69L/R76G/V89D	720	797, 873
I12V/I13V/H15Q/T47A/Q82R/V89D	721	798, 874
I13V/H15Q/R76G/D77N/Q82R/V89D	722	799, 875
I13V/H15Q/T47A/R76G/V89D	723	800, 876
I13V/H15Q/T47A/Q82R/V89D	724	801, 877
I13V/H15Q/N24D/Q82R/V89D	725	802, 878
I13V/H15Q/I36V/T47A/S67L/V89D	727	803, 879
H15Q/T47A/K65R/S67L/Q82R/V89D	728	804, 880
H15Q/L33P/T47A/S67L/P71S/V89D	729	805, 881
I13V/H15Q/Q72H/R76G/I86T	730	806, 882
H15Q/T47A/S67L/Q82R/V89D	731	807, 883
F2L/H15Q/D46E/T47A/Q72H/R76G/Q82R/V89D	732	808, 884
I13V/H15Q/L33F/T47A/Q82R/V89D	733	809, 885
I13V/H15Q/T47A/E58G/S67L/Q82R/V89D	734	810, 886
H15Q/N24S/T47A/Q72H/R76G/V89D	735	811, 887
I13V/H15Q/E44V/T47A/Q82R/V89D	736	812, 888
H15Q/N18D/T47A/Q72H/V73A/R76G/I86T/V89D	737	813, 889
I13V/H15Q/T37A/E44D/S48C/S67L/Q82R/V89D	738	814, 890
H15Q/L33H/S67L/R76G/Q82R/V89D	739	815, 891
I13V/H15Q/T47A/Q72H/R76G/I86T	740	816, 892
H15Q/S39I/E44D/Q72H/V75G/R76G/Q82R/V89D	741	817, 893
H15Q/T47A/S67L/R76G/Q82R/V89D	742	818, 894
I13V/H15Q/T47A/S67L/Q72H/R76G/Q82R/V89D	743	819, 895

TABLE 6

Exemplary variant NKp30 polypeptides

Mutation(s)	ECD SEQ ID NO	IgC-like domain SEQ ID NO
Wild-type	54	1161
L30V/A60V/S64P/S86G	1156	1162
L30V	1157	1163
A60V	1158	1164

TABLE 6-continued

Exemplary variant NKp30 polypeptides

Mutation(s)	ECD SEQ ID NO	IgC-like domain SEQ ID NO
S64P	1159	1165
S86G	1160	1166

TABLE 7

Exemplary variant CD86 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
Wild-type	29	1173
Q35H/H90L/Q102H	1169	1174
Q35H	1170	1175
H90L	1171	1176
Q102H	1172	1177

TABLE 8

Exemplary variant CD112 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
Wild-type	48	1272, 1367
Y33H/A112V/G117D	1273	1320, 1368
V19A/Y33H/S64G/S80G/G98S/N106Y/A112V	1274	1321, 1369
L32P/A112V	1275	1322, 1370
A95V/A112I	1276	1323, 1371
P28S/A112V	1277	1324, 1372
P27A/T38N/V101A/A112V	1278	1325, 1373
S118F	1279	1326, 1374
R12W/H48Y/F54S/S118F	1280	1327, 1375
R12W/Q79R/S118F	1281	1328, 1376
T113S/S118Y	1282	1329, 1377
S118Y	1283	1330, 1378
N106I/S118Y	1284	1331, 1379
N106I/S118F	1285	1332, 1380
A95T/L96P/S118Y	1286	1333, 1381
Y33H/P67S/N106Y/A112V	1287	1334, 1382
N106Y/A112V	1288	1335, 1383
T18S/Y33H/A112V	1289	1336, 1384
P9S/Y33H/N47S/A112V	1290	1337, 1385
P42S/P67H/A112V	1291	1338, 1386
P27L/L32P/P42S/A112V	1292	1339, 1387
G98D/A112V	1293	1340, 1388
Y33H/S35P/N106Y/A112V	1294	1341, 1389
L32P/P42S/T100A/A112V	1295	1342, 1390
P27S/P45S/N106I/A112V	1296	1343, 1391
Y33H/N47K/A112V	1297	1344, 1392
Y33H/N106Y/A112V	1298	1345, 1393
K78R/D84G/A112V/F114S	1299	1346, 1394
Y33H/N47K/F54L/A112V	1300	1347, 1395
Y33H/A112V	1301	1348, 1396
A95V/A112V	1302	1349, 1397
R12W/A112V	1303	1350, 1398
R12W/P27S/A112V	1304	1351, 1399
Y33H/V51M/A112V	1305	1352, 1400
Y33H/A112V/S118T	1306	1353, 1401
Y33H/V101A/A112V/P115S	1307	1354, 1402
H24R/T38N/D43G/A112V	1308	1355, 1403
A112V	1309	1356, 1404
P27A/A112V	1310	1357, 1405
A112V/S118T	1311	1358, 1406
R12W/A112V/M1221	1312	1359, 1407

TABLE 8-continued

Exemplary variant CD112 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
Q83K/N106Y/A112V	1313	1360, 1408
R12W/P27S/A112V/S118T	1314	1361, 1409
P28S/Y33H/A112V	1315	1362, 1410
P27S/Q90R/A112V	1316	1363, 1411
L15V/P27A/A112V/S118T	1317	1364, 1412
Y33H/N106Y/T108I/A112V	1318	1365, 1413
Y33H/P56L/V75M/V101M/A112V	1319	1366, 1414
N47K/Q79R/S118F	1415	1456, 1497
Q40R/P60T/A112V/S118T	1416	1457, 1498
F114Y/S118F	1417	1458, 1499
Y33H/K78R/S118Y	1418	1459, 1500
R12W/A46T/K66M/Q79R/N106I/T113A/S118F	1419	1460, 1501
Y33H/A112V/S118F	1420	1461, 1502
R12W/Y33H/N106I/S118F	1421	1462, 1503
L15V/Q90R/S118F	1422	1463, 1504
N47K/D84G/N106I/S118Y	1423	1464, 1505
L32P/S118F	1424	1465, 1506
Y33H/Q79R/A112V/S118Y	1425	1466, 1507
T18A/N106I/S118T	1426	1467, 1508
L15V/Y33H/N106Y/A112V/S118F	1427	1468, 1509
V37M/S118F	1428	1469, 1510
N47K/A112V/S118Y	1429	1470, 1511
A461/A112V	1430	1471, 1512
P28S/Y33H/N106I/S118Y	1431	1472, 1513
P30S/Y33H/N47K/V75M/Q79R/N106I/S118Y	1432	1473, 1514
V19A/N47K/N106Y/K116E/S118Y	1433	1474, 1515
Q79R/T85A/A112V/S118Y	1434	1475, 1516
V101M/N106I/S118Y	1435	1476, 1517
Y33H/Q79R/N106I/A112V/S118T	1436	1477, 1518
Q79R/A112V	1437	1478, 1519
Y33H/A46I/Q79R/N106I/S118F	1438	1479, 1520
A112V/G121S	1439	1480, 1521
Y33H/Q79R/N106I/S118Y	1440	1481, 1522
Y33H/N106I/A112V	1441	1482, 1523
Y33H/A46T/V101M/A112V/S118T	1442	1483, 1524
L32P/L99M/N106I/S118F	1443	1484, 1525
L32P/T108A/S118F	1444	1485, 1526
R12W/Q79R/A112V	1445	1486, 1527
Y33H/N106Y/E110G/A112V	1446	1487, 1528
Y33H/N106I/S118Y	1447	1488, 1529
Q79R/S118F	1448	1489, 1530
Y33H/Q79R/G98D/V101M/A112V	1449	1490, 1531
N47K/T81S/V101M/A112V/S118F	1450	1491, 1532
G82S/S118Y	1451	1492, 1533
Y33H/A112V/S118Y	1452	1493, 1534
Y33H/N47K/Q79R/N106Y/A112V	1453	1494, 1535
Y33H/S118T	1454	1495, 1536
R12W/Y33H/Q79R/V101M/A112V	1455	1496, 1537
Y33H/Q83K/A112V/S118T	1538	1562, 1586
V29M/Y33H/N106I/S118F	1539	1563, 1587
Y33H/A46T/A112V	1540	1564, 1588
Y33H/Q79R/S118F	1541	1565, 1589
Y33H/N47K/F74L/S118F	1542	1566, 1590
R12W/V101M/N106I/S118Y	1543	1567, 1591
A46T/V101A/N106I/S118Y	1544	1568, 1592
N106Y/A112V/S118T	1545	1569, 1593
S76P/T811/V101M/N106Y/A112V/S118F	1546	1570, 1594
P9R/L21V/P22L/134M/S69F/F74L/ A87V/A112V/L125A	1547	1571, 1595
Y33H/V101M/A112V	1548	1572, 1596
V29A/L32P/S118F	1549	1573, 1597
Y33H/V101M/N106I/A112V	1550	1574, 1598
R12W/Y33H/N47K/Q79R/S118Y	1551	1575, 1599
Y33H/A46T/A112V/S118T	1552	1576, 1600
Y33H/A112V/F114L/S118T	1553	1577, 1601
Y33H/T38A/A46T/V101M/A112V	1554	1578, 1602
P28S/Y33H/S69P/N106I/A112V/S118Y	1555	1579, 1603
Y33H/P42L/N47K/V101M/A112V	1556	1580, 1604
Y33H/N47K/F74S/Q83K/N106I/F111L/ A112V/S118T	1557	1581, 1605

TABLE 8-continued

Exemplary variant CD112 polypeptides

Mutation(s)	ECD SEQ ID NO	IgV SEQ ID NO
Y33H/A112V/S118T/V119A	1558	1582, 1606
Y33H/N106I/A112V/S118F	1559	1583, 1607
Y33H/K66M/S118F/W124L	1560	1584, 1608
N106I/A112V	1561	1585, 1609

[0227] In some embodiments, the two or more IgSF domain, including a vIgD of CD155 and one or more additional IgSF domain (e.g. second or third variant IgSF domain) from another IgSF family member, are covalently or non-covalently linked. A plurality of non-affinity modified and/or affinity modified IgSF domains in a stacked immunomodulatory protein polypeptide chain need not be covalently linked directly to one another. In some embodiments, the two or more IgSF domains are linked directly or indirectly, such as via a linker. In some embodiments, an intervening span of one or more amino acid residues indirectly covalently bonds IgSF domains to each other. The linkage can be via the N-terminal to C-terminal residues. In some embodiments, the linkage can be made via side chains of amino acid residues that are not located at the N-terminus or C-terminus of the IgSF domain(s). Thus, linkages can be made via terminal or internal amino acid residues or combinations thereof.

[0228] In some embodiments, the immunomodulatory protein contains at least two IgSF domains, each linked directly or indirectly via a linker. In some embodiments, the immunomodulatory protein contains at least three immunomodulatory proteins, each linked directly or indirectly via a linker. Various configurations are shown in FIGS. **5**A and **5**B.

[0229] In some embodiments, one or more "peptide linkers" link the vIgD of CD155 and one or more additional IgSF domain (e.g. second or third variant IgSF domain). In some embodiments, a peptide linker can be a single amino acid residue or greater in length. In some embodiments, the peptide linker has at least one amino acid residue but is no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues in length. In some embodiments, the linker is (in one-letter amino acid code): GGGGS ("4GS"; SEQ ID NO: 1752) or multimers of the 4GS linker, such as repeats of 2, 3, 4, or 5 4GS linkers. In some embodiments, the peptide linker is $(GGGGS)_2$ SEQ ID NO: 1182 or $(GGGGS)_3$ SEQ ID NO: 1181. In some embodiments, the linker also can include a series of alanine residues alone or in addition to another peptide linker (such as a 4GS linker or multimer thereof). In some embodiments, the number of alanine residues in each series is: 2, 3, 4, 5, or 6 alanines.

[0230] In some embodiments, the non-affinity modified and/or affinity modified IgSF domains are linked by "wild-type peptide linkers" inserted at the N-terminus and/or C-terminus of a second non-affinity modified and/or affinity modified IgSF domains. These linkers are also called leading sequences (N-terminal to non-affinity modified or affinity modified IgSF domain) or trailing sequences (C-terminal to non-affinity modified or affinity modified IgSF domain), and sequences that exist in the wild-type protein that span immediately outside the structural prediction of the Ig fold of the IgSF. In some embodiments, the "wild-type linker" is

an amino acid sequence that exists after the signal sequence, but before in the IgSF domain, such as the defined IgV domain, in the amino acid sequence of the wild-type protein. In some embodiments, the "wild-type" linker is an amino acid sequence that exists immediately after the IgSF domain, such as immediately after the defined IgV domain but before the IgC domain, in the amino acid sequence of the wild-type protein. These linker sequences can contribute to the proper folding and function of the neighboring IgSF domain(s). In some embodiments, there is present a leading peptide linker inserted at the N-terminus of the first IgSF domain and/or a trailing sequence inserted at the C-terminus of the first non-affinity modified and/or affinity modified IgSF domain. In some embodiments, there is present a second leading peptide linker inserted at the N-terminus of the second IgSF domain and/or a second trailing sequence inserted at the C-terminus of the second non-affinity modified and/or affinity modified IgSF domain. When the first and second non-affinity modified and/or affinity modified IgSF domains are derived from the same parental protein and are connected in the same orientation, wild-type peptide linkers between the first and second non-affinity modified and/or affinity modified IgSF domains are not duplicated. For example, when the first trailing wild-type peptide linker and the second leading wild-type peptide linker are the same, the Type II immunomodulatory protein does not comprise either the first trailing wild-type peptide linker or the second leading wild-type peptide linker.

[0231] In some embodiments, the Type II immunomodulatory protein comprises a first leading wild-type peptide linker inserted at the N-terminus of the first non-affinity modified and/or affinity modified IgSF domain, wherein the first leading wild-type peptide linker comprises at least 5 (such as at least about any of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more) consecutive amino acids from the intervening sequence in the wild-type protein from which the first non-affinity modified and/or affinity modified IgSF domain is derived between the parental IgSF domain and the immediately preceding domain (such as a signal peptide or an IgSF domain). In some embodiments, the first leading wild-type peptide linker comprises the entire intervening sequence in the wild-type protein from which the first non-affinity modified and/or affinity modified IgSF domain is derived between the parental IgSF domain and the immediately preceding domain (such as a signal peptide or an IgSF domain).

[0232] In some embodiments, the Type II immunomodulatory protein further comprises a first trailing wild-type peptide linker inserted at the C-terminus of the first non-affinity modified and/or affinity modified IgSF domain, wherein the first trailing wild-type peptide linker comprises at least 5 (such as at least about any of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more) consecutive amino acids from the intervening sequence in the wild-type protein from which the first non-affinity modified and/or affinity modified IgSF domain is derived between the parental IgSF domain and the immediately following domain (such as an IgSF domain or a transmembrane domain). In some embodiments, the first trailing wild-type peptide linker comprises the entire intervening sequence in the wild-type protein from which the first non-affinity modified and/or affinity modified IgSF domain is derived between the parental IgSF domain and the immediately following domain (such as an IgSF domain or a transmembrane domain).

[0233] In some embodiments, the Type II immunomodulatory protein further comprises a second leading wild-type peptide linker inserted at the N-terminus of the second non-affinity modified and/or affinity modified IgSF domain, wherein the second leading wild-type peptide linker comprises at least 5 (such as at least about any of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more) consecutive amino acids from the intervening sequence in the wild-type protein from which the second non-affinity modified and/or affinity modified IgSF domain is derived between the parental IgSF domain and the immediately preceding domain (such as a signal peptide or an IgSF domain). In some embodiments, the second leading wild-type peptide linker comprises the entire intervening sequence in the wild-type protein from which the second non-affinity modified and/or affinity modified IgSF domain is derived between the parental IgSF domain and the immediately preceding domain (such as a signal peptide or an IgSF domain).

[0234] In some embodiments, the Type II immunomodulatory protein further comprises a second trailing wild-type peptide linker inserted at the C-terminus of the second non-affinity modified and/or affinity modified IgSF domain, wherein the second trailing wild-type peptide linker comprises at least 5 (such as at least about any of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more) consecutive amino acids from the intervening sequence in the wild-type protein from which the second non-affinity modified and/or affinity modified IgSF domain is derived between the parental IgSF domain and the immediately following domain (such as an IgSF domain or a transmembrane domain). In some embodiments, the second trailing wild-type peptide linker comprises the entire intervening sequence in the wild-type protein from which the second non-affinity modified and/or affinity modified IgSF domain is derived between the parental IgSF domain and the immediately following domain (such as an IgSF domain or a transmembrane domain).

[0235] In some embodiments, the two or more IgSF domain, including a vIgD of CD155 and one or more additional IgSF domain (e.g. second and/or third variant IgSF domain) from another IgSF family member, are linked or attached to an Fc to form an Fc fusion, which, upon expression in a cell can, in some aspects, produce a dimeric multi-domain stack immunomodulatory protein. Thus, also provided are dimeric multi-domain immunomodulatory proteins.

[0236] In some embodiments, the variant CD155 polypeptide and one or more additional IgSF domain are independently linked, directly or indirectly, to the N- or C-terminus of an Fc region. In some embodiments, the variant CD155 polypeptide and at least one of the one or more additional IgSF domain are linked, directly or indirectly, and one of the variant CD155 and one of the one or more additional IgSF domain is also linked, directly or indirectly, to the N- or C-terminus of an Fc region. In some embodiments, the N- or C-terminus of the Fc region is linked to the variant CD155 polypeptide or the one or more additional IgSF domain and the other of the N- or C-terminus of the Fc region is linked to the other of the CD155 variant or another of the one or more additional IgSF domain. In some embodiments, linkage to the Fc is via a peptide linker, e.g. a peptide linker, such as described above. In some embodiments, linkage between the variant CD155 and second IgSF domain is via a peptide linker, e.g. a peptide linker, such as described above. In some embodiments, linkage between the variant

CD155 and the one or more additional IgSF domain is via a peptide linker, e.g., a peptide linker, such as described above. In some embodiments, the vIgD of CD155, the one or more additional IgSF domains, and the Fc domain can be linked together in any of numerous configurations as depicted in FIGS. **5**A and **5**B. Exemplary configurations are described in the Examples.

[0237] In some embodiments, the stacked immunomodulatory protein is a dimer formed by two immunomodulatory Fc fusion polypeptides. Also provided are nucleic acid molecules encoding any of the stacked immunomodulatory proteins. In some embodiments, the dimeric multi-domain stack immunomodulatory protein can be produced in cells by expression, or in some cases co-expression, of stack immunomodulatory Fc region polypeptides, such as described above in accord with generating dimeric Fc fusion proteins.

[0238] In some embodiments, the dimeric multi-domain stack immunomodulatory protein is divalent for each Fc subunit, monovalent for each subunit, or divalent for one subunit and tetravalent for the other.

[0239] In some embodiments, the dimeric multi-domain stack immunomodulatory protein is a homodimeric multi-domain stack Fc protein. In some embodiments, the dimeric multi-domain stack immunomodulatory protein comprises a first stack immunomodulatory Fc fusion polypeptide and a second stack immunomodulatory Fc fusion polypeptide in which the first and second polypeptide are the same. In some embodiments, the multi-domain stack molecule contains a first Fc fusion polypeptide containing a variant CD155 and a second IgSF domain and a second Fc fusion polypeptide containing the variant CD155 and the second IgSF domain. In some embodiments, the multi-domain stack molecule contains a first Fc fusion polypeptide containing a variant CD155, a second IgSF domain, and a third IgSF domain and a second Fc fusion polypeptide containing the variant CD155, the second IgSF domain, and the third IgSF domain. In some embodiments, the Fc portion of the first and/or second fusion polypeptide can be any Fc as described above. In some embodiments, the Fc portion or region of the first and second fusion polypeptide is the same.

[0240] In some embodiments, the multi-domain stack molecule is heterodimeric, comprising two different Fc fusion polypeptides, e.g. a first and a second Fc polypeptide, wherein at least one is an Fc fusion polypeptide containing at least one variant CD155 polypeptide and/or at least one is an Fc polypeptide containing a second IgSF domain (e.g. second variant IgSF domain). In some embodiments, the first or second Fc fusion polypeptide further contains a third IgSF domain (e.g. third variant IgSF domain). In some embodiments, the mult-domain stack molecule contains a first Fc fusion polypeptide containing a variant CD155 and a second Fc fusion polypeptide containing at a second IgSF domain, in which, in some cases, the first or second Fc fusion polypeptide additionally contains a third IgSF domain. In some embodiments, the multi-domain stack molecule contains a first Fc fusion polypeptide containing a variant CD155, a second IgSF domain, and in some cases, a third IgSF domain and a second Fc fusion polypeptide that is not linked to either a variant CD155 polypeptide or an additional IgSF domain. In some embodiments, the Fc portion or region of the first and second fusion polypeptide is the same. In some embodiments, the Fc portion or region of the first and second fusion polypeptide is different. In some embodiments, the multi-domain stack molecule contains a first fusion Fc polypeptides containing 1, 2, 3, 4 or more variant CD155 polypeptides and 1, 2, 3, 4 or more additional IgSF domains, wherein the total number of IgSF domains in the first stack Fc fusion polypeptide is greater than 2, 3, 4, 5, 6 or more. In one example of such an embodiment, the second stack Fc fusion polypeptide contains 1, 2, 3, 4 or more variant CD155 polypeptides and 1, 2, 3, 4 or more additional IgSF domains, wherein the total number of IgSF domains in the second stack Fc fusion polypeptide is greater than 2, 3, 4, 5, 6 or more. In another example of such embodiments, the second Fc polypeptide is not linked to either a variant CD155 polypeptide or additional IgSF domain.

[0241] In some embodiments, the heterodimeric stack molecule contains a first stack immunomodulatory Fc fusion polypeptide and a second stack immunomodulatory Fc fusion polypeptide in which the first and second polypeptide are different. In some embodiments, a heterodimeric stack molecule contains a first Fc polypeptide fusion containing an Fc region and a first variant CD155 polypeptide and/or second IgSF domain (e.g. second variant IgSF domain) and a second Fc polypeptide fusion containing an Fc region and the other of the first variant CD155 polypeptide or the second IgSF domain. In some embodiments, a heterodimeric stack molecule contains a first Fc polypeptide fusion containing an Fc region and a first variant CD155 polypeptide and/or second IgSF domain (e.g. second variant IgSF domain) and a second Fc polypeptide fusion containing an Fc region and both the first variant CD155 polypeptide and second IgSF domain (e.g. second variant IgSF domain) but in a different orientation or configuration from the first Fc region. In some embodiments, the first and/or second Fc fusion polypeptide also contains a third IgSF domain (e.g. third variant IgSF domain).

[0242] In some embodiments, the Fc domain of one or both of the first and second stacked immunomodulatory Fc fusion polypeptide comprises a modification (e.g. substitution) such that the interface of the Fc molecule is modified to facilitate and/or promote heterodimerization. In some embodiments, modifications include introduction of a protuberance (knob) into a first Fc polypeptide and a cavity (hole) into a second Fc polypeptide such that the protuberance is positionable in the cavity to promote complexing of the first and second Fc-containing polypeptides. Amino acids targeted for replacement and/or modification to create protuberances or cavities in a polypeptide are typically interface amino acids that interact or contact with one or more amino acids in the interface of a second polypeptide.

[0243] In some embodiments, a sequence of amino acids is added preceding the Fc sequence for constructs in which the Fc sequence is the N-terminal portion of the sequence. In some cases, the sequence of amino acids HMSSVSAQ (SEQ ID NO:1027) is added immediately preceding the Fc sequence for constructs in which the Fc sequence is the N-terminal portion of the sequence. In some embodiments, a heterodimeric stack molecule contains a first Fc polypeptide fusion containing an Fc region (knob) and a first variant CD155 polypeptide and/or second IgSF domain (e.g. second variant IgSF domain) and a second Fc polypeptide fusion containing an Fc region (hole) and a stuffer sequence HMSSVSAQ (SEQ ID NO:1027) added immediately preceding both Fc regions of the first and second Fc polypeptide fusion.

[0244] In some embodiments, a first polypeptide that is modified to contain protuberance (hole) amino acids include replacement of a native or original amino acid with an amino acid that has at least one side chain which projects from the interface of the first polypeptide and is therefore positionable in a compensatory cavity (hole) in an adjacent interface of a second polypeptide. Most often, the replacement amino acid is one which has a larger side chain volume than the original amino acid residue. One of skill in the art knows how to determine and/or assess the properties of amino acid residues to identify those that are ideal replacement amino acids to create a protuberance. In some embodiments, the replacement residues for the formation of a protuberance are naturally occurring amino acid residues and include, for example, arginine (R), phenylalanine (F), tyrosine (Y), or tryptophan (W). In some examples, the original residue identified for replacement is an amino acid residue that has a small side chain such as, for example, alanine, asparagine, aspartic acid, glycine, serine, threonine, or valine.

[0245] In some embodiments, a second polypeptide that is modified to contain a cavity (hole) is one that includes replacement of a native or original amino acid with an amino acid that has at least one side chain that is recessed from the interface of the second polypeptide and thus is able to accommodate a corresponding protuberance from the interface of a first polypeptide. Most often, the replacement amino acid is one which has a smaller side chain volume than the original amino acid residue. One of skill in the art knows how to determine and/or assess the properties of amino acid residues to identify those that are ideal replacement residues for the formation of a cavity. Generally, the replacement residues for the formation of a cavity are naturally occurring amino acids and include, for example, alanine (A), serine (S), threonine (T) and valine (V). In some examples, the original amino acid identified for replacement is an amino acid that has a large side chain such as, for example, tyrosine, arginine, phenylalanine, or tryptophan.

[0246] The CH3 interface of human IgG1, for example, involves sixteen residues on each domain located on four anti-parallel β-strands which buries 1090 A2 from each surface (see e.g., Deisenhofer et al. (1981) Biochemistry, 20:2361-2370; Miller et al., (1990) J Mol. Biol., 216, 965-973; Ridgway et al., (1996) Prot. Engin., 9: 617-621; U.S. Pat. No. 5,731,168). Modifications of a CH3 domain to create protuberances or cavities are described, for example, in U.S. Pat. No. 5,731,168; International Patent Applications WO98/50431 and WO 2005/063816; and Ridgway et al., (1996) Prot. Engin., 9: 617-621. In some examples, modifications of a CH3 domain to create protuberances or cavities are typically targeted to residues located on the two central anti-parallel β-strands. The aim is to minimize the risk that the protuberances which are created can be accommodated by protruding into the surrounding solvent rather than being accommodated by a compensatory cavity in the partner CH3 domain.

[0247] In some embodiments, the heterodimeric molecule contains a T366W mutation in the CH3 domain of the "knobs chain" and T366S, L368A, Y407V mutations in the CH3 domain of the "hole chain". In some cases, an additional interchain disulfide bridge between the CH3 domains can also be used (Merchant, A. M., et al., Nature Biotech. 16 (1998) 677-681) e.g. by introducing a Y349C mutation into the CH3 domain of the "knobs" or "hole" chain and a E356C mutation or a S354C mutation into the CH3 domain of the other chain. In some embodiments, the heterodimeric molecule contains S354C, T366W mutations in one of the two CH3 domains and Y349C, T366S, L368A, Y407V mutations in the other of the two CH3 domains. In some embodiments, the heterodimeric molecule comprises E356C, T366W mutations in one of the two CH3 domains and Y349C, T366S, L368A, Y407V mutations in the other of the two CH3 domains. In some embodiments, the heterodimeric molecule comprises Y349C, T366W mutations in one of the two CH3 domains and E356C, T366S, L368A, Y407V mutations in the other of the two CH3 domains. In some embodiments, the heterodimeric molecule comprises Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains. Examples of other knobs-in-holes technologies are known in the art, e.g. as described by EP 1 870 459 A1.

[0248] In some embodiments, the Fc regions of the heterodimeric molecule additionally can contain one or more other Fc mutation, such as any described above. In some embodiments, the heterodimer molecule contains an Fc region with a mutation that reduces effector function.

[0249] In some embodiments, an Fc variant containing CH3 protuberance (knob) or cavity(hole) modifications can be joined to a stacked immunomodulatory polypeptide anywhere, but typically via its N- or C-terminus, to the N- or C-terminus of a first and/or second stacked immunomodulatory polypeptide, such as to form a fusion polypeptide. The linkage can be direct or indirect via a linker. Typically, a knob and hole molecule is generated by co-expression of a first stacked immunomodulatory polypeptide linked to an Fc variant containing CH3 protuberance modification(s) with a second stacked immunomodulatory polypeptide linked to an Fc variant containing CH3 cavity modification(s).

[0250] There is provided herein a homodimeric multi-domain stack molecule produced from a stack immunomodulatory Fc fusion polypeptide containing an IgSF domain, e.g. IgV domain, of a variant CD155 polypeptide and a second IgSF domain, e.g. IgV, of a variant CD112 polypeptide. In some embodiments, the first and second immunomodulatory Fc fusion polypeptide of the multi-domain stack molecule has the sequence set forth in any of SEQ ID NOS: 1260, 1261, 1262, 1263, 1264, or 1265 or a sequence of amino acids that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 1260, 1261, 1262, 1263, 1264, or 1265 and contains the one more amino acid modifications in the variant CD112 and/or CD155 IgSF domain. In some embodiments, the resulting multi-domain stack molecules bind to both TIGIT and CD112R. In some aspects, the binding to TIGIT is to the same or similar degree or, in some cases, is increased, compared to the binding to TIGIT of the corresponding IgSF domain of unmodified or wild-type CD112 or CD155. In some aspects, the binding to CD112R is to the same or similar degree or, in some cases, is increased, compared to the binding to CD112R of the corresponding IgSF domain of unmodified or wild-type CD112. In some embodiments, the binding to TIGIT or CD112R is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of the binding to TIGIT or CD112R of the non-stacked form of the variant CD112 IgSF-Fc. In some embodiments, the binding to TIGIT is at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of the binding to TIGIT of the non-stacked form of the variant

CD155 IgSF-Fc. In some embodiments, the resulting multi-domain stack molecule increases T cell immune responses compared to the non-stack variant CD112 IgSF-Fc and/or variant CD155-IgSF-Fc, such as determined in a reporter assay. In some embodiments, the increase is greater than 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold or more.

[0251] There is provided herein a homodimeric multi-domain stack molecule produced from a stack immunomodulatory Fc fusion polypeptide containing an IgSF domain, e.g. IgV domain, of a variant CD155 polypeptide and a second IgSF domain, e.g. IgV, of a variant PD-L1 polypeptide. In some embodiments, the first and second immunomodulatory Fc fusion polypeptide of the multi-domain stack molecule has the sequence set forth in any of SEQ ID NOS: 1254, 1255, 1256, 1257, 1258, or 1259 or a sequence of amino acids that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 1254, 1255, 1256, 1257, 1258, or 1259 and contains the one more amino acid modifications in the variant PD-L1 and/or CD155 IgSF domain. In some embodiments, the resulting multi-domain stack molecules bind to both TIGIT and PD-1. In some aspects, the binding to TIGIT is to the same or similar degree or, in some cases, is increased, compared to the binding to TIGIT of the corresponding IgSF domain of unmodified or wild-type CD155. In some aspects, the binding to PD-1 is to the same or similar degree or, in some cases, is increased, compared to the binding to PD-1 of the corresponding IgSF domain of unmodified or wild-type PD-L1. In some embodiments, the binding to TIGIT or PD-1 is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of the binding to TIGIT or PD-1 of the non-stacked form of the variant CD155 IgSF-Fc. In some embodiments, the binding to TIGIT is at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of the binding to TIGIT of the non-stacked form of the variant CD155 IgSF-Fc. In some embodiments, the resulting multi-domain stack molecule increases T cell immune responses compared to the non-stack variant PD-L1 IgSF-Fc and/or variant CD155-IgSF-Fc, such as determined in a reporter assay. In some embodiments, the increase is greater than 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold or more.

[0252] There is provided herein a homodimeric multi-domain stack molecule produced from a stack immunomodulatory Fc fusion polypeptide containing an IgSF domain, e.g. IgV domain, of a variant CD155 polypeptide and a second IgSF domain, e.g. IgV, of a variant PD-L2 polypeptide. In some embodiments, the first and second immunomodulatory Fc fusion polypeptide of the multi-domain stack molecule has the sequence set forth in any of SEQ ID NOS: 1121, 1122, 1123, 1124, 1125, or 1126 or a sequence of amino acids that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 1121, 1122, 1123, 1124, 1125, or 1126 and contains the one more amino acid modifications in the variant PD-L2 and/or CD155 IgSF domain. In some embodiments, the resulting multi-domain stack molecules bind to both TIGIT and PD-1. In some aspects, the binding to TIGIT is to the same or similar degree or, in some cases, is increased, compared to the binding to TIGIT of the corresponding IgSF domain of unmodified or wild-type CD155. In some aspects, the binding to PD-1 is to the same or similar degree or, in some cases, is increased, compared to the binding to PD-1 of the corresponding IgSF domain of unmodified or wild-type PD-L1. In some embodiments, the binding to TIGIT or PD-1 is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of the binding to TIGIT or PD-1 of the non-stacked form of the variant CD155 IgSF-Fc. In some embodiments, the binding to TIGIT is at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of the binding to TIGIT of the non-stacked form of the variant CD155 IgSF-Fc. In some embodiments, the resulting multi-domain stack molecule increases T cell immune responses compared to the non-stack variant PD-L2 IgSF-Fc and/or variant CD155-IgSF-Fc, such as determined in a reporter assay. In some embodiments, the increase is greater than 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold or more.

[0253] There is provided herein a heterodimeric multi-domain stack molecule produced from a stack immunomodulatory Fc fusion polypeptide containing an IgSF domain, e.g. IgV domain, of a variant CD155 polypeptide and a second IgSF domain, e.g. IgV, of a variant PD-L2 polypeptide. In some embodiments, the first and second immunomodulatory Fc fusion polypeptide of the multi-domain stack molecule comprises a knob molecule that has the sequence set forth in any of SEQ ID NOS: 1127, 1128, 1129, 1130, 1131, or 1133, or a sequence of amino acids that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 1127, 1128, 1129, 1130, 1131, or 1133, and contains the one more amino acid modifications in the variant PD-L2 and/or CD155 IgSF domain. In some embodiments, the first and second immunomodulatory Fc fusion polypeptide of the multi-domain stack molecule comprises a hole molecule that has the sequence set forth in any of SEQ ID NOS: 1118, 1132, or 1134, or a sequence of amino acids that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 1118, 1132, or 1134, and contains the one more amino acid modifications in the variant PD-L2 and/or CD155 IgSF domain. In some embodiments, the resulting multi-domain stack molecules bind to both TIGIT and PD-1. In some embodiments, the knob and hole molecules are expressed in various combinations and is generated by co-expression of a first stacked immunomodulatory polypeptide linked to an Fc variant containing CH3 protuberance modification(s) with a second stacked immunomodulatory polypeptide linked to an Fc variant containing CH3 cavity modification(s). For example, the first and second immunomodulatory Fc fusion polypeptide of the multi-domain stack molecule comprises a knob and hole molecule that has the pair of sequences set forth in any of SEQ ID NOS: 1127+1118, 1128+1118, 1129+1118, 1130+1118, 1133+1134, 1131+1132, 1129+1132, 1129+1134, 1130+1132, 1130+1134, or a sequence of amino acids that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of the pairs of SEQ ID NOS: 1127+1118, 1128+1118, 1129+1118, 1130+1118, 1133+1134, 1131+1132, 1129+1132, 1129+1134, 1130+1132, 1130+1134, and contains the one more amino acid modifications in the variant PD-L2 and/or CD155 IgSF domain. In some cases, the knob or hole molecule includes a N-terminal HMSSVSAQ set forth in SEQ ID NO:1120. In some aspects, the binding to TIGIT is to the same or similar degree or, in some cases, is increased,

compared to the binding to TIGIT of the corresponding IgSF domain of unmodified or wild-type CD155. In some aspects, the binding to PD-1 is to the same or similar degree or, in some cases, is increased, compared to the binding to PD-1 of the corresponding IgSF domain of unmodified or wild-type PD-L2. In some embodiments, the binding to TIGIT or PD-1 is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of the binding to TIGIT or PD-1 of the non-stacked form of the variant CD155 IgSF-Fc. In some embodiments, the binding to TIGIT is at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of the binding to TIGIT of the non-stacked form of the variant CD155 IgSF-Fc. In some embodiments, the resulting multi-domain stack molecule increases T cell immune responses compared to the non-stack variant PD-L2 IgSF-Fc and/or variant CD155-IgSF-Fc, such as determined in a reporter assay. In some embodiments, the increase is greater than 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold or more.

[0254] There is provided herein a homodimeric multi-domain stack molecule produced from a stack immunomodulatory Fc fusion polypeptide containing an IgSF domain, e.g. IgV domain, of a variant CD112 polypeptide, a second IgSF domain, e.g. IgV, of a variant CD155 polypeptide and a third IgSF domain, e.g. IgV, of a variant PD-L1 polypeptide. In some embodiments, the first and second immunomodulatory Fc fusion polypeptide of the multi-domain stack molecule has the sequence set forth in any of SEQ ID NOS: 1266, 1267, and 1268 or a sequence of amino acids that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any of SEQ ID NOS: 1266, 1267, and 1268 and contains the one more amino acid modifications in the variant CD112, CD155 and/or PD-L1 IgSF domain. In some embodiments, the resulting multi-domain stack molecules bind to both TIGIT, CD112R and PD-1. In some aspects, the binding to TIGIT is to the same or similar degree or, in some cases, is increased, compared to the binding to TIGIT of the corresponding IgSF domain of unmodified or wild-type CD112 or CD155. In some aspects, the binding to CD112R is to the same or similar degree, or, in some cases, is increased, compared to the binding to CD112R of the corresponding IgSF domain of unmodified or wild-type CD112. In some aspects, the binding to PD-1 is to the same or similar degree, or, in some cases, is increased, compared to the binding to PD-1 of the corresponding IgSF domain of unmodified or wild-type PD-L1. In some embodiments, the binding to TIGIT or CD112R is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of the binding to TIGIT or CD112R of the non-stacked form of the variant CD112 IgSF-Fc. In some embodiments, the binding to TIGIT is at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of the binding to TIGIT of the non-stacked form of the variant CD155 IgSF-Fc. In some embodiments, the binding to PD-1 is at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of the binding to PD-1 of the non-stacked form of the variant PD-1 IgSF-Fc. In some embodiments, the resulting multi-domain stack molecule increases T cell immune responses compared to the non-stack variant CD112 IgSF-Fc, variant CD155-IgSF-Fc and/or variant PD-L1-IgSF-Fc, such as determined in a reporter assay. In some embodiments, the increase is greater than 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold or more.

[0255] C. Conjugates and Fusions of Variant Polypeptides and Immunomodulatory Proteins

[0256] In some embodiments, the variant polypeptides provided herein, which are immunomodulatory proteins comprising variants of an Ig domain of the IgSF family (vIgD), can be conjugated with or fused with a moiety, such as an effector moiety, such as another protein, directly or indirectly, to form a conjugate ("IgSF conjugate"). In some embodiments, the attachment can be covalent or non-covalent, e.g., via a biotin-streptavidin non-covalent interaction. In some embodiments of a CD155-Fc variant fusion, any one or combination of any two or more of the foregoing conjugates can be attached to the Fc or to the variant CD155 polypeptide or to both.

[0257] In some embodiments, the moiety can be a targeting moiety, a small molecule drug (non-polypeptide drug of less than 500 daltons molar mass), a toxin, a cytostatic agent, a cytotoxic agent, an immunosuppressive agent, a radioactive agent suitable for diagnostic purposes, a radioactive metal ion for therapeutic purposes, a prodrug-activating enzyme, an agent that increases biological half-life, or a diagnostic or detectable agent.

[0258] In some embodiments the effector moiety is a therapeutic agent, such as a cancer therapeutic agent, which is either cytotoxic, cytostatic or otherwise provides some therapeutic benefit. In some embodiments, the effector moiety is a targeting moiety or agent, such as an agent that targets a cell surface antigen, e.g., an antigen on the surface of a tumor cell. In some embodiments, the effector moiety is a label, which can generate a detectable signal, either directly or indirectly. In some embodiments, the effector moiety is a toxin. In some embodiments, the effector moiety is a protein, peptide, nucleic acid, small molecule or nanoparticle.

[0259] In some embodiments, 1, 2, 3, 4, 5 or more effector moieties, which can be the same or different, are conjugated, linked or fused to the variant polypeptide or protein to form an IgSF conjugate. In some embodiments, such effector moieties can be attached to the variant polypeptide or immunomodulatory protein using various molecular biological or chemical conjugation and linkage methods known in the art and described below. In some embodiments, linkers such as peptide linkers, cleavable linkers, non-cleavable linkers or linkers that aid in the conjugation reaction, can be used to link or conjugate the effector moieties to the variant polypeptide or immunomodulatory protein.

[0260] In some embodiments, the IgSF conjugate comprises the following components: (protein or polypeptide), $(L)_q$ and $(\text{effector moiety})_m$, wherein the protein or polypeptide is any of the described variant polypeptides or immunomodulatory proteins capable of binding one or more cognate counter structure ligands as described; L is a linker for linking the protein or polypeptide to the moiety; m is at least 1; q is 0 or more; and the resulting IgSF conjugate binds to the one or more counter structure ligands. In particular embodiments, m is 1 to 4 and q is 0 to 8.

[0261] In some embodiments, there is provided an IgSF conjugate comprising a variant polypeptide or immunomodulatory protein provided herein conjugated with a targeting agent that binds to a cell surface molecule, for example, for targeted delivery of the variant polypeptide or immunomodulatory protein to a specific cell. In some embodiments, the targeting agent is a molecule(s) that has

the ability to localize and bind to a molecule present on a normal cell/tissue and/or tumor cell/tumor in a subject. In other words, IgSF conjugates comprising a targeting agent can bind to a ligand (directly or indirectly), which is present on a cell, such as a tumor cell. The targeting agents of the invention contemplated for use include antibodies, polypeptides, peptides, aptamers, other ligands, or any combination thereof, that can bind a component of a target cell or molecule.

[0262] In some embodiments, the targeting agent binds a tumor cell(s) or can bind in the vicinity of a tumor cell(s) (e.g., tumor vasculature or tumor microenvironment) following administration to the subject. The targeting agent may bind to a receptor or ligand on the surface of the cancer cell. In another aspect of the invention, a targeting agent is selected which is specific for a noncancerous cells or tissue. For example, a targeting agent can be specific for a molecule present normally on a particular cell or tissue. Furthermore, in some embodiments, the same molecule can be present on normal and cancer cells. Various cellular components and molecules are known. For example, if a targeting agent is specific for EGFR, the resulting IgSF conjugate can target cancer cells expressing EGFR as well as normal skin epidermal cells expressing EGFR. Therefore, in some embodiments, an IgSF conjugate of the invention can operate by two separate mechanisms (targeting cancer and non-cancer cells).

[0263] In various aspects of the invention disclosed herein an IgSF conjugate of the invention comprises a targeting agent which can bind/target a cellular component, such as a tumor antigen, a bacterial antigen, a viral antigen, a mycoplasm antigen, a fungal antigen, a prion antigen, an antigen from a parasite. In some aspects, a cellular component, antigen or molecule can each be used to mean, a desired target for a targeting agent. For example, in various embodiments, a targeting agent is specific for or binds to a component, which includes but is not limited to, epidermal growth factor receptor (EGFR, ErbB-1, HER1), ErbB-2 (HER2/neu), ErbB-3/HER3, ErbB-4/HER4, EGFR ligand family; insulin-like growth factor receptor (IGFR) family, IGF-binding proteins (IGFBPs), IGFR ligand family; platelet derived growth factor receptor (PDGFR) family, PDGFR ligand family; fibroblast growth factor receptor (FGFR) family, FGFR ligand family, vascular endothelial growth factor receptor (VEGFR) family, VEGF family; HGF receptor family; TRK receptor family; ephrin (EPH) receptor family; AXL receptor family; leukocyte tyrosine kinase (LTK) receptor family; TIE receptor family, angiopoietin 1,2; receptor tyrosine kinase-like orphan receptor (ROR) receptor family, e.g. ROR1; CD171 (LICAM); B7-H6 (NCR3LG1); CD155, tumor glycosylation antigen, e.g. sTn or Tn, such as sTn Ag of MUC1; LHR (LHCGR); phosphatidylserine, discoidin domain receptor (DDR) family; RET receptor family; KLG receptor family; RYK receptor family; MuSK receptor family; Transforming growth factor-α (TGF-α) receptors, TGF-β; Cytokine receptors, Class I (hematopoietin family) and Class II (interferon/IL-10 family) receptors, tumor necrosis factor (TNF) receptor superfamily (TNFRSF), death receptor family; cancer-testis (CT) antigens, lineage-specific antigens, differentiation antigens, alpha-actinin-4, ARTCl, breakpoint cluster region-Abelson (Bcr-abl) fusion products, B-RAF, caspase-5 (CASP-5), caspase-8 (CASP-8), β-catenin (CTNNBl), cell division cycle 27 (CDC27), cyclin-dependent kinase 4 (CDK4), CDKN2A, COA-I, dek-can fusion protein, EFTUD-2, Elongation factor 2 (ELF2), Ets variant gene 6/acute myeloid leukemia 1 gene ETS (ETC6-AML1) fusion protein, fibronectin (FN), e.g. the extradomain A (EDA) of fibronectin, GPNMB, low density lipid receptor/GDP-L fucose: β-D-galactose 2-α-L-fucosyltransferase (LDLR/FUT) fusion protein, HLA-A2. arginine to isoleucine exchange at residue 170 of the a-helix of the α2-domain in the HLA-A2gene (HLA-A*201-R170I), HLA-Al 1, heat shock protein 70-2 mutated (HSP70-2M), K1AA0205, MART2, melanoma ubiquitous mutated 1, 2, 3 (MUM-I, 2, 3), prostatic acid phosphatase (PAP), neo-PAP, Myosin class I, NFYC, OGT, OS-9, pml-RARα fusion protein, PRDX5, PTPRK, K-ras (KRAS2), N-ras (NRAS), HRAS, RBAF600, SIRT2, SNRPD1, SYT-SSXl or -SSX2 fusion protein, Triosephosphate Isomerase, BAGE, BAGK-1, BAGE-2,3,4,5, GAGE-1,2,3,4,5,6,7,8, GnT-V (aberrant N-acetyl glucosaminyl transferase V, MGAT5), HERV-K-MEL, KK-LC, KM-HN-I, LAGE, LAGE-I, CTL-recognized antigen on melanoma (CAMEL), MAGE-A1 (MAGE-I), MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A8, MAGE-A9, MAGE-AlO, MAGE-AI 1, MAGE-A12, MAGE-3, MAGE-B1, MAGE-B2, MAGE-B5, MAGE-B6, MAGE-Cl, MAGE-C2, mucin 1 (MUCl), MART-1/Melan-A (MLANA), gplOO, gplOO/Pmell7 (SILV), tyrosinase (TYR), TRP-I, HAGE, NA-88, NY-ESO-I, NY-ESO-1/LAGE-2, SAGE, Spl7, SSX-1,2,3,4, TRP2-INT2, carcinoembryonic antigen (CEA), Kallikrein 4, mammaglobin-A, OAl, prostate specific antigen (PSA), TRP-1/gp75, TRP-2, adipophilin, interferon inducible protein absent in melanoma 2 (AIM-2), BING-4, CPSF, cyclin Dl, epithelial cell adhesion molecule (Ep-CAM), EphA3, fibroblast growth factor-5 (FGF-5), glycoprotein 250 (gp250), EGFR (ERBBl), HER-2/neu (ERBB2), interleukin 13 receptor α2 chain (IL13Ra2), IL-6 receptor, intestinal carboxyl esterase (iCE), alpha-feto protein (AFP), M-CSF, mdm-2, MUCI, p53 (TP53), PBF, PRAME, PSMA, RAGE-I, RNF43, RU2AS, SOX10, STEAPI, survivin (BIRC5), human telomerase reverse transcriptase (hTERT), telomerase, Wilms' tumor gene (WTl), SYCP1, BRDT, SPANX, XAGE, ADAM2, PAGE-5, LIP1, CTAGE-I, CSAGE, MMAl, CAGE, BORIS, HOM-TES-85, AF15ql4, HCA661, LDHC, MORC, SGY-I, SPOl 1, TPX1, NY-SAR-35, FTHL17, NXF2, TDRD1, TEX15, FATE, TPTE, immunoglobulin idiotypes, Bence-Jones protein, estrogen receptors (ER), androgen receptors (AR), CD40, CD30, CD20, CD 19, CD33, cancer antigen 72-4 (CA 72-4), cancer antigen 15-3 (CA 15-3), cancer antigen 27-29 (CA 27-29), cancer antigen 125 (CA 125), cancer antigen 19-9 (CA 19-9), β-human chorionic gonadotropin, β-2 microglobulin, squamous cell carcinoma antigen, neuron-specific enolase, heat shock protein gp96, GM2, sargramostim, CTLA-4, 707 alanine proline (707-AP), adenocarcinoma antigen recognized by T cells 4 (ART-4), carcinoembryogenic antigen peptide-1 (CAP-I), calcium-activated chloride channel-2 (CLCA2), cyclophilin B (Cyp-B), human signet ring tumor-2 (HST-2), Human papilloma virus (HPV) proteins (HPV-E6, HPV-E7, major or minor capsid antigens, others), Epstein-Barr virus (EBV) proteins (EBV latent membrane proteins—LMP1, LMP2; others), Hepatitis B or C virus proteins, and HIV proteins.

[0264] In some embodiments, an IgSF conjugate, through its targeting agent, will bind a cellular component of a tumor cell, tumor vasculature or tumor microenvironment, thereby promoting killing of targeted cells via modulation of the

immune response, (e.g., by activation of co-stimulatory molecules or inhibition of negative regulatory molecules of immune cell activation), inhibition of survival signals (e.g., growth factor or cytokine or hormone receptor antagonists), activation of death signals, and/or immune-mediated cytotoxicity, such as through antibody dependent cellular cytotoxicity. Such IgSF conjugates can function through several mechanisms to prevent, reduce or eliminate tumor cells, such as to facilitate delivery of conjugated effector moieties to the tumor target, such as through receptor-mediated endocytosis of the IgSF conjugate; or such conjugates can recruit, bind, and/or activate immune cells (e.g. NK cells, monocytes/macrophages, dendritic cells, T cells, B cells). Moreover, in some instances one or more of the foregoing pathways may operate upon administration of one or more IgSF conjugates of the invention.

[0265] In some embodiments, an IgSF conjugate, through its targeting agent, will be localized to, such as bind to, a cellular component of a tumor cell, tumor vasculature or tumor microenvironment, thereby modulating cells of the immune response in the vicinity of the tumor. In some embodiments, the targeting agent facilitates delivery of the conjugated IgSF (e.g. vIgD) to the tumor target, such as to interact with its cognate binding partner to alter signaling of immune cells (e.g. NK cells, monocytes/macrophages, dendritic cells, T cells, B cells) bearing the cognate binding partner. In some embodiments, localized delivery mediates an antagonizing or blocking activity of the TIGIT inhibitory receptor. In some embodiments, localized delivery agonizes the TIGIT inhibitory receptor, which, in some cases, can occur where there is proximal clustering of an activating receptor.

[0266] In some embodiments, the targeting agent is an immunoglobulin. As used herein, the term "immunoglobulin" includes natural or artificial mono- or polyvalent antibodies including, but not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, single chain Fv (scFv); anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, e.g., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) or subclass of immunoglobulin molecule.

[0267] In some embodiments, an IgSF conjugate, through its antibody targeting moiety, will bind a cellular component of a tumor cell, tumor vasculature or tumor microenvironment, thereby promoting apoptosis of targeted cells via modulation of the immune response, (e.g., by activation of co-stimulatory molecules or inhibition of negative regulatory molecules of immune cell activation), inhibition of survival signals (e.g., growth factor or cytokine or hormone receptor antagonists), activation of death signals, and/or immune-mediated cytotoxicity, such as through antibody dependent cellular cytotoxicity. Such IgSF conjugates can function through several mechanisms to prevent, reduce or eliminate tumor cells, such as to facilitate delivery of conjugated effector moieties to the tumor target, such as through receptor-mediated endocytosis of the IgSF conjugate; or such conjugates can recruit, bind, and/or activate immune cells (e.g. NK cells, monocytes/macrophages, dendritic cells, T cells, B cells).

[0268] In some embodiments, an IgSF conjugate, through its antibody targeting moiety, will bind a cellular component of a tumor cell, tumor vasculature or tumor microenvironment, thereby modulating the immune response (e.g., by activation of co-stimulatory molecules or inhibition of negative regulatory molecules of immune cell activation). In some embodiments, such conjugates can recognize, bind, and/or modulate (e.g. inhibit or activate) immune cells (e.g. NK cells, monocytes/macrophages, dendritic cells, T cells, B cells).

[0269] Antibody targeting moieties of the invention include antibody fragments that include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. Also included in the invention are Fc fragments, antigen-Fc fusion proteins, and Fc-targeting moiety conjugates or fusion products (Fc-peptide, Fc-aptamer). The antibody targeting moieties of the invention may be from any animal origin including birds and mammals. In one aspect, the antibody targeting moieties are human, murine (e.g., mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken. Further, such antibodies may be humanized versions of animal antibodies. The antibody targeting moieties of the invention may be monospecific, bispecific, trispecific, or of greater multispecificity.

[0270] In various embodiments, an antibody/targeting moiety recruits, binds, and/or activates immune cells (e.g. NK cells, monocytes/macrophages, dendritic cells) via interactions between Fc (in antibodies) and Fc receptors (on immune cells) and via the conjugated variant polypeptides or immunomodulatory proteins provided herein. In some embodiments, an antibody/targeting moiety recognizes or binds a tumor agent via and localizes to the tumor cell the conjugated variant polypeptides or immunomodulatory proteins provided herein to facilitate modulation of immune cells in the vicinity of the tumor.

[0271] Examples of antibodies which can be incorporated into IgSF conjugates include but are not limited to antibodies such as Cetuximab (IMC-C225; Erbitux®), Trastuzumab (Herceptin®), Rituximab (Rituxan®; MabThera®), Bevacizumab (Avastin®), Alemtuzumab (Campath®; Campath-1H®; Mabcampath®), Panitumumab (ABX-EGF; Vectibix®), Ranibizumab (Lucentis®), Ibritumomab, Ibritumomab tiuxetan, (Zevalin @), Tositumomab, Iodine 1131 Tositumomab (BEXXAR®), Catumaxomab (Removab®), Gemtuzumab, Gemtuzumab ozogamicine (Mylotarg®), Abatacept (CTLA4-Ig; Orencia®), Belatacept (L104EA29YIg; LEA29Y; LEA), Ipilimumab (MDX-010; MDX-101), Tremelimumab (ticilimumab; CP-675,206), PRS-010, PRS-050, Aflibercept (VEGF Trap, AVE005), Volociximab (M200), F200, MORAb-009, SS1P (CAT-5001), Cixutumumab (IMC-A12), Matuzumab

(EMD72000), Nimotuzumab (h-R3), Zalutumumab (HuMax-EGFR), Necitumumab IMC-11F8, mAb806/ch806, Sym004, mAb-425, Panorex @ (17-1A) (murine monoclonal antibody); Panorex @ (17-1A) (chimeric murine monoclonal antibody); IDEC-Y2B8 (murine, anti-CD20 MAb); BEC2 (anti-idiotypic MAb, mimics the GD epitope) (with BCG); Oncolym (Lym-1 monoclonal antibody); SMART MI95 Ab, humanized 13' I LYM-I (Oncolym), Ovarex (B43.13, anti-idiotypic mouse MAb); MDX-210 (humanized anti-HER-2 bispecific antibody); 3622W94 MAb that binds to EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas; Anti-VEGF, Zenapax (SMART Anti-Tac (IL-2 receptor); SMART MI95 Ab, humanized Ab, humanized); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-447 (humanized anti-EGF receptor bispecific antibody); NovoMAb-G2 (pancarcinoma specific Ab); TNT (chimeric MAb to histone antigens); TNT (chimeric MAb to histone antigens); Gliomab-H (Monoclon s—Humanized Abs); GNI-250 Mab; EMD-72000 (chimeric-EGF antagonist); LymphoCide (humanized LL2 antibody); and MDX-260 bispecific, targets GD-2, ANA Ab, SMART IDlO Ab, SMART ABL 364 Ab or ImmuRAIT-CEA. As illustrated by the forgoing list, it is conventional to make antibodies to a particular target epitope.

[0272] In some embodiments, the antibody targeting moiety is a full length antibody, or antigen-binding fragment thereof, containing an Fe domain. In some embodiments, the variant polypeptide or immunomodulatory protein is conjugated to the Fc portion of the antibody targeting moiety, such as by conjugation to the N-terminus of the Fc portion of the antibody.

[0273] In some embodiments, the vIgD is linked, directly or indirectly, to the N- or C-terminus of the light and/or heavy chain of the antibody. In some embodiments, linkage can be via a peptide linker, such as any described above. Various configurations can be constructed. FIG. **7**A-**7**C depict exemplary configurations. In some embodiments, the antibody conjugate can be produced by co-expression of the heavy and light chain of the antibody in a cell.

[0274] In one aspect of the invention, the targeting agent is an aptamer molecule. For example, in some embodiments, the aptamer is comprised of nucleic acids that function as a targeting agent. In various embodiments, an IgSF conjugate of the invention comprises an aptamer that is specific for a molecule on a tumor cell, tumor vasculature, and/or a tumor microenvironment. In some embodiments, the aptamer itself can comprise a biologically active sequence, in addition to the targeting module (sequence), wherein the biologically active sequence can induce an immune response to the target cell. In other words, such an aptamer molecule is a dual use agent. In some embodiments, an IgSF conjugate of the invention comprises conjugation of an aptamer to an antibody, wherein the aptamer and the antibody are specific for binding to separate molecules on a tumor cell, tumor vasculature, tumor microenvironment, and/or immune cells.

[0275] The term "aptamer" includes DNA, RNA or peptides that are selected based on specific binding properties to a particular molecule. For example, an aptamer(s) can be selected for binding a particular gene or gene product in a tumor cell, tumor vasculature, tumor microenvironment, and/or an immune cell, as disclosed herein, where selection is made by methods known in the art and familiar to one of skill in the art.

[0276] In some aspects of the invention the targeting agent is a peptide. For example, the variant polypeptides or immunomodulatory proteins provided herein can be conjugated to a peptide which can bind with a component of a cancer or tumor cells. Therefore, such IgSF conjugates of the invention comprise peptide targeting agents which binds to a cellular component of a tumor cell, tumor vasculature, and/or a component of a tumor microenvironment. In some embodiments, targeting agent peptides can be an antagonist or agonist of an integrin. Integrins, which comprise an alpha and a beta subunit, include numerous types well known to a skilled artisan.

[0277] In one embodiment, the targeting agent is Vvβ3. Integrin Vvβ3 is expressed on a variety of cells and has been shown to mediate several biologically relevant processes, including adhesion of osteoclasts to bone matrix, migration of vascular smooth muscle cells, and angiogenesis. Suitable targeting molecules for integrins include RGD peptides or peptidomimetics as well as non-RGD peptides or peptidomimetics (see, e.g., U.S. Pat. Nos. 5,767,071 and 5,780,426) for other integrins such as V4.βi (VLA-4), V4-P7 (see, e.g., U.S. Pat. No. 6,365,619; Chang et al, Bioorganic & Medicinal Chem Lett, 12:159-163 (2002); Lin et al., Bioorganic & Medicinal Chem Lett, 12:133-136 (2002)), and the like.

[0278] In some embodiments, there is provided an IgSF conjugate comprising a variant polypeptide or immunomodulatory protein provided herein conjugated with a therapeutic agent. In some embodiments, the therapeutic agent includes, for example, daunomycin, doxorubicin, methotrexate, and vindesine (Rowland et al., Cancer Immunol. Immunother. 21:183-187, 1986). In some embodiments, the therapeutic agent has an intracellular activity. In some embodiments, the IgSF conjugate is internalized and the therapeutic agent is a cytotoxin that blocks the protein synthesis of the cell, therein leading to cell death. In some embodiments, the therapeutic agent is a cytotoxin comprising a polypeptide having ribosome-inactivating activity including, for example, gelonin, bouganin, saporin, ricin, ricin A chain, bryodin, diphtheria toxin, restrictocin, *Pseudomonas* exotoxin A and variants thereof. In some embodiments, where the therapeutic agent is a cytotoxin comprising a polypeptide having a ribosome-inactivating activity, the IgSF conjugate must be internalized upon binding to the target cell in order for the protein to be cytotoxic to the cells.

[0279] In some embodiments, there is provided an IgSF conjugate comprising a variant polypeptide or immunomodulatory protein provided herein conjugated with a toxin. In some embodiments, the toxin includes, for example, bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Mandler et al., J. Nat. Cancer Inst. 92(19):1573-1581 (2000); Mandler et al., Bioorganic & Med. Chem. Letters 10:1025-1028 (2000); Mandler et al., Bioconjugate Chem. 13:786-791 (2002)), maytansinoids (EP 1391213; Liu et al., Proc. Natl. Acad. Sci. USA 93:8618-8623 (1996)), and calicheamicin (Lode et al., Cancer Res. 58:2928 (1998); Hinman et al., Cancer Res. 53:3336-3342 (1993)). The toxins may exert their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition.

[0280] In some embodiments, there is provided an IgSF conjugate comprising a variant polypeptide or immunomodulatory protein provided herein conjugated with a label,

which can generate a detectable signal, indirectly or directly. These IgSF conjugates can be used for research or diagnostic applications, such as for the in vivo detection of cancer. The label is preferably capable of producing, either directly or indirectly, a detectable signal. For example, the label may be radio-opaque or a radioisotope, such as 3H, 14C, 32P, 35S, 123I, 125I, 131I; a fluorescent (fluorophore) or chemiluminescent (chromophore) compound, such as fluorescein isothiocyanate, rhodamine or luciferin; an enzyme, such as alkaline phosphatase, β-galactosidase or horseradish peroxidase; an imaging agent; or a metal ion. In some embodiments, the label is a radioactive atom for scintigraphic studies, for example 99Tc or 123I, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as zirconium-89, iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Zirconium-89 may be complexed to various metal chelating agents and conjugated to antibodies, e.g., for PET imaging (WO 2011/056983). In some embodiments, the IgSF conjugate is detectable indirectly. For example, a secondary antibody that is specific for the IgSF conjugate and contains a detectable label can be used to detect the IgSF conjugate.

[0281] The IgSF conjugates may be prepared using any methods known in the art. See, e.g., WO 2009/067800, WO 2011/133886, and U.S. Patent Application Publication No. 2014322129, incorporated by reference herein in their entirety.

[0282] The variant polypeptides or immunomodulatory proteins of an IgSF conjugate may be "attached to" the effector moiety by any means by which the variant polypeptides or immunomodulatory proteins can be associated with, or linked to, the effector moiety. For example, the variant polypeptides or immunomodulatory proteins of an IgSF conjugate may be attached to the effector moiety by chemical or recombinant means. Chemical means for preparing fusions or conjugates are known in the art and can be used to prepare the IgSF conjugate. The method used to conjugate the variant polypeptides or immunomodulatory proteins and effector moiety must be capable of joining the variant polypeptides or immunomodulatory proteins with the effector moiety without interfering with the ability of the variant polypeptides or immunomodulatory proteins to bind to their one or more counter structure ligands.

[0283] The variant polypeptides or immunomodulatory proteins of an IgSF conjugate may be linked indirectly to the effector moiety. For example, the variant polypeptides or immunomodulatory proteins of an IgSF conjugate may be directly linked to a liposome containing the effector moiety of one of several types. The effector moiety(s) and/or the variant polypeptides or immunomodulatory proteins may also be bound to a solid surface.

[0284] In some embodiments, the variant polypeptides or immunomodulatory proteins of an IgSF conjugate and the effector moiety are both proteins and can be conjugated using techniques well known in the art. There are several hundred crosslinkers available that can conjugate two proteins. (See for example "Chemistry of Protein Conjugation and Crosslinking," 1991, Shans Wong, CRC Press, Ann Arbor). The crosslinker is generally chosen based on the reactive functional groups available or inserted on the variant polypeptides or immunomodulatory proteins and/or effector moiety. In addition, if there are no reactive groups, a photoactivatible crosslinker can be used. In certain instances, it may be desirable to include a spacer between the variant polypeptides or immunomodulatory proteins and the effector moiety. Crosslinking agents known to the art include the homobifunctional agents: glutaraldehyde, dimethyladipimidate and Bis(diazobenzidine) and the heterobifunctional agents: m Maleimidobenzoyl-N-Hydroxysuccinimide and Sulfo-m Maleimidobenzoyl-N-Hydroxysuccinimide.

[0285] In some embodiments, the variant polypeptides or immunomodulatory proteins of an IgSF conjugate may be engineered with specific residues for chemical attachment of the effector moiety. Specific residues used for chemical attachment of molecule known to the art include lysine and cysteine. The crosslinker is chosen based on the reactive functional groups inserted on the variant polypeptides or immunomodulatory proteins, and available on the effector moiety.

[0286] An IgSF conjugate may also be prepared using recombinant DNA techniques. In such a case a DNA sequence encoding the variant polypeptides or immunomodulatory proteins is fused to a DNA sequence encoding the effector moiety, resulting in a chimeric DNA molecule. The chimeric DNA sequence is transfected into a host cell that expresses the fusion protein. The fusion protein can be recovered from the cell culture and purified using techniques known in the art.

[0287] Examples of attaching an effector moiety, which is a label, to the variant polypeptides or immunomodulatory proteins include the methods described in Hunter, et al., Nature 144:945 (1962); David, et al., Biochemistry 13:1014 (1974); Pain, et al., J. Immunol. Meth. 40:219 (1981); Nygren, J. Histochem. and Cytochem. 30:407 (1982); Wensel and Meares, Radioimmunoimaging And Radioimmunotherapy, Elsevier, N.Y. (1983); and Colcher et al., "Use Of Monoclonal Antibodies As Radiopharmaceuticals For The Localization Of Human Carcinoma Xenografts In Athymic Mice", Meth. Enzymol., 121:802-16 (1986).

[0288] The radio- or other labels may be incorporated in the conjugate in known ways. For example, the peptide may be biosynthesized or may be synthesized by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as 99Tc or 123I, 186Re, 188Re and 111In can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker et al., Biochem. Biophys. Res. Commun. 80:49-57 (1978)) can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

[0289] Conjugates of the variant polypeptides or immunomodulatory proteins and a cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098

(1987). Carbon-14-labeled 1-p-isothiocyanatobenzyl-3-methyldiethylenetriaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See, e.g., WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., Cancer Research 52:127-131 (1992); U.S. Pat. No. 5,208,020) may be used.

[0290] The IgSF conjugates of the invention expressly contemplate, but are not limited to, drug conjugates prepared with cross-linker reagents: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL, U.S.A). See pages 467-498, 2003-2004 Applications Handbook and Catalog.

[0291] D. Transmembrane and Secretable Immunomodulatory Proteins and Engineered Cells

[0292] Provided herein are engineered cells which express the immunomodulatory variant CD155 polypeptides (alternatively, "engineered cells"). In some embodiments, the expressed immunomodulatory variant CD155 polypeptide is a transmembrane proteins and is surface expressed. In some embodiments, the expressed immunomodulatory variant CD155 polypeptide is expressed and secreted from the cell.

[0293] 1. Transmembrane Immunomodulatory Proteins

[0294] In some embodiments, an immunomodulatory polypeptide comprising a variant CD155 can be a membrane bound protein. As described in more detail below, the immunomodulatory polypeptide can be a transmembrane immunomodulatory polypeptide comprising a variant CD155 in which is contained: an ectodomain containing at least one affinity modified IgSF domain (IgV or IgC), a transmembrane domain and, optionally, a cytoplasmic domain. In some embodiments, the transmembrane immunomodulatory protein can be expressed on the surface of an immune cell, such as a mammalian cell, including on the surface of a lymphocyte (e.g. T cell or NK cell) or antigen presenting cell. In some embodiments, the transmembrane immunomodulatory protein is expressed on the surface of a mammalian T-cell, including such T-cells as: a T helper cell, a cytotoxic T-cell (alternatively, cytotoxic T lymphocyte or CTL), a natural killer T-cell, a regulatory T-cell, a memory T-cell, or a gamma delta T-cell. In some embodiments, the mammalian cell is an antigen presenting cell (APC). Typically, but not exclusively, the ectodomain (alternatively, "extracellular domain") of comprises the one or more amino acid variations (e.g. amino acid substitutions) of the variant CD155 of the invention. Thus, for example, in some embodiments a transmembrane protein will comprise an ectodomain that comprises one or more amino acid substitutions of a variant CD155 of the invention.

[0295] In some embodiments, the engineered cells express a variant CD155 polypeptides are transmembrane immunomodulatory polypeptides (TIPs) that can be a membrane protein such as a transmembrane protein. In typical embodiments, the ectodomain of a membrane protein comprises an extracellular domain or IgSF domain thereof of a variant CD155 provided herein in which is contained one or more amino acid substitutions in at least one IgSF domain as described. The transmembrane immunomodulatory proteins provided herein further contain a transmembrane domain linked to the ectodomain. In some embodiments, the transmembrane domain results in an encoded protein for cell surface expression on a cell. In some embodiments, the transmembrane domain is linked directly to the ectodomain. In some embodiments, the transmembrane domain is linked indirectly to the ectodomain via one or more linkers or spacers. In some embodiments, the transmembrane domain contains predominantly hydrophobic amino acid residues, such as leucine and valine.

[0296] In some embodiments, a full length transmembrane anchor domain can be used to ensure that the TIPs will be expressed on the surface of the engineered cell, such as engineered T cell. Conveniently, this could be from a particular native protein that is being affinity modified (e.g. CD155 or other native IgSF protein), and simply fused to the sequence of the first membrane proximal domain in a similar fashion as the native IgSF protein (e.g. CD155). In some embodiments, the transmembrane immunomodulatory protein comprises a transmembrane domain of the corresponding wild-type or unmodified IgSF member, such as a transmembrane domain contained in the sequence of amino acids set forth in SEQ ID NO:20 (Table 2). In some embodiments, the membrane bound form comprises a transmembrane domain of the corresponding wild-type or unmodified polypeptide, such as corresponding to residues 221-241 of SEQ ID NO:20.

[0297] In some embodiments, the transmembrane domain is a non-native transmembrane domain that is not the transmembrane domain of native CD155. In some embodiments, the transmembrane domain is derived from a transmembrane domain from another non-CD155 family member polypeptide that is a membrane-bound or is a transmembrane protein. In some embodiments, a transmembrane anchor domain from another protein on T cells can be used. In some embodiments, the transmembrane domain is derived from CD8. In some embodiments, the transmembrane domain can further contain an extracellular portion of CD8 that serves as a spacer domain. An exemplary CD8 derived transmembrane domain is set forth in SEQ ID NO: 130 or 1142 or a portion thereof containing the CD8 transmembrane domain. In some embodiments, the transmembrane domain is a synthetic transmembrane domain.

[0298] In some embodiments, the transmembrane immunomodulatory protein further contains an endodomain, such as a cytoplasmic signaling domain, linked to the transmembrane domain. In some embodiments, the cytoplasmic signaling domain induces cell signaling. In some embodiments, the endodomain of the transmembrane immunomodulatory protein comprises the cytoplasmic domain of the corresponding wild-type or unmodified polypeptide, such as a cytoplasmic domain contained in the sequence of amino acids set forth in SEQ ID NO:20 (see Table 2).

[0299] In some embodiments, a provided transmembrane immunomodulatory protein that is or comprises a variant CD155 comprises a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 122 and contains an ectodomain comprising at least one affinity-modified CD155 IgSF domain as described and a transmembrane domain. In some embodiments, the transmembrane immunomodulatory protein contains any one or more amino acid substitutions in an IgSF domain (e.g.

IgV domain) as described, including any set forth in Table 1. In some embodiments, the transmembrane immunomodulatory protein can further comprise a cytoplasmic domain as described. In some embodiments, the transmembrane immunomodulatory protein can further contain a signal peptide. In some embodiments, the signal peptide is the native signal peptide of wild-type IgSF member, such as contained in the sequence of amino acids set forth in SEQ ID NO:20 (see e.g. Table 2).

[0300] Also provided is a nucleic acid molecule encoding such transmembrane immunomodulatory proteins. In some embodiments, a nucleic acid molecule encoding a transmembrane immunomodulatory protein comprises a nucleotide sequence that encodes a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NOS: 122 and contains an ectodomain comprising at least one affinity-modified IgSF domain as described, a transmembrane domain and, optionally, a cytoplasmic domain. In some embodiments, the nucleic acid molecule can further comprise a sequence of nucleotides encoding a signal peptide. In some embodiments, the signal peptide is the native signal peptide of the corresponding wild-type IgSF member (see e.g. Table 2).

[0301] In some embodiments, provided are CAR-related transmembrane immunomodulatory proteins in which the endodomain of a transmembrane immunomodulatory protein comprises a cytoplasmic signaling domain that comprises at least one ITAM (immunoreceptor tyrosine-based activation motif)-containing signaling domain. ITAM is a conserved motif found in a number of protein signaling domains involved in signal transduction of immune cells, including in the CD3-zeta chain ("CD3-z") involved in T-cell receptor signal transduction. In some embodiments, the endodomain comprises at CD3-zeta signaling domain. In some embodiments, the CD3-zeta signaling domain comprises the sequence of amino acids set forth in SEQ ID NO: 131 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% to SEQ ID NO:131 and retains the activity of T cell signaling. In some embodiments, the endodomain of a CAR-related transmembrane immunomodulatory protein can further comprise a costimulatory signaling domain to further modulate immunomodulatory responses of the T-cell. In some embodiments, the costimulatory signaling domain is CD28, ICOS, 41BB or OX40. In some embodiments, the costimulatory signaling domain is a derived from CD28 or 4-1BB and comprises the sequence of amino acids set forth in any of SEQ ID NOS: 1143-1146 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% to SEQ ID NO:1143-1146 and retains the activity of T cell costimulatory signaling. In some embodiments, the provided CAR-related transmembrane immunomodulatory proteins have features of CARs to stimulate T cell signaling upon binding of an affinity modified IgSF domain to a cognate binding partner or counter structure. In some embodiments, upon specific binding by the affinity-modified IgSF domain to its counter structure can lead to changes in the immunological activity of the T-cell activity as reflected by changes in cytotoxicity, proliferation or cytokine production.

[0302] In some embodiments, the transmembrane immunomodulatory protein does not contain an endodomain capable of mediating cytoplasmic signaling. In some embodiments, the transmembrane immunomodulatory protein lacks the signal transduction mechanism of the wild-type or unmodified polypeptide and therefore does not itself induce cell signaling. In some embodiments, the transmembrane immunomodulatory protein lacks an intracellular (cytoplasmic) domain or a portion of the intracellular domain of the corresponding wild-type or unmodified polypeptide, such as a cytoplasmic signaling domain contained in the sequence of amino acids set forth in SEQ ID NO:20 (see Table 2). In some embodiments, the transmembrane immunomodulatory protein does not contain an ITIM (immunoreceptor tyrosine-based inhibition motif), such as contained in certain inhibitory receptors, including inhibitory receptors of the IgSF family (e.g. TIGIT or TIGIT). Thus, in some embodiments, the transmembrane immunomodulatory protein only contains the ectodomain and the transmembrane domain, such as any as described.

[0303] 2. Secreted Immunomodulatory Proteins and Engineered Cells

[0304] In some embodiments, the CD155 variant immunomodulatory polypeptide containing any one or more of the amino acid mutations as described herein, is secretable, such as when expressed from a cell. Such a variant CD155 immunomodulatory protein does not comprise a transmembrane domain. In some embodiments, the variant CD155 immunomodulatory protein is not conjugated to a half-life extending moiety (such as an Fc domain or a multimerization domain). In some embodiments, the variant CD155 immunomodulatory protein comprises a signal peptide, e.g. an antibody signal peptide or other efficient signal sequence to get domains outside of cell. When the immunomodulatory protein comprises a signal peptide and is expressed by an engineered cell, the signal peptide causes the immunomodulatory protein to be secreted by the engineered cell. Generally, the signal peptide, or a portion of the signal peptide, is cleaved from the immunomodulatory protein with secretion. The immunomodulatory protein can be encoded by a nucleic acid (which can be part of an expression vector). In some embodiments, the immunomodulatory protein is expressed and secreted by a cell (such as an immune cell, for example a primary immune cell).

[0305] Thus, in some embodiments, there are provided variant CD155 immunomodulatory proteins that further comprise a signal peptide. In some embodiments, provided herein is a nucleic acid molecule encoding the variant CD155 immunomodulatory protein operably connected to a secretion sequence encoding the signal peptide.

[0306] A signal peptide is a sequence on the N-terminus of an immunomodulatory protein that signals secretion of the immunomodulatory protein from a cell. In some embodiments, the signal peptide is about 5 to about 40 amino acids in length (such as about 5 to about 7, about 7 to about 10, about 10 to about 15, about 15 to about 20, about 20 to about 25, or about 25 to about 30, about 30 to about 35, or about 35 to about 40 amino acids in length).

[0307] In some embodiments, the signal peptide is a native signal peptide from the corresponding wild-type CD155 (see Table 2). In some embodiments, the signal peptide is a non-native signal peptide. For example, in some embodiments, the non-native signal peptide is a mutant native signal peptide from the corresponding wild-type CD155, and can include one or more (such as 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more) substitutions insertions or deletions. In some embodi-

ments, the non-native signal peptide is a signal peptide or mutant thereof of a family member from the same gSF family as the wild-type IgSF family member. In some embodiments, the non-native signal peptide is a signal peptide or mutant thereof from an gSF family member from a different IgSF family that the wild-type IgSF family member. In some embodiments, the signal peptide is a signal peptide or mutant thereof from a non-IgSF protein family, such as a signal peptide from an immunoglobulin (such as IgG heavy chain or IgG-kappa light chain), a cytokine (such as interleukin-2 (IL-2), or CD33), a serum albumin protein (e.g. HSA or albumin), a human azurocidin preprotein signal sequence, a luciferase, a trypsinogen (e.g. chymotrypsinogen or trypsinogen) or other signal peptide able to efficiently secrete a protein from a cell. Exemplary signal peptides include any described in the Table 9.

sequence for secretion. In some embodiments, the encoded immunomodulatory protein is secreted when expressed from a cell. In some embodiments, the immunomodulatory protein encoded by the nucleic acid molecule does not comprise a transmembrane domain. In some embodiments, the immunomodulatory protein encoded by the nucleic acid molecule does not comprise a half-life extending moiety (such as an Fc domain or a multimerization domain). In some embodiments, the immunomodulatory protein encoded by the nucleic acid molecule comprises a signal peptide. In some embodiments, a nucleic acid of the invention further comprises nucleotide sequence that encodes a secretory or signal peptide operably linked to the nucleic acid encoding the immunomodulatory protein, thereby allowing for secretion of the immunomodulatory protein

TABLE 9

Exemplary Signal Peptides

SEQ ID NO	Signal Peptide	Peptide Sequence
SEQ ID NO: 137	HSA signal peptide	MKWVTFISLLFLFSSAYS
SEQ ID NO: 138	Ig kappa light chain	MDMRAPAGIFGFLLVLFPGYRS
SEQ ID NO: 139	human azurocidin preprotein signal sequence	MTRLTVLALLAGLLASSRA
SEQ ID NO: 140	IgG heavy chain signal peptide	MELGLSWIFLLAILKGVQC
SEQ ID NO: 141	IgG heavy chain signal peptide	MELGLRWVFLVAILEGVQC
SEQ ID NO: 142	IgG heavy chain signal peptide	MKHLWFFLLLVAAPRWVLS
SEQ ID NO: 143	IgG heavy chain signal peptide	MDWTWRILFLVAAATGAHS
SEQ ID NO: 144	IgG heavy chain signal peptide	MDWTWRFLFVVAAATGVQS
SEQ ID NO: 145	IgG heavy chain signal peptide	MEFGLSWLFLVAILKGVQC
SEQ ID NO: 146	IgG heavy chain signal peptide	MEFGLSWVFLVALFRGVQC
SEQ ID NO: 147	IgG heavy chain signal peptide	MDLLHKNMKHLWFFLLLVAAPRWVLS
SEQ ID NO: 148	IgG Kappa light chain signal sequences:	MDMRVPAQLLGLLLLWLSGARC
SEQ ID NO: 149	IgG Kappa light chain signal sequences:	MKYLLPTAAAGLLLLAAQPAMA
SEQ ID NO: 150	Gaussia luciferase	MGVKVLFALICIAVAEA
SEQ ID NO: 151	Human albumin	MKWVTFISLLFLFSSAYS
SEQ ID NO: 152	Human chymotrypsinogen	MAFLWLLSCWALLGTTFG
SEQ ID NO: 153	Human interleukin-2	MQLLSCIALILALV
SEQ ID NO: 154	Human trypsinogen-2	MNLLLILTFVAAAVA

[0308] In some embodiments of a secretable variant CD155 immunomodulatory protein, the immunomodulatory protein comprises a signal peptide when expressed, and the signal peptide (or a portion thereof) is cleaved from the immunomodulatory protein upon secretion.

[0309] In some embodiments, the engineered cells express a variant CD155 polypeptides that are secreted from the cell. In some embodiments, such a variant CD155 polypeptide is encoded by a nucleic acid molecule encoding an immunomodulatory protein under the operable control of a signal

[0310] 3. Cells and Engineering Cells

[0311] Provided herein are engineered cells expressing any of the provided immunomodulatory polypeptides. In some embodiments, the engineered cells express on their surface any of the provided transmembrane immunomodulatory polypeptides. In some embodiments, the engineered cells express and are capable of or are able to secrete the immunomodulatory protein from the cells under conditions suitable for secretion of the protein. In some embodiments, the immunomodulatory protein is expressed on or in a

lymphocyte such as a tumor infiltrating lymphocyte (TIL), T-cell or NK cell, or on a myeloid cell. In some embodiments, the engineered cells are antigen presenting cells (APCs). In some embodiments, the engineered cells are engineered mammalian T-cells or engineered mammalian antigen presenting cells (APCs). In some embodiments, the engineered T-cells or APCs are human or murine cells.

[0312] In some embodiments, engineered T-cells include, but are not limited to, T helper cell, cytotoxic T-cell (alternatively, cytotoxic T lymphocyte or CTL), natural killer T-cell, regulatory T-cell, memory T-cell, or gamma delta T-cell. In some embodiments, the engineered T cells are CD4+ or CD8+.

[0313] In some embodiments, the engineered APCs include, for example, MHC II expressing APCs such as macrophages, B cells, and dendritic cells, as well as artificial APCs (aAPCs) including both cellular and acellular (e.g., biodegradable polymeric microparticles) aAPCs. Artificial APCs (aAPCs) are synthetic versions of APCs that can act in a similar manner to APCs in that they present antigens to T-cells as well as activate them. Antigen presentation is performed by the MHC (Class I or Class II). In some embodiments, in engineered APCs such as aAPCs, the antigen that is loaded onto the MHC is, in some embodiments, a tumor specific antigen or a tumor associated antigen. The antigen loaded onto the MHC is recognized by a T-cell receptor (TCR) of a T cell, which, in some cases, can express TIGIT or other molecule recognized by the variant CD155 polypeptides provided herein. Materials which can be used to engineer an aAPC include: poly (glycolic acid), poly(lactic-co-glycolic acid), iron-oxide, liposomes, lipid bilayers, sepharose, and polystyrene.

[0314] In some embodiments a cellular aAPC can be engineered to contain a TIP and TCR agonist which is used in adoptive cellular therapy. In some embodiments, a cellular aAPC can be engineered to contain a TIP and TCR agonist which is used in ex vivo expansion of human T cells, such as prior to administration, e.g. for reintroduction into the patient. In some aspects, the aAPC may include expression of at least one anti-CD3 antibody clone, e.g. such as, for example, OKT3 and/or UCHT1. In some aspects, the aAPCs may be inactivated (e.g. irradiated). In some embodiment, the TIP can include any variant IgSF domain that exhibits binding affinity for a cognate binding partner on a T cell.

[0315] In some embodiments, an immunomodulatory protein provided herein, such as a transmembrane immunomodulatory protein or a secretable immunomodulatory protein, is co-expressed or engineered into a cell that expresses an antigen-binding receptor, such as a recombinant receptor, such as a chimeric antigen receptor (CAR) or T cell receptor (TCR). In some embodiments, the engineered cell, such as an engineered T cell, recognizes a desired antigen associated with cancer, inflammatory and autoimmune disorders, or a viral infection. In specific embodiments, the antigen-binding receptor contains an antigen-binding moiety that specifically binds a tumor specific antigen or a tumor associated antigen. In some embodiments, the engineered T-cell is a CAR (chimeric antigen receptor) T-cell that contains an antigen-binding domain (e.g. scFv) that specifically binds to an antigen, such as a tumor specific antigen or tumor associated antigen. In some embodiments, the TIP protein is expressed in an engineered T-cell receptor cell or an engineered chimeric antigen receptor cell. In such embodiments, the engineered cell co-expresses the TIP and the CAR or TCR. In some embodiments, the SIP protein is expressed in an engineered T-cell receptor cell or an engineered chimeric antigen receptor cell. In such embodiments, the engineered cell co-expresses the SIP and the CAR or TCR.

[0316] Chimeric antigen receptors (CARs) are recombinant receptors that include an antigen-binding domain (ectodomain), a transmembrane domain and an intracellular signaling region (endodomain) that is capable of inducing or mediating an activation signal to the T cell after the antigen is bound. In some example, CAR-expressing cells are engineered to express an extracellular single chain variable fragment (scFv) with specificity for a particular tumor antigen linked to an intracellular signaling part comprising an activating domain and, in some cases, a costimulatory domain. The costimulatory domain can be derived from, e.g., CD28, OX-40, 4-1BB/CD137 or inducible T cell costimulator (ICOS). The activating domain can be derived from, e.g., CD3, such as CD3 zeta, epsilon, delta, gamma, or the like. In certain embodiments, the CAR is designed to have two, three, four, or more costimulatory domains. The CAR scFv can be designed to target an antigen expressed on a cell associated with a disease or condition, e.g. a tumor antigen, such as, for example, CD19, which is a transmembrane protein expressed by cells in the B cell lineage, including all normal B cells and B cell malignances, including but not limited to NHL, CLL, and non-T cell ALL. Example CAR+ T cell therapies and constructs are described in U.S. Patent Publication Nos. 2013/0287748, 2014/0227237, 2014/0099309, and 2014/0050708, and these references are incorporated by reference in their entirety.

[0317] In some aspects, the antigen-binding domain is an antibody or antigen-binding fragment thereof, such as a single chain fragment (scFv). In some embodiments, the antigen is expressed on a tumor or cancer cell. Exemplary of an antigen is CD19. Exemplary of a CAR is an anti-CD19 CAR, such as a CAR containing an anti-CD19 scFv set forth in SEQ ID NO:1152. In some embodiments, the CAR further contains a spacer, a transmembrane domain, and an intracellular signaling domain or region comprising an ITAM signaling domain, such as a CD3zeta signaling domain. In some embodiments, the CAR further includes a costimulatory signaling domain.

[0318] In some embodiments, the spacer and transmembrane domain are the hinge and transmembrane domain derived from CD8 such as having an exemplary sequence set forth in SEQ ID NO: 130, 1142, 2032 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:130, 1142, 2032. In some embodiments, the endodomain comprises at CD3-zeta signaling domain. In some embodiments, the CD3-zeta signaling domain comprises the sequence of amino acids set forth in SEQ ID NO: 131 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or more sequence identity to SEQ ID NO:131 and retains the activity of T cell signaling. In some embodiments, the endodomain of a CAR, can further comprise a costimulatory signaling domain or region to further modulate immunomodulatory responses of the T-cell. In some embodiments, the costimulatory signaling domain is or comprises a costimulatory region, or is derived from a costimulatory region, of CD28, ICOS, 41BB or OX40. In some embodiments, the costimulatory signaling domain is a derived from CD28 or 4-1BB and comprises the sequence of

amino acids set forth in any of SEQ ID NOS: 1143-1146 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or more sequence identity to SEQ ID NO:1143-1146 and retains the activity of T cell costimulatory signaling.

[0319] In some embodiments, the construct encoding the CAR further encodes a second protein, such as a marker, e.g. detectable protein, separated from the CAR by a self-cleaving peptide sequence. In some embodiments, the self-cleaving peptide sequence is an F2A, T2A, E2A or P2A self-cleaving peptide. Exemplary sequences of a T2A self-cleaving peptide are set for the in any one of SEQ ID NOS: 1147, 1155 or 2039 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or more sequence identity to any of SEQ ID NOS: 1147, 1155 or 2039. In some embodiments, the T2A is encoded by the sequence of nucleotides set forth in SEQ ID NO:1155 or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or more sequence identity to any of SEQ ID NO: 1155. In some embodiments, the marker is a detectable protein, such as a fluorescent protein, e.g. a green fluorescent protein (GFP) or blue fluorescent protein (BFP). Exemplary sequences of a fluorescent protein marker are set forth in SEQ ID NO:1148 or 2038 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or more sequence identity to SEQ ID NO:1148 or 2038.

[0320] In some embodiments, the CAR is an anti-CD19 CAR that has the sequence of amino acids set forth in any of SEQ ID NOS: 1138, 1149, 1150, 1151, 2033, 2034, 2036 or 2037 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or more sequence identity to any one of SEQ ID NOS: 1138, 1149, 1150, 1151, 2033, 2034, 2036 or 2037. In some embodiments, the CAR is encoded by a sequence of nucleotides set forth in SEQ ID NO: 1153 or 2035 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or more sequence identity to any one of SEQ ID NO: 1153 or 2035.

[0321] In another embodiment, the engineered T-cell possesses a TCR, including a recombinant or engineered TCR. In some embodiments, the TCR can be a native TCR. Those of skill in the art will recognize that generally native mammalian T-cell receptors comprise an alpha and a beta chain (or a gamma and a delta chain) involved in antigen specific recognition and binding. In some embodiments, the TCR is an engineered TCR that is modified. In some embodiments, the TCR of an engineered T-cell specifically binds to a tumor associated or tumor specific antigen presented by an APC.

[0322] In some embodiments, the immunomodulatory polypeptides, such as transmembrane immunomodulatory polypeptides or secretable immunomodulatory polypeptides, can be incorporated into engineered cells, such as engineered T cells or engineered APCs, by a variety of strategies such as those employed for recombinant host cells. A variety of methods to introduce a DNA construct into primary T cells are known in the art. In some embodiments, viral transduction or plasmid electroporation are employed. In typical embodiments, the nucleic acid molecule encoding the immunomodulatory protein, or the expression vector, comprises a signal peptide that localizes the expressed transmembrane immunomodulatory proteins to the cellular membrane or for secretion. In some embodiments, a nucleic acid encoding a transmembrane immunomodulatory proteins of the invention is sub-cloned into a viral vector, such as a retroviral vector, which allows expression in the host mammalian cell. The expression vector can be introduced into a mammalian host cell and, under host cell culture conditions, the immunomodulatory protein is expressed on the surface or is secreted.

[0323] In an exemplary example, primary T-cells can be purified ex vivo (CD4 cells or CD8 cells or both) and stimulated with an activation protocol consisting of various TCR/CD28 agonists, such as anti-CD3/anti-CD28 coated beads. After a 2 or 3 day activation process, a recombinant expression vector containing an immunomodulatory polypeptide can be stably introduced into the primary T cells through art standard lentiviral or retroviral transduction protocols or plasmid electroporation strategies. Cells can be monitored for immunomodulatory polypeptide expression by, for example, flow cytometry using anti-epitope tag or antibodies that cross-react with native parental molecule and polypeptides comprising variant CD155. T-cells that express the immunomodulatory polypeptide can be enriched through sorting with anti-epitope tag antibodies or enriched for high or low expression depending on the application.

[0324] Upon immunomodulatory polypeptide expression the engineered T-cell can be assayed for appropriate function by a variety of means. The engineered CAR or TCR co-expression can be validated to show that this part of the engineered T cell was not significantly impacted by the expression of the immunomodulatory protein. Once validated, standard in vitro cytotoxicity, proliferation, or cytokine assays (e.g., IFN-gamma expression) can be used to assess the function of engineered T-cells. Exemplary standard endpoints are percent lysis of the tumor line, proliferation of the engineered T-cell, or IFN-gamma protein expression in culture supernatants. An engineered construct which results in statistically significant increased lysis of tumor line, increased proliferation of the engineered T-cell, or increased IFN-gamma expression over the control construct can be selected for. Additionally, non-engineered, such as native primary or endogenous T-cells could also be incorporated into the same in vitro assay to measure the ability of the immunomodulatory polypeptide construct expressed on the engineered cells, such as engineered T-cells, to modulate activity, including, in some cases, to activate and generate effector function in bystander, native T-cells. Increased expression of activation markers such as CD69, CD44, or CD62L could be monitored on endogenous T cells, and increased proliferation and/or cytokine production could indicate desired activity of the immunomodulatory protein expressed on the engineered T cells.

[0325] In some embodiments, the similar assays can be used to compare the function of engineered T cells containing the CAR or TCR alone to those containing the CAR or TCR and a TIP construct. Typically, these in vitro assays are performed by plating various ratios of the engineered T cell and a "tumor" cell line containing the cognate CAR or TCR antigen together in culture. Standard endpoints are percent lysis of the tumor line, proliferation of the engineered T cell, or IFN-gamma production in culture supernatants. An engineered immunomodulatory protein which resulted in statis-

tically significant increased lysis of tumor line, increased proliferation of the engineered T cell, or increased IFN-gamma production over the same TCR or CAR construct alone can be selected for. Engineered human T cells can be analyzed in immunocompromised mice, like the NSG strain, which lacks mouse T, NK and B cells. Engineered human T cells in which the CAR or TCR binds a target counter-structure on the xenograft and is co-expressed with the TIP affinity modified IgSF domain can be adoptively transferred in vivo at different cell numbers and ratios compared to the xenograft. For example, engraftment of CD19+ leukemia tumor lines containing a luciferase/GFP vector can be monitored through bioluminescence or ex vivo by flow cytometry. In a common embodiment, the xenograft is introduced into the murine model, followed by the engineered T cells several days later. Engineered T cells containing the immunomodulatory protein can be assayed for increased survival, tumor clearance, or expanded engineered T cells numbers relative to engineered T cells containing the CAR or TCR alone. As in the in vitro assay, endogenous, native (i.e., non-engineered) human T cells could be co-adoptively transferred to look for successful epitope spreading in that population, resulting in better survival or tumor clearance.

[0326] E. Infectious Agents for Expressing Variant Polypeptides and Immunomodulatory Proteins

[0327] Also provided are infectious agents that contain nucleic acids encoding any of the variant polypeptides, such as CD155 vIgD polypeptides, including secretable or transmembrane immunomodulatory proteins described herein. In some embodiments, such infectious agents can deliver the nucleic acids encoding the variant immunomodulatory polypeptides described herein, such as CD155 vIgD polypeptides, to a target cell in a subject, e.g., immune cell and/or antigen-presenting cell (APC) or tumor cell in a subject. Also provided are nucleic acids contained in such infectious agents, and/or nucleic acids for generation or modification of such infectious agents, such as vectors and/or plasmids, and compositions containing such infectious agents.

[0328] In some embodiments, the infectious agent is a microorganism or a microbe. In some embodiments, the infectious agent is a virus or a bacterium. In some embodiments, the infectious agent is a virus. In some embodiments, the infectious agent is a bacterium. In some embodiments, such infectious agents can deliver nucleic acid sequences encoding any of the variant polypeptides, such as CD155 vIgD polypeptides, including secretable or transmembrane immunomodulatory proteins, described herein. Thus, in some embodiments, the cell in a subject that is infected or contacted by the infectious agents can be rendered to express on the cell surface or secrete, the variant immunomodulatory polypeptides. In some embodiments, the infectious agent can also deliver one or more other therapeutics or nucleic acids encoding other therapeutics to the cell and/or to an environment within the subject. In some embodiments, other therapeutics that can be delivered by the infectious agents include cytokines or other immunomodulatory molecules.

[0329] In some embodiments, the infectious agent, e.g., virus or bacteria, contains nucleic acid sequences that encode any of the variant polypeptides, such as CD155 vIgD polypeptides, including secretable or transmembrane immunomodulatory proteins, described herein, and by virtue of contact and/or infection of a cell in the subject, the cell expresses the variant polypeptides, such as CD155 vIgD polypeptides, including secretable or transmembrane immunomodulatory proteins, encoded by the nucleic acid sequences contained in the infectious agent. In some embodiments, the infectious agent can be administered to the subject. In some embodiments, the infectious agent can be contacted with cells from the subject ex vivo.

[0330] In some embodiments, the variant polypeptides, such as CD155 vIgD polypeptides, including transmembrane immunomodulatory proteins, expressed by the cell infected by the infectious agent is a transmembrane protein and is surface expressed. In some embodiments, the variant polypeptides, such as CD155 vIgD polypeptides, including secretable immunomodulatory proteins, expressed by the cell infected by the infectious agent is expressed and secreted from the cell. The transmembrane immunomodulatory protein or secreted immunomodulatory protein can be any described herein.

[0331] In some embodiments, the cells in the subject that are targeted by the infectious agent include a tumor cell, an immune cell, and/or an antigen-presenting cell (APC). In some embodiments, the infectious agent targets a cell in the tumor microenvironment (TME). In some embodiments, the infectious agent delivers the nucleic acids encoding the variant polypeptides, such as CD155 vIgD polypeptides, including secretable or transmembrane immunomodulatory proteins, to an appropriate cell (for example, an APC, such as a cell that displays a peptide/MHC complex on its cell surface, such as a dendritic cell) or tissue (e.g., lymphoid tissue) that will induce and/or augment the desired effect, e.g., immunomodulation and/or a specific cell-medicated immune response, e.g., CD4 and/or CD8 T cell response, which CD8 T cell response may include a cytotoxic T cell (CTL) response. In some embodiments, the infectious agent targets an APC, such as a dendritic cell (DC). In some embodiments, the nucleic acid molecule delivered by the infectious agents described herein include appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequences encoding the variant immunomodulatory polypeptides, in a particular target cell, e.g., regulatory elements such as promoters.

[0332] In some embodiments, the infectious agent that contains nucleic acid sequences encoding the immunomodulatory polypeptides can also contain nucleic acid sequences that encode one or more additional gene products, e.g., cytokines, prodrug converting enzymes, cytotoxins and/or detectable gene products. For example, in some embodiments, the infectious agent is an oncolytic virus and the virus can include nucleic acid sequences encoding additional therapeutic gene products (see, e.g., Kim et al., (2009) Nat Rev Cancer 9:64-71; Garcia-Aragoncillo et al., (2010) Curr Opin Mol Ther 12:403-411; see U.S. Pat. Nos. 7,588,767, 7,588,771, 7,662,398 and 7,754,221 and U.S. Pat. Publ. Nos. 2007/0202572, 2007/0212727, 2010/0062016, 2009/0098529, 2009/0053244, 2009/0155287, 2009/0117034, 2010/0233078, 2009/0162288, 2010/0196325, 2009/0136917 and 2011/0064650. In some embodiments, the additional gene product can be a therapeutic gene product that can result in death of the target cell (e.g., tumor cell) or gene products that can augment or boost or regulate an immune response (e.g., cytokine). Exemplary gene products also include among an anticancer agent, an anti-metastatic agent, an antiangiogenic agent, an immunomodulatory molecule, an immune checkpoint inhibitor, an antibody, a cytokine, a growth factor, an antigen, a cytotoxic gene product, a pro-apoptotic gene product, an anti-apoptotic gene prod-

uct, a cell matrix degradative gene, genes for tissue regeneration and reprogramming human somatic cells to pluripotency, and other genes described herein or known to one of skill in the art. In some embodiments, the additional gene product is Granulocyte-macrophage colony-stimulating factor (GM-CSF).

[0333] 1. Viruses

[0334] In some embodiments, the infectious agent is a virus. In some embodiments, the infectious agent is an oncolytic virus, or a virus that targets particular cells, e.g., immune cells. In some embodiments, the infectious agent targets a tumor cell and/or cancer cell in the subject. In some embodiments, the infectious agent targets an immune cell or an antigen-presenting cell (APC).

[0335] In some embodiments, the infectious agent is an oncolytic virus. Oncolytic viruses are viruses that accumulate in tumor cells and replicate in tumor cells. By virtue of replication in the cells, and optional delivery of nucleic acids encoding variant CD155 polypeptides or immunomodulatory polypeptides described herein, tumor cells are lysed, and the tumor shrinks and can be eliminated. Oncolytic viruses can also have a broad host and cell type range. For example, oncolytic viruses can accumulate in immunoprivileged cells or immunoprivileged tissues, including tumors and/or metastases, and also including wounded tissues and cells, thus allowing the delivery and expression of nucleic acids encoding the variant immunomodulatory polypeptides described herein in a broad range of cell types. Oncolytic viruses can also replicate in a tumor cell specific manner, resulting in tumor cell lysis and efficient tumor regression.

[0336] Exemplary oncolytic viruses include adenoviruses, adeno-associated viruses, herpes viruses, Herpes Simplex Virus, vesticular stomatic virus, Reovirus, Newcastle Disease virus, parvovirus, measles virus, vesticular stomatitis virus (VSV), Coxsackie virus and Vaccinia virus. In some embodiments, oncolytic viruses can specifically colonize solid tumors, while not infecting other organs, and can be used as an infectious agent to deliver the nucleic acids encoding the variant immunomodulatory polypeptides described herein to such solid tumors.

[0337] Oncolytic viruses for use in delivering the nucleic acids encoding variant CD155 polypeptides or immunomodulatory polypeptides described herein, can be any of those known to one of skill in the art and include, for example, vesicular stomatitis virus, see, e.g., U.S. Pat. Nos. 7,731,974, 7,153,510, 6,653,103 and U.S. Pat. Pub. Nos. 2010/0178684, 2010/0172877, 2010/0113567, 2007/0098743, 20050260601, 20050220818 and EP Pat. Nos. 1385466, 1606411 and 1520175; herpes simplex virus, see, e.g., U.S. Pat. Nos. 7,897,146, 7,731,952, 7,550,296, 7,537,924, 6,723,316, 6,428,968 and U.S. Pat. Pub. Nos., 2014/0154216, 2011/0177032, 2011/0158948, 2010/0092515, 2009/0274728, 2009/0285860, 2009/0215147, 2009/0010889, 2007/0110720, 2006/0039894, 2004/0009604, 2004/0063094, International Patent Pub. Nos., WO 2007/052029, WO 1999/038955; retroviruses, see, e.g., U.S. Pat. Nos. 6,689,871, 6,635,472, 5,851,529, 5,716,826, 5,716,613 and U.S. Pat. Pub. No. 20110212530; vaccinia viruses, see, e.g., 2016/0339066, and adeno-associated viruses, see, e.g., U.S. Pat. Nos. 8,007,780, 7,968,340, 7,943,374, 7,906,111, 7,927,585, 7,811,814, 7,662,627, 7,241,447, 7,238,526, 7,172,893, 7,033,826, 7,001,765, 6,897,045, and 6,632,670.

[0338] Oncolytic viruses also include viruses that have been genetically altered to attenuate their virulence, to improve their safety profile, enhance their tumor specificity, and they have also been equipped with additional genes, for example cytotoxins, cytokines, prodrug converting enzymes to improve the overall efficacy of the viruses (see, e.g., Kirn et al., (2009) Nat Rev Cancer 9:64-71; Garcia-Aragoncillo et al., (2010) Curr Opin Mol Ther 12:403-411; see U.S. Pat. Nos. 7,588,767, 7,588,771, 7,662,398 and 7,754,221 and U.S. Pat. Publ. Nos. 2007/0202572, 2007/0212727, 2010/0062016, 2009/0098529, 2009/0053244, 2009/0155287, 2009/0117034, 2010/0233078, 2009/0162288, 2010/0196325, 2009/0136917 and 2011/0064650). In some embodiments, the oncolytic viruses can be those that have been modified so that they selectively replicate in cancerous cells, and, thus, are oncolytic. For example, the oncolytic virus is an adenovirus that has been engineered to have modified tropism for tumor therapy and also as gene therapy vectors. Exemplary of such is ONYX-015, H101 and Ad5ΔCR (Hallden and Portella (2012) Expert Opin Ther Targets, 16:945-58) and TNFerade (McLoughlin et al. (2005) Ann. Surg. Oncol., 12:825-30), or a conditionally replicative adenovirus Oncorine®.

[0339] In some embodiments, the infectious agent is a modified herpes simplex virus. In some embodiments, the infectious agent is a modified version of Talimogene laherparepvec (also known as T-Vec, Imlygic or OncoVex GM-CSF), that is modified to contain nucleic acids encoding any of the variant immunomodulatory polypeptides described herein, such as variant CD155 polypeptide described herein. In some embodiments, the infectious agent is a modified herpes simplex virus that is described, e.g., in WO 2007/052029, WO 1999/038955, US 2004/0063094, US 2014/0154216, or, variants thereof.

[0340] In some embodiments, the infectious agent is a virus that targets a particular type of cells in a subject that is administered the virus, e.g., a virus that targets immune cells or antigen-presenting cells (APCs). Dendritic cells (DCs) are essential APCs for the initiation and control of immune responses. DCs can capture and process antigens, migrate from the periphery to a lymphoid organ, and present the antigens to resting T cells in a major histocompatibility complex (MHC)-restricted fashion. In some embodiments, the infectious agent is a virus that specifically can target DCs to deliver nucleic acids encoding the variant CD155 polypeptide or immunomodulatory polypeptides for expression in DCs. In some embodiments, the virus is a lentivirus or a variant or derivative thereof, such as an integration-deficient lentiviral vector. In some embodiments, the virus is a lentivirus that is pseudotyped to efficiently bind to and productively infect cells expressing the cell surface marker dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), such as DCs. In some embodiments, the virus is a lentivirus pseudotyped with a Sindbis virus E2 glycoprotein or modified form thereof, such as those described in WO 2013/149167. In some embodiments, the virus allows for delivery and expression of a sequence of interest (e.g., a nucleic acid encoding any of the variant CD155 polypeptide or immunomodulatory polypeptides described herein) to a DC. In some embodiments, the virus includes those described in WO 2008/011636 or US 2011/0064763, Tareen et al. (2014) Mol. Ther., 22:575-587, or variants thereof. Exemplary of a dendritic cell-tropic vector platform is ZVex™.

[0341] 2. Bacteria

[0342] In some embodiments, the infectious agent is a bacterium. For example, in some embodiments, the bacteria can deliver nucleic acids encoding any of the variant CD155 polypeptide or immunomodulatory polypeptides described herein to a target cell in the subject, such as a tumor cell, an immune cell, an antigen-presenting cell and/or a phagocytic cell. In some embodiments, the bacterium can be preferentially targeted to a specific environment within a subject, such as a tumor microenvironment (TME), for expression and/or secretion of the variant immunomodulatory polypeptides and/or to target specific cells in the environment for expression of the variant immunomodulatory polypeptides.

[0343] In some embodiments, the bacterium delivers the nucleic acids to the cells via bacterial-mediated transfer of plasmid DNA to mammalian cells (also referred to as "bactofection"). For example, in some embodiments, delivery of genetic material is achieved through entry of the entire bacterium into target cells. In some embodiments, spontaneous or induced bacterial lysis can lead to the release of plasmid for subsequent eukaryotic cell expression. In some embodiments, the bacterium can deliver nucleic acids to non-phagocytic mammalian cells (e.g., tumor cells) and/or to phagocytic cells, e.g., certain immune cells and/or APCs. In some embodiments, the nucleic acids delivered by the bacterium can be transferred to the nucleus of the cell in the subject for expression. In some embodiments, the nucleic acids also include appropriate nucleic acid sequences necessary for the expression of the operably linked sequences encoding the variant immunomodulatory polypeptides in a particular host cell, e.g., regulatory elements such as promoters or enhancers. In some embodiments, the infectious agent that is a bacterium can deliver nucleic acids encoding the immunomodulatory proteins in the form of an RNA, such as a pre-made translation-competent RNA delivered to the cytoplasm of the target cell for translation by the target cell's machinery.

[0344] In some embodiments, the bacterium can replicate and lyse the target cells, e.g., tumor cells. In some embodiments, the bacterium can contain and/or release nucleic acid sequences and/or gene products in the cytoplasm of the target cells, thereby killing the target cell, e.g., tumor cell. In some embodiments, the infectious agent is bacterium that can replicate specifically in a particular environment in the subject, e.g., tumor microenvironment (TME). For example, in some embodiments, the bacterium can replicate specifically in anaerobic or hypoxic microenvironments. In some embodiments, conditions or factors present in particular environments, e.g., aspartate, serine, citrate, ribose or galactose produced by cells in the TME, can act as chemoattractants to attract the bacterium to the environment. In some embodiments, the bacterium can express and/or secrete the immunomodulatory proteins described herein in the environment, e.g., TME.

[0345] In some embodiments, the infectious agent is a bacterium that is a *Listeria* sp., a *Bifidobacterium* sp., an *Escherichia* sp., a *Clostridium* sp., a *Salmonella* sp., a *Shigella* sp., a *Vibrio* sp. or a *Yersinia* sp. In some embodiments, the bacterium is selected from among one or more of *Listeria monocytogenes, Salmonella typhimurium, Salmonella choleraesuis, Escherichia coli, Vibrio cholera, Clostridium perfringens, Clostridium butyricum, Clostridium novyi, Clostridium acetobutylicum, Bifidobacterium infantis, Bifidobacterium longum* and *Bifidobacterium adolescentis*. In some embodiments, the bacterium is an engineered bacterium. In some embodiments, the bacterium is an engineered bacterium such as those described in, e.g., Seow and Wood (2009) Molecular Therapy 17(5):767-777; Baban et al. (2010) Bioengineered Bugs 1:6, 385-394; Patyar et al. (2010) J Biomed Sci 17:21; Tangney et al. (2010) Bioengineered Bugs 1:4, 284-287; van Pijkeren et al. (2010) Hum Gene Ther. 21(4):405-416; WO 2012/149364; WO 2014/198002; U.S. Pat. Nos. 9,103,831; 9,453,227; US 2014/0186401; US 2004/0146488; US 2011/0293705; US 2015/0359909 and EP 3020816. The bacterium can be modified to deliver nucleic acid sequences encoding any of the variant immunomodulatory polypeptides, conjugates and/or fusions provided herein, and/or to express such variant immunomodulatory polypeptides in the subject.

[0346] F. Nucleic Acids, Vectors and Methods for Producing the Polypeptides or Cells

[0347] Provided herein are isolated or recombinant nucleic acids collectively referred to as "nucleic acids" which encode any of the various provided embodiments of the variant CD155 polypeptides or immunomodulatory polypeptides provided herein. In some embodiments, nucleic acids provided herein, including all described below, are useful in recombinant production (e.g., expression) of variant CD155 polypeptides or immunomodulatory polypeptides provided herein. In some embodiments, nucleic acids provided herein, including all described below, are useful in expression of variant CD155 polypeptides or immunomodulatory polypeptides provided herein in cells, such as in engineered cells, e.g. immune cells, or infectious agent cells. The nucleic acids provided herein can be in the form of RNA or in the form of DNA, and include mRNA, cRNA, recombinant or synthetic RNA and DNA, and cDNA. The nucleic acids provided herein are typically DNA molecules, and usually double-stranded DNA molecules. However, single-stranded DNA, single-stranded RNA, double-stranded RNA, and hybrid DNA/RNA nucleic acids or combinations thereof comprising any of the nucleotide sequences of the invention also are provided.

[0348] Also provided herein are recombinant expression vectors and recombinant host cells useful in producing the variant CD155 polypeptides or immunomodulatory polypeptides provided herein.

[0349] Also provided herein are engineered cells, such as engineered immune cells, containing any of the provided nucleic acids or encoded variant CD155 polypeptides or immunomodulatory polypeptides, such as any of the transmembrane immunomodulatory polypeptides or secretable immunomodulatory polypeptides.

[0350] Also provided herein are infectious agents, such as bacterial or viral cells, containing any of the provided nucleic acids or encoded variant CD155 polypeptides or immunomodulatory polypeptides, such as any of the transmembrane immunomodulatory polypeptides or secretable immunomodulatory polypeptides.

[0351] In any of the above provided embodiments, the nucleic acids encoding the immunomodulatory polypeptides provided herein can be introduced into cells using recombinant DNA and cloning techniques. To do so, a recombinant DNA molecule encoding an immunomodulatory polypeptide is prepared. Methods of preparing such DNA molecules are well known in the art. For instance, sequences coding for the peptides could be excised from DNA using suitable restriction enzymes. Alternatively, the DNA molecule could be synthesized using chemical synthesis techniques, such as

the phosphoramidite method. Also, a combination of these techniques could be used. In some instances, a recombinant or synthetic nucleic acid may be generated through polymerase chain reaction (PCR). In some embodiments, a DNA insert can be generated encoding one or more variant CD155 polypeptides containing at least one affinity-modified IgSF domain and, in some embodiments, a signal peptide, a transmembrane domain and/or an endodomain in accord with the provided description. This DNA insert can be cloned into an appropriate transduction/transfection vector as is known to those of skill in the art. Also provided are expression vectors containing the nucleic acid molecules.

[0352] In some embodiments, the expression vectors are capable of expressing the immunomodulatory proteins in an appropriate cell under conditions suited to expression of the protein. In some aspects, nucleic acid molecule or an expression vector comprises the DNA molecule that encodes the immunomodulatory protein operatively linked to appropriate expression control sequences. Methods of effecting this operative linking, either before or after the DNA molecule is inserted into the vector, are well known. Expression control sequences include promoters, activators, enhancers, operators, ribosomal binding sites, start signals, stop signals, cap signals, polyadenylation signals, and other signals involved with the control of transcription or translation.

[0353] In some embodiments, expression of the immunomodulatory protein is controlled by a promoter or enhancer to control or regulate expression. The promoter is operably linked to the portion of the nucleic acid molecule encoding the variant polypeptide or immunomodulatory protein. In some embodiments, the promotor is a constitutively active promotor (such as a tissue-specific constitutively active promotor or other constitutive promotor). In some embodiments, the promotor is an inducible promotor, which may be responsive to an inducing agent (such as a T cell activation signal).

[0354] In some embodiments, a constitutive promoter is operatively linked to the nucleic acid molecule encoding the variant polypeptide or immunomodulatory protein. Exemplary constitutive promoters include the Simian vacuolating virus 40 (SV40) promoter, the cytomegalovirus (CMV) promoter, the ubiquitin C (UbC) promoter, and the EF-1 alpha (EF1a) promoter. In some embodiments, the constitutive promoter is tissue specific. For example, in some embodiments, the promoter allows for constitutive expression of the immunomodulatory protein in specific tissues, such as immune cells, lymphocytes, or T cells. Exemplary tissue-specific promoters are described in U.S. Pat. No. 5,998,205, including, for example, a fetoprotein, DF3, tyrosinase, CEA, surfactant protein, and ErbB2 promoters.

[0355] In some embodiments, an inducible promoter is operatively linked to the nucleic acid molecule encoding the variant polypeptide or immunomodulatory protein such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. For example, the promoter can be a regulated promoter and transcription factor expression system, such as the published tetracycline-regulated systems or other regulatable systems (see, e.g. published International PCT Appl. No. WO 01/30843), to allow regulated expression of the encoded polypeptide. An exemplary regulatable promoter system is the Tet-On (and Tet-Off) system available, for example, from Clontech (Palo Alto, CA). This promoter system allows the regulated expression of the transgene controlled by tetracycline or tetracycline derivatives, such as doxycycline. Other regulatable promoter systems are known (see e.g., published U.S. Application No. 2002-0168714, entitled "Regulation of Gene Expression Using Single-Chain, Monomeric, Ligand Dependent Polypeptide Switches," which describes gene switches that contain ligand binding domains and transcriptional regulating domains, such as those from hormone receptors).

[0356] In some embodiments, the promotor is responsive to an element responsive to T-cell activation signaling. Solely by way of example, in some embodiments, an engineered T cell comprises an expression vector encoding the immunomodulatory protein and a promotor operatively linked to control expression of the immunomodulatory protein. The engineered T cell can be activated, for example by signaling through an engineered T cell receptor (TCR) or a chimeric antigen rector (CAR), and thereby triggering expression and secretion of the immunomodulatory protein through the responsive promotor.

[0357] In some embodiments, an inducible promoter is operatively linked to the nucleic acid molecule encoding the immunomodulatory protein such that the immunomodulatory protein is expressed in response to a nuclear factor of activated T-cells (NFAT) or nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB). For example, in some embodiments, the inducible promoter comprises a binding site for NFAT or NF-κB. For example, in some embodiments, the promoter is an NFAT or NF-κB promoter or a functional variant thereof. Thus, in some embodiments, the nucleic acids make it possible to control the expression of immunomodulatory protein while also reducing or eliminating the toxicity of the immunomodulatory protein. In particular, engineered immune cells comprising the nucleic acids of the invention express and secrete the immunomodulatory protein only when the cell (e.g., a T-cell receptor (TCR) or a chimeric antigen receptor (CAR) expressed by the cell) is specifically stimulated by an antigen and/or the cell (e.g., the calcium signaling pathway of the cell) is non-specifically stimulated by, e.g., phorbol myristate acetate (PMA)/Ionomycin. Accordingly, the expression and, in some cases, secretion, of immunomodulatory protein can be controlled to occur only when and where it is needed (e.g., in the presence of an infectious disease-causing agent, cancer, or at a tumor site), which can decrease or avoid undesired immunomodulatory protein interactions.

[0358] In some embodiments, the nucleic acid encoding an immunomodulatory protein described herein comprises a suitable nucleotide sequence that encodes a NFAT promoter, NF-κB promoter, or a functional variant thereof. "NFAT promoter" as used herein means one or more NFAT responsive elements linked to a minimal promoter. "NF-κB promoter" refers to one or more NF-κB responsive elements linked to a minimal promoter. In some embodiments, the minimal promoter of a gene is a minimal human IL-2 promoter or a CMV promoter. The NFAT responsive elements may comprise, e.g., NFAT1, NFAT2, NFAT3, and/or NFAT4 responsive elements. The NFAT promoter, NF-κB promoter, or a functional variant thereof may comprise any number of binding motifs, e.g., at least two, at least three, at least four, at least five, or at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or up to twelve binding motifs.

[0359] The resulting recombinant expression vector having the DNA molecule thereon is used to transform an

appropriate host. This transformation can be performed using methods well known in the art. In some embodiments, a nucleic acid provided herein further comprises nucleotide sequence that encodes a secretory or signal peptide operably linked to the nucleic acid encoding an immunomodulatory polypeptide such that a resultant soluble immunomodulatory polypeptide is recovered from the culture medium, host cell, or host cell periplasm. In other embodiments, the appropriate expression control signals are chosen to allow for membrane expression of an immunomodulatory polypeptide. Furthermore, commercially available kits as well as contract manufacturing companies can also be utilized to make engineered cells or recombinant host cells provided herein.

[0360] In some embodiments, the resulting expression vector having the DNA molecule thereon is used to transform, such as transduce, an appropriate cell. The introduction can be performed using methods well known in the art. Exemplary methods include those for transfer of nucleic acids encoding the receptors, including via viral, e.g., retroviral or lentiviral, transduction, transposons, and electroporation. In some embodiments, the expression vector is a viral vector. In some embodiments, the nucleic acid is transferred into cells by lentiviral or retroviral transduction methods.

[0361] Any of a large number of publicly available and well-known mammalian host cells, including mammalian T-cells or APCs, can be used in the preparing the polypeptides or engineered cells. The selection of a cell is dependent upon a number of factors recognized by the art. These include, for example, compatibility with the chosen expression vector, toxicity of the peptides encoded by the DNA molecule, rate of transformation, ease of recovery of the peptides, expression characteristics, bio-safety and costs. A balance of these factors must be struck with the understanding that not all cells can be equally effective for the expression of a particular DNA sequence.

[0362] In some embodiments, the host cells can be a variety of eukaryotic cells, such as in yeast cells, or with mammalian cells such as Chinese hamster ovary (CHO) or HEK293 cells. In some embodiments, the host cell is a suspension cell and the polypeptide is engineered or produced in cultured suspension, such as in cultured suspension CHO cells, e.g. CHO-S cells. In some examples, the cell line is a CHO cell line that is deficient in DHFR (DHFR-), such as DG44 and DUXB11. In some embodiments, the cell is deficient in glutamine synthase (GS), e.g. CHO-S cells, CHOK1 SV cells, and CHOZN((R)) GS−/− cells. In some embodiments, the CHO cells, such as suspension CHO cells, may be CHO-S-2H2 cells, CHO-S-clone 14 cells, or ExpiCHO-S cells.

[0363] In some embodiments, host cells can also be prokaryotic cells, such as with *E. coli*.

[0364] The transformed recombinant host is cultured under polypeptide expressing conditions, and then purified to obtain a soluble protein. Recombinant host cells can be cultured under conventional fermentation conditions so that the desired polypeptides are expressed. Such fermentation conditions are well known in the art. Finally, the polypeptides provided herein can be recovered and purified from recombinant cell cultures by any of a number of methods well known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, and affinity chromatography. Protein refolding steps can be used, as desired, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed in the final purification steps.

[0365] In some embodiments, the cell is an immune cell, such as any described above in connection with preparing engineered cells. In some embodiments, such engineered cells are primary cells. In some embodiments, the engineered cells are autologous to the subject. In some embodiment, the engineered cells are allogeneic to the subject. In some embodiments, the engineered cells are obtained from a subject, such as by leukapheresis, and transformed ex vivo for expression of the immunomodulatory polypeptide, e.g. transmembrane immunomodulatory polypeptide or secretable immunomodulatory polypeptide.

[0366] Also provided are nucleic acids encoding any of the variant immunomodulatory polypeptides contained in infectious agents described herein. In some embodiments, the infectious agents deliver the nucleic acids to a cell in the subject, and/or permit expression of the encoded variant polypeptides in the cell. Also provided are nucleic acids that are used to generate, produce or modify such infectious agents. For example, in some embodiments, provided are vectors and/or plasmids that contain nucleic acids encoding the variant immunomodulatory polypeptides, for generation of the infectious agents, delivery to the cells in a subject and/or expression of the variant immunomodulatory polypeptides in the cells in the subject.

[0367] In some embodiments, the provided nucleic acids are recombinant viral or bacterial vectors containing nucleic acid sequences encoding the variant immunomodulatory polypeptides. In some embodiments, the recombinant vectors can be used to produce an infectious agent that contains nucleic acid sequences encoding the variant immunomodulatory polypeptides and/or to be delivered to a target cell in the subject for expression by the target cell. In some embodiments, the recombinant vector is an expression vector. In some embodiments, the recombinant vector includes appropriate sequences necessary for generation and/or production of the infectious agent and expression in the target cell.

[0368] In some embodiments, the recombinant vector is a plasmid or cosmid. Plasmid or cosmid containing nucleic acid sequences encoding the variant immunomodulatory polypeptides, as described herein, is readily constructed using standard techniques well known in the art. For generation of the infectious agent, the vector or genome can be constructed in a plasmid form that can then be transfected into a packaging or producer cell line or a host bacterium. The recombinant vectors can be generated using any of the recombinant techniques known in the art. In some embodiments, the vectors can include a prokaryotic origin of replication and/or a gene whose expression confers a detectable or selectable marker such as a drug resistance for propagation and/or selection in prokaryotic systems.

[0369] In some embodiments, the recombinant vector is a viral vector. Exemplary recombinant viral vectors include a lentiviral vector genome, poxvirus vector genome, vaccinia virus vector genome, adenovirus vector genome, adenovirus-associated virus vector genome, herpes virus vector genome, and alpha virus vector genome. Viral vectors can be live, attenuated, replication conditional or replication deficient, non-pathogenic (defective), replication competent

viral vector, and/or is modified to express a heterologous gene product, e.g., the variant immunomodulatory polypeptides provided herein. Vectors for generation of viruses also can be modified to alter attenuation of the virus, which includes any method of increasing or decreasing the transcriptional or translational load.

[0370] Exemplary viral vectors that can be used include modified vaccinia virus vectors (see, e.g., Guerra et al., J. Virol. 80:985-98 (2006); Tartaglia et al., AIDS Research and Human Retroviruses 8: 1445-47 (1992); Gheradi et al., J. Gen. Virol. 86:2925-36 (2005); Mayr et al., Infection 3:6-14 (1975); Hu et al., J. Virol. 75: 10300-308 (2001); U.S. Pat. Nos. 5,698,530, 6,998,252, 5,443,964, 7,247,615 and 7,368,116); adenovirus vector or adenovirus-associated virus vectors (see., e.g., Molin et al., J. Virol. 72:8358-61 (1998); Narumi et al., Am J. Respir. Cell Mol. Biol. 19:936-41 (1998); Mercier et al., Proc. Natl. Acad. Sci. USA 101:6188-93 (2004); U.S. Pat. Nos. 6,143,290; 6,596,535; 6,855,317; 6,936,257; 7,125,717; 7,378,087; 7,550,296); retroviral vectors including those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), ecotropic retroviruses, simian immunodeficiency virus (SIV), human immunodeficiency virus (HIV), and combinations (see, e.g., Buchscher et al., J. Virol. 66:2731-39 (1992); Johann et al., J. Virol. 66: 1635-40 (1992); Sommerfelt et al., Virology 176:58-59 (1990); Wilson et al., J. Virol. 63:2374-78 (1989); Miller et al., J. Virol. 65:2220-24 (1991); Miller et al., Mol. Cell Biol. 10:4239 (1990); Kolberg, NIH Res. 4:43 1992; Cornetta et al., Hum. Gene Ther. 2:215 (1991)); lentiviral vectors including those based upon Human Immunodeficiency Virus (HIV-1), HIV-2, feline immunodeficiency virus (FIV), equine infectious anemia virus, Simian Immunodeficiency Virus (SIV), and maedi/visna virus (see, e.g., Pfeifer et al., Annu. Rev. Genomics Hum. Genet. 2: 177-211 (2001); Zufferey et al., J. Virol. 72: 9873, 1998; Miyoshi et al., J. Virol. 72:8150, 1998; Philpott and Thrasher, Human Gene Therapy 18:483, 2007; Engelman et al., J. Virol. 69: 2729, 1995; Nightingale et al., Mol. Therapy, 13: 1121, 2006; Brown et al., J. Virol. 73:9011 (1999); WO 2009/076524; WO 2012/141984; WO 2016/011083; McWilliams et al., J. Virol. 77: 11150, 2003; Powell et al., J. Virol. 70:5288, 1996) or any, variants thereof, and/or vectors that can be used to generate any of the viruses described above. In some embodiments, the recombinant vector can include regulatory sequences, such as promoter or enhancer sequences, that can regulate the expression of the viral genome, such as in the case for RNA viruses, in the packaging cell line (see, e.g., U.S. Pat. Nos. 5,385,839 and 5,168,062).

[0371] In some embodiments, the recombinant vector is an expression vector, e.g., an expression vector that permits expression of the encoded gene product when delivered into the target cell, e.g., a cell in the subject, e.g., a tumor cell, an immune cell and/or an APC. In some embodiments, the recombinant expression vectors contained in the infectious agent are capable of expressing the immunomodulatory proteins in the target cell in the subject, under conditions suited to expression of the protein.

[0372] In some aspects, nucleic acids or an expression vector comprises a nucleic acid sequence that encodes the immunomodulatory protein operatively linked to appropriate expression control sequences. Methods of affecting this operative linking, either before or after the nucleic acid sequence encoding the immunomodulatory protein is inserted into the vector, are well known. Expression control sequences include promoters, activators, enhancers, operators, ribosomal binding sites, start signals, stop signals, cap signals, polyadenylation signals, and other signals involved with the control of transcription or translation. The promoter can be operably linked to the portion of the nucleic acid sequence encoding the immunomodulatory protein. In some embodiments, the promotor is a constitutively active promotor in the target cell (such as a tissue-specific constitutively active promotor or other constitutive promotor). For example, the recombinant expression vector may also include, lymphoid tissue-specific transcriptional regulatory elements (TRE) such as a B lymphocyte, T lymphocyte, or dendritic cell specific TRE. Lymphoid tissue specific TRE are known in the art (see, e.g., Thompson et al., Mol. Cell. Biol. 12:1043-53 (1992); Todd et al., J. Exp. Med. 177: 1663-74 (1993); Penix et al., J. Exp. Med. 178:1483-96 (1993)). In some embodiments, the promotor is an inducible promotor, which may be responsive to an inducing agent (such as a T cell activation signal). In some embodiments, nucleic acids delivered to the target cell in the subject, e.g., tumor cell, immune cell and/or APC, can be operably linked to any of the regulatory elements described above.

[0373] In some embodiments, the vector is a bacterial vector, e.g., a bacterial plasmid or cosmid. In some embodiments, the bacterial vector is delivered to the target cell, e.g., tumor cells, immune cells and/or APCs, via bacterial-mediated transfer of plasmid DNA to mammalian cells (also referred to as "bactofection"). In some embodiments, the delivered bacterial vector also contains appropriate expression control sequences for expression in the target cells, such as a promoter sequence and/or enhancer sequences, or any regulatory or control sequences described above. In some embodiments, the bacterial vector contains appropriate expression control sequences for expression and/or secretion of the encoded variant polypeptides in the infectious agent, e.g., the bacterium.

[0374] In some embodiments, polypeptides provided herein can also be made by synthetic methods. Solid phase synthesis is the preferred technique of making individual peptides since it is the most cost-effective method of making small peptides. For example, well known solid phase synthesis techniques include the use of protecting groups, linkers, and solid phase supports, as well as specific protection and deprotection reaction conditions, linker cleavage conditions, use of scavengers, and other aspects of solid phase peptide synthesis. Peptides can then be assembled into the polypeptides as provided herein.

IV. Methods of Assessing Activity Immune Modulation of Variant CD155 Polypeptides and Immunomodulatory Proteins

[0375] In some embodiments, the variant CD155 polypeptides provided herein (e.g. full-length and/or specific binding fragments or conjugates, stack constructs or fusion thereof or engineered cells) exhibit immunomodulatory activity to modulate T cell activation. In some embodiments, CD155 polypeptides modulate IFN-gamma expression in a T cell assay relative to a wild-type or unmodified CD155 control. In some cases, modulation of IFN-gamma expression can increase or decrease IFN-gamma expression relative to the control. Assays to determine specific binding and IFN-gamma expression are well-known in the art and include the MLR (mixed lymphocyte reaction) assays measuring interferon-gamma cytokine levels in culture supernatants (Wang

et al., Cancer Immunol Res. 2014 September: 2(9):846-56), SEB (staphylococcal enterotoxin B) T cell stimulation assay (Wang et al., Cancer Immunol Res. 2014 September: 2(9): 846-56), and anti-CD3 T cell stimulation assays (Li and Kurlander, J Transl Med. 2010: 8: 104).

[0376] In some embodiments, a variant CD155 polypeptide can in some embodiments increase or, in alternative embodiments, decrease IFN-gamma (interferon-gamma) expression in a primary T-cell assay relative to a wild-type CD155 control. In some embodiments, such activity may depend on whether the variant CD155 polypeptide is provided in a form for antagonist activity or in a form for agonist activity. In some embodiments, a variant CD155 polypeptide or immunomodulatory protein is an antagonist of the inhibitory receptor, such as blocks an inhibitory signal in the cell that may occur to decrease response to activating stimuli, e.g., CD3 and/or CD28 costimulatory signal or a mitogenic signal. Those of skill will recognize that different formats of the primary T-cell assay used to determine an increase or decrease in IFN-gamma expression exist.

[0377] In assaying for the ability of a variant CD155 to increase or decrease IFN-gamma expression in a primary T-cell assay, a Mixed Lymphocyte Reaction (MLR) assay can be used. In some cases, a soluble form of a variant CD155 that preferentially or specifically binds CD226 can be employed to determine the ability of the variant CD155 to antagonize T cells and thereby decrease the IFN-gamma expression in a MLR.

[0378] Alternatively, in some embodiments, a variant CD155 polypeptide or immunomodulatory protein provided in antagonist form, such as soluble form, e.g. variant CD155-Fc or secretable immunomodulatory protein, block activity of the TIGIT inhibitory receptor and thereby increase MLR activity in the assay, such as observed by increased production of IFN-gamma in the assay. Thus, in some embodiments, soluble form of a variant CD155 which preferentially binds TIGIT can be employed to determine the ability of the variant CD155 to block inhibitory signal in T cells and thereby increase the IFN-gamma expression in a MLR.

[0379] In some embodiments, a variant CD155 polypeptide or immunomodulatory protein provided in agonist form, such as a localizing vIgD stack or conjugate containing a tumor-localizing moiety or an engineered cell expressing a transmembrane immunomodulatory protein as provided, may stimulate activity of the TIGIT inhibitory receptor and thereby decrease MLR activity, such as evidenced by decreased IFN-gamma production. In some embodiments, a variant CD155 polypeptide or immunomodulatory protein provided in agonist form, such as a localizing vIgD stack or conjugate containing a tumor-localizing moiety or an engineered cell expressing a transmembrane immunomodulatory protein as provided, may block activity of the TIGIT inhibitory receptor and thereby increase MLR activity, such as increase IFN-gamma production.

[0380] Alternatively, in assaying for the ability of a variant CD155 to modulate an increase or decrease IFN-gamma expression in a primary T-cell assay, a co-immobilization assay can be used. In a co-immobilization assay, a TCR signal, provided in some embodiments by anti-CD3 antibody, is used in conjunction with a co-immobilized variant CD155 to determine the ability to increase or decrease IFN-gamma expression relative to a CD155 unmodified or wild-type control. In some embodiments, a variant CD155 polypeptide or immunomodulatory protein, e.g. a co-immobilized variant CD155 (e.g., CD155-Fc), decreases IFN-gamma production in a co-immobilization assay.

[0381] In some embodiments, in assaying for the ability of a variant CD155 to modulate an increase or decrease IFN-gamma expression a T cell reporter assay can be used. In some embodiments, the T cell is a Jurkat T cell line or is derived from Jurkat T cell lines. In reporter assays, the reporter cell line (e.g. Jurkat reporter cell) also is generated to overexpress an inhibitory receptor that is the cognate binding partner of the variant IgSF domain polypeptide. For example, in the case of a variant CD155, the reporter cell line (e.g. Jurkat reporter cell) is generated to overexpress TIGIT. In some embodiments, the reporter T cells also contain a reporter construct containing an inducible promoter responsive to T cell activation operably linked to a reporter. In some embodiments, the reporter is a fluorescent or luminescent reporter. In some embodiments, the reporter is luciferase. In some embodiments, the promoter is responsive to CD3 signaling. In some embodiments, the promoter is an NFAT promoter. In some embodiments, the promoter is responsive to costimulatory signaling, e.g. CD28 costimulatory signaling. In some embodiments, the promoter is an IL-2 promoter.

[0382] In aspects of a reporter assay, a reporter cell line is stimulated, such as by co-incubation with antigen presenting cells (APCs) expressing the wild-type ligand of the inhibitory receptor, e.g. CD155. In some embodiments, the APCs are artificial APCs. Artificial APCs are well known to a skilled artisan. In some embodiments, artificial APCs are derived from one or more mammalian cell line, such as K562, CHO or 293 cells.

[0383] In some embodiments, the Jurkat reporter cells are co-incubated with artificial APCs overexpressing the inhibitory ligand in the presence of the variant IgSF domain molecule or immunomodulatory protein, e.g., variant CD155 polypeptide or immunomodulatory protein. In some embodiments, reporter expression is monitored, such as by determining the luminescence or fluorescence of the cells. In some embodiments, normal interactions between its inhibitory receptor and ligand result in a repression of or decrease in the reporter signal, such as compared to control, e.g. reporter expression by co-incubation of control T cells and APCs in which the inhibitory receptor and ligand interaction is not present, e.g. APCs that do not overexpress CD155. In some embodiments, a variant CD155 polypeptide or immunomodulatory protein provided herein antagonizes the interaction, e.g. when provided in soluble form as a variant CD155-Fc or when expressed from the APC as a secretable immunomodulatory protein, thereby resulting in an increase in the reporter signal compared to the absence of the variant CD155 polypeptide or immunomodulatory protein. In some cases, certain formats of a variant CD155 polypeptide or immunomodulatory protein as provided herein may provide an agonist activity, thereby decreasing reporter expression compared to the absence of the variant CD155 polypeptide or immunomodulatory protein.,

[0384] Use of proper controls is known to those of skill in the art, however, in the aforementioned embodiments, a control typically involves use of the unmodified CD155, such as a wild-type of native CD155 isoform from the same mammalian species from which the variant CD155 was derived or developed. In some embodiments, the wild-type or native CD155 is of the same form or corresponding form

as the variant. For example, if the variant CD155 is a soluble form containing a variant ECD fused to an Fc protein, then the control is a soluble form containing the wild-type or native ECD of CD155 fused to the Fc protein. Irrespective of whether the binding affinity and/or selectivity to TIGIT is increased or decreased, a variant CD155 in some embodiments will increase IFN-gamma expression and, in alternative embodiments, decrease IFN-gamma expression in a T-cell assay relative to a wild-type CD155 control.

[0385] In some embodiments, a variant CD155 polypeptide or immunomodulatory protein, increases IFN-gamma expression (i.e., protein expression) relative to a wild-type or unmodified CD155 control by at least: 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or higher. In other embodiments, a variant CD155 or immunomodulatory protein decreases IFN-gamma expression (i.e. protein expression) relative to a wild-type or unmodified CD155 control by at least: 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or higher. In some embodiments, the wild-type CD155 control is murine CD155, such as would typically be used for a variant CD155 altered in sequence from that of a wild-type murine CD155 sequence. In some embodiments, the wild-type CD155 control is human CD155, such as would typically be used for a variant CD155 altered in sequence from that of a wild-type human CD155 sequence, such as an CD155 sequence comprising the sequence of amino acids of SEQ ID NO: 47, SEQ ID NO: 58 or SEQ ID NO: 155.

V. Pharmaceutical Formulations

[0386] Provided herein are compositions containing any of the variant CD155 polypeptides, immunomodulatory proteins, conjugates, engineered cells or infectious agents described herein. The pharmaceutical composition can further comprise a pharmaceutically acceptable excipient. For example, the pharmaceutical composition can contain one or more excipients for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. In some aspects, a skilled artisan understands that a pharmaceutical composition containing cells may differ from a pharmaceutical composition containing a protein.

[0387] In some embodiments, the pharmaceutical composition is a solid, such as a powder, capsule, or tablet. For example, the components of the pharmaceutical composition can be lyophilized. In some embodiments, the solid pharmaceutical composition is reconstituted or dissolved in a liquid prior to administration.

[0388] In some embodiments, the pharmaceutical composition is a liquid, for example variant CD155 polypeptides dissolved in an aqueous solution (such as physiological saline or Ringer's solution). In some embodiments, the pH of the pharmaceutical composition is between about 4.0 and about 8.5 (such as between about 4.0 and about 5.0, between about 4.5 and about 5.5, between about 5.0 and about 6.0, between about 5.5 and about 6.5, between about 6.0 and about 7.0, between about 6.5 and about 7.5, between about 7.0 and about 8.0, or between about 7.5 and about 8.5).

[0389] In some embodiments, the pharmaceutical composition comprises a pharmaceutically-acceptable excipient, for example a filler, binder, coating, preservative, lubricant, flavoring agent, sweetening agent, coloring agent, a solvent, a buffering agent, a chelating agent, or stabilizer. Examples of pharmaceutically-acceptable fillers include cellulose, dibasic calcium phosphate, calcium carbonate, microcrystalline cellulose, sucrose, lactose, glucose, mannitol, sorbitol, maltol, pregelatinized starch, corn starch, or potato starch. Examples of pharmaceutically-acceptable binders include polyvinylpyrrolidone, starch, lactose, xylitol, sorbitol, maltitol, gelatin, sucrose, polyethylene glycol, methyl cellulose, or cellulose. Examples of pharmaceutically-acceptable coatings include hydroxypropyl methylcellulose (HPMC), shellac, corn protein zein, or gelatin. Examples of pharmaceutically-acceptable disintegrants include polyvinylpyrrolidone, carboxymethyl cellulose, or sodium starch glycolate. Examples of pharmaceutically-acceptable lubricants include polyethylene glycol, magnesium stearate, or stearic acid. Examples of pharmaceutically-acceptable preservatives include methyl parabens, ethyl parabens, propyl paraben, benzoic acid, or sorbic acid. Examples of pharmaceutically-acceptable sweetening agents include sucrose, saccharine, aspartame, or sorbitol. Examples of pharmaceutically-acceptable buffering agents include carbonates, citrates, gluconates, acetates, phosphates, or tartrates.

[0390] In some embodiments, the pharmaceutical composition further comprises an agent for the controlled or sustained release of the product, such as injectable microspheres, bio-erodible particles, polymeric compounds (polylactic acid, polyglycolic acid), beads, or liposomes.

[0391] In some embodiments, the pharmaceutical composition is sterile. Sterilization may be accomplished by filtration through sterile filtration membranes or radiation. Where the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0392] In some embodiments, provided are pharmaceutical compositions containing the transmembrane immunomodulatory proteins, including engineered cells expressing such transmembrane immunomodulatory proteins. In some embodiments, the pharmaceutical compositions and formulations include one or more optional pharmaceutically acceptable carrier or excipient. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present invention are preferably formulated for intravenous administration.

[0393] In some embodiments, the pharmaceutical composition contains infectious agents containing nucleic acid sequences encoding the immunomodulatory variant polypeptides. In some embodiments, the pharmaceutical composition contains a dose of infectious agents suitable for administration to a subject that is suitable for treatment. In some embodiments, the pharmaceutical composition contains an infectious agent that is a virus, at a single or multiple dosage amount, of between about between or between about 1×10^5 and about 1×10^{12} plaque-forming units (pfu), 1×10^6 and 1×10^{10} pfu, or 1×10^7 and 1×10^{10} pfu, each inclusive, such as at least or at least about or at about 1×10^6, 1×10^7,

1×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, 5×10^9pfu or about 1×10^{10} pfu. In some embodiments, the pharmaceutical composition can contain a virus concentration of from or from about 10^5 to about 10^{10} pfu/mL, for example, 5×10^6 to 5×10^9 or 1×10^7 to 1×10^9 pfu/mL, such as at least or at least about or at about 10^6 pfu/mL, 10^7 pfu/mL, 10^8 pfu/mL or 10^9 pfu/mL. In some embodiments, the pharmaceutical composition contains an infectious agent that is a bacterium, at a single or multiple dosage amount, of between about between or between about 1×10^3 and about 1×10^9 colony-forming units (cfu), 1×10^4 and 1×10^9 cfu, or 1×10^5 and 1×10^7 cfu, each inclusive, such as at least or at least about or at about 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 or 1×10^9 cfu. In some embodiments, the pharmaceutical composition can contain a bacterial concentration of from or from about 10^3 to about 10^8 cfu/mL, for example, 5×10^5 to 5×10^7 or 1×10^6 to 1×10^7 cfu/mL, such as at least or at least about or at about 10^5 cfu/mL, 10^6 cfu/mL, 10^7 cfu/mL or 10^8 cfu/mL

[0394] Such a formulation may, for example, be in a form suitable for intravenous infusion. A pharmaceutically acceptable carrier may be a pharmaceutically acceptable material, composition, or vehicle that is involved in carrying or transporting cells of interest from one tissue, organ, or portion of the body to another tissue, organ, or portion of the body. For example, the carrier may be a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or some combination thereof. Each component of the carrier must be "pharmaceutically acceptable" in that it must be compatible with the other ingredients of the formulation. It also must be suitable for contact with any tissue, organ, or portion of the body that it may encounter, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits.

[0395] In some embodiments, the pharmaceutical composition is administered to a subject. Generally, dosages and routes of administration of the pharmaceutical composition are determined according to the size and condition of the subject, according to standard pharmaceutical practice. For example, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models such as mice, rats, rabbits, dogs, pigs, or monkeys. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. The exact dosage will be determined in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active compound or to maintain the desired effect. Factors that may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy.

[0396] Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation. The frequency of dosing will depend upon the pharmacokinetic parameters of the molecule in the formulation used. Typically, a composition is administered until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as multiple doses (at the same or different concentrations/dosages) over time, or as a continuous infusion. Further refinement of the appropriate dosage is routinely made. Appropriate dosages may be ascertained through use of appropriate dose-response data. A number of biomarkers or physiological markers for therapeutic effect can be monitored including T cell activation or proliferation, cytokine synthesis or production (e.g., production of TNF-α, IFN-γ, IL-2), induction of various activation markers (e.g., CD25, IL-2 receptor), inflammation, joint swelling or tenderness, serum level of C-reactive protein, anti-collagen antibody production, and/or T cell-dependent antibody response(s).

[0397] In some embodiments, the pharmaceutical composition is administered to a subject through any route, including orally, transdermally, by inhalation, intravenously, intra-arterially, intramuscularly, direct application to a wound site, application to a surgical site, intraperitoneally, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intrapleurally, intraventricularly, intra-articularly, intraocularly, or intraspinally.

[0398] In some embodiments, the dosage of the pharmaceutical composition is a single dose or a repeated dose. In some embodiments, the doses are given to a subject once per day, twice per day, three times per day, or four or more times per day. In some embodiments, about 1 or more (such as about 2 or more, about 3 or more, about 4 or more, about 5 or more, about 6 or more, or about 7 or more) doses are given in a week. In some embodiments, multiple doses are given over the course of days, weeks, months, or years. In some embodiments, a course of treatment is about 1 or more doses (such as about 2 or more does, about 3 or more doses, about 4 or more doses, about 5 or more doses, about 7 or more doses, about 10 or more doses, about 15 or more doses, about 25 or more doses, about 40 or more doses, about 50 or more doses, or about 100 or more doses).

[0399] In some embodiments, an administered dose of the pharmaceutical composition is about 1 μg of protein per kg subject body mass or more (such as about 2 μg of protein per kg subject body mass or more, about 5 μg of protein per kg subject body mass or more, about 10 μg of protein per kg subject body mass or more, about 25 μg of protein per kg subject body mass or more, about 50 μg of protein per kg subject body mass or more, about 100 μg of protein per kg subject body mass or more, about 250 μg of protein per kg subject body mass or more, about 500 pg of protein per kg subject body mass or more, about 1 mg of protein per kg subject body mass or more, about 2 mg of protein per kg subject body mass or more, or about 5 mg of protein per kg subject body mass or more).

[0400] In some embodiments, a therapeutic amount of a cell composition is administered. Typically, precise amount of the compositions of the present invention to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject). It can generally be stated that a pharmaceutical composition comprising engineered cells, e.g. T cells, as described herein may be administered at a dosage of 10^4 to 10^9 cells/kg body weight, such as 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. Engineered cell compositions, such as T cell compositions, may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, e.g., Rosenberg et al, New Eng. J. of Med. 319: 1676, 1988). The optimal dosage and treatment regime for a particular patient

can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

[0401] A variety of means are known for determining if administration of a therapeutic composition of the invention sufficiently modulates immunological activity by eliminating, sequestering, or inactivating immune cells mediating or capable of mediating an undesired immune response; inducing, generating, or turning on immune cells that mediate or are capable of mediating a protective immune response; changing the physical or functional properties of immune cells; or a combination of these effects. Examples of measurements of the modulation of immunological activity include, but are not limited to, examination of the presence or absence of immune cell populations (using flow cytometry, immunohistochemistry, histology, electron microscopy, polymerase chain reaction (PCR)); measurement of the functional capacity of immune cells including ability or resistance to proliferate or divide in response to a signal (such as using T-cell proliferation assays and pepscan analysis based on 3H-thymidine incorporation following stimulation with anti-CD3 antibody, anti-T-cell receptor antibody, anti-CD28 antibody, calcium ionophores, PMA (phorbol 12-myristate 13-acetate) antigen presenting cells loaded with a peptide or protein antigen; B cell proliferation assays); measurement of the ability to kill or lyse other cells (such as cytotoxic T cell assays); measurements of the cytokines, chemokines, cell surface molecules, antibodies and other products of the cells (e.g., by flow cytometry, enzyme-linked immunosorbent assays, Western blot analysis, protein microarray analysis, immunoprecipitation analysis); measurement of biochemical markers of activation of immune cells or signaling pathways within immune cells (e.g., Western blot and immunoprecipitation analysis of tyrosine, serine or threonine phosphorylation, polypeptide cleavage, and formation or dissociation of protein complexes; protein array analysis; DNA transcriptional, profiling using DNA arrays or subtractive hybridization); measurements of cell death by apoptosis, necrosis, or other mechanisms (e.g., annexin V staining, TUNEL assays, gel electrophoresis to measure DNA laddering, histology; fluorogenic caspase assays, Western blot analysis of caspase substrates); measurement of the genes, proteins, and other molecules produced by immune cells (e.g., Northern blot analysis, polymerase chain reaction, DNA microarrays, protein microarrays, 2-dimensional gel electrophoresis, Western blot analysis, enzyme linked immunosorbent assays, flow cytometry); and measurement of clinical symptoms or outcomes such as improvement of autoimmune, neurodegenerative, and other diseases involving self-proteins or self-polypeptides (clinical scores, requirements for use of additional therapies, functional status, imaging studies) for example, by measuring relapse rate or disease severity (using clinical scores known to the ordinarily skilled artisan) in the case of multiple sclerosis, measuring blood glucose in the case of type I diabetes, or joint inflammation in the case of rheumatoid arthritis.

VI. Articles of Manufacture and Kits

[0402] Also provided herein are articles of manufacture comprising the pharmaceutical compositions described herein in suitable packaging. Suitable packaging for compositions (such as ophthalmic compositions) described herein are known in the art, and include, for example, vials (such as sealed vials), vessels, ampules, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. These articles of manufacture may further be sterilized and/or sealed.

[0403] Further provided are kits comprising the pharmaceutical compositions (or articles of manufacture) described herein, which may further comprise instruction(s) on methods of using the composition, such as uses described herein. The kits described herein may also include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein.

VII. Therapeutic Applications

[0404] The pharmaceutical compositions described herein (including pharmaceutical composition comprising the variant CD155 polypeptides, the immunomodulatory proteins, the conjugates, and the engineered cells described herein) can be used in a variety of therapeutic applications, such as the treatment of a disease. For example, in some embodiments the pharmaceutical composition is used to treat inflammatory or autoimmune disorders, cancer, organ transplantation, viral infections, and/or bacterial infections in a mammal. The pharmaceutical composition can modulate an immune response to treat the disease.

[0405] In some embodiments, the provided methods are applicable to therapeutic administration of variant CD155 polypeptides, the immunomodulatory proteins, the conjugates, the engineered cells and infectious agents described herein. It is within the level of a skilled artisan, in view of the provided disclosure, to choose a format for the indication depending on the type of modulation of the immune response, e.g. increase or decrease that is desired.

[0406] In some embodiments, a pharmaceutical composition provided herein that stimulates the immune response is administered, which can be useful, for example, in the treatment of cancer, viral infections, or bacterial infections. In some embodiments, the pharmaceutical composition contains a variant CD155 polypeptide in a format that exhibits antagonist activity of its cognate binding partner TIGIT and/or that inhibits signaling via TIGIT. Exemplary formats of CD155 polypeptide for use in connection with such therapeutic applications include, for example, a variant CD155 polypeptide that is soluble (e.g. variant CD155-Fc fusion protein), an immunomodulatory protein or "stack" of a variant CD155 polypeptide and another IgSF domain, including soluble forms thereof that are Fc fusions, an engineered cell expressing a secretable immunomodulatory protein, or an infectious agent comprising a nucleic acid molecule encoding a secretable immunomodulatory protein, such as for expression and secretion of the secretable immunomodulatory protein in an infected cell (e.g. tumor cell or APC, e.g. dendritic cell).

[0407] In some embodiments, the pharmaceutical composition can be used to inhibit growth of mammalian cancer cells (such as human cancer cells). A method of treating cancer can include administering an effective amount of any of the pharmaceutical compositions described herein to a subject with cancer. The effective amount of the pharmaceutical composition can be administered to inhibit, halt, or reverse progression of cancers, including cancers that are sensitive to modulation of immunological activity, such as by the provided variants or immunomodulatory proteins.

Human cancer cells can be treated in vivo, or ex vivo. In ex vivo treatment of a human patient, tissue or fluids containing cancer cells are treated outside the body and then the tissue or fluids are reintroduced back into the patient. In some embodiments, the cancer is treated in a human patient in vivo by administration of the therapeutic composition into the patient. Thus, the present invention provides ex vivo and in vivo methods to inhibit, halt, or reverse progression of the tumor, or otherwise result in a statistically significant increase in progression-free survival (i.e., the length of time during and after treatment in which a patient is living with cancer that does not get worse), or overall survival (also called "survival rate;" i.e., the percentage of people in a study or treatment group who are alive for a certain period of time after they were diagnosed with or treated for cancer) relative to treatment with a control.

[0408] The cancers that can be treated by the pharmaceutical compositions and the treatment methods described herein include, but are not limited to, melanoma, bladder cancer, hematological malignancies (leukemia, lymphoma, myeloma), liver cancer, brain cancer, renal cancer, breast cancer, pancreatic cancer (adenocarcinoma), colorectal cancer, lung cancer (small cell lung cancer and non-small-cell lung cancer), spleen cancer, cancer of the thymus or blood cells (i.e., leukemia), prostate cancer, testicular cancer, ovarian cancer, uterine cancer, gastric carcinoma, a musculoskeletal cancer, a head and neck cancer, a gastrointestinal cancer, a germ cell cancer, or an endocrine and neuroendocrine cancer. In some embodiments, the cancer is Ewing's sarcoma. In some embodiments, the cancer is selected from melanoma, lung cancer, bladder cancer, and a hematological malignancy. In some embodiments, the cancer is a lymphoma, lymphoid leukemia, myeloid leukemia, cervical cancer, neuroblastoma, or multiple myeloma.

[0409] In some embodiments, the pharmaceutical composition is administered as a monotherapy (i.e., as a single agent) or as a combination therapy (i.e., in combination with one or more additional anticancer agents, such as a chemotherapeutic drug, a cancer vaccine, or an immune checkpoint inhibitor. In some embodiments, the pharmaceutical composition can also be administered with radiation therapy. In some aspects of the present disclosure, the immune checkpoint inhibitor is nivolumab, tremelimumab, pembrolizumab, ipilimumab, or the like.

[0410] In some embodiments, the pharmaceutical composition suppresses an immune response, which can be useful in the treatment of inflammatory or autoimmune disorders, or organ transplantation. In some embodiments, the pharmaceutical composition contains a variant CD155 polypeptide in a format that exhibits agonist activity of its cognate binding partner TIGIT and/or that stimulates inhibitory signaling via TIGIT. Exemplary formats of a CD155 polypeptide for use in connection with such therapeutic applications include, for example, an immunomodulatory protein or "stack" of a variant CD155 polypeptide and an IgSF domain or variant thereof that localizes to a cell or tissue of an inflammatory environment, a conjugate containing a variant CD155 polypeptide linked to a moiety that localizes to a cell or tissue of an inflammatory environment, an engineered cell expressing a transmembrane immunomodulatory protein, or an infectious agent comprising a nucleic acid molecule encoding a transmembrane immunomodulatory protein, such as for expression of the transmembrane immunomodulatory protein in an infected cell.

[0411] In some embodiments, the inflammatory or autoimmune disorder is antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, a vasculitis, an autoimmune skin disease, transplantation, a Rheumatic disease, an inflammatory gastrointestinal disease, an inflammatory eye disease, an inflammatory neurological disease, an inflammatory pulmonary disease, an inflammatory endocrine disease, or an autoimmune hematological disease.

[0412] In some embodiments, the inflammatory and autoimmune disorders that can be treated by the pharmaceutical composition described herein is Addison's Disease, allergies, alopecia areata, Alzheimer's, antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, ankylosing spondylitis, antiphospholipid syndrome (Hughes Syndrome), asthma, atherosclerosis, rheumatoid arthritis, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, autoimmune lymphoproliferative syndrome, autoimmune myocarditis, autoimmune oophoritis, autoimmune orchitis, azoospermia, Behcet's Disease, Berger's Disease, bullous pemphigoid, cardiomyopathy, cardiovascular disease, celiac Sprue/coeliac disease, chronic fatigue immune dysfunction syndrome (CFIDS), chronic idiopathic polyneuritis, chronic inflammatory demyelinating, polyradicalneuropathy (CIDP), chronic relapsing polyneuropathy (Guillain-Barré syndrome), Churg-Strauss Syndrome (CSS), cicatricial pemphigoid, cold agglutinin disease (CAD), COPD (chronic obstructive pulmonary disease), CREST syndrome, Crohn's disease, dermatitis, herpetiformus, dermatomyositis, diabetes, discoid lupus, eczema, epidermolysis bullosa acquisita, essential mixed cryoglobulinemia, Evan's Syndrome, exopthalmos, fibromyalgia, Goodpasture's Syndrome, Graves' Disease, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA nephropathy, immunoproliferative disease or disorder, inflammatory bowel disease (IBD), interstitial lung disease, juvenile arthritis, juvenile idiopathic arthritis (JIA), Kawasaki's Disease, Lambert-Eaton Myasthenic Syndrome, lichen planus, lupus nephritis, lymphocytic hypophysitis, Meniere's Disease, Miller Fish Syndrome/acute disseminated encephalomyeloradiculopathy, mixed connective tissue disease, multiple sclerosis (MS), muscular rheumatism, myalgic encephalomyelitis (ME), myasthenia gravis, ocular inflammation, *Pemphigus foliaceus*, pemphigus vulgaris, pernicious anaemia, polyarteritis nodosa, polychondritis, polyglandular syndromes (Whitaker's syndrome), polymyalgia rheumatica, polymyositis, primary agammaglobulinemia, primary biliary cirrhosis/autoimmune cholangiopathy, psoriasis, psoriatic arthritis, Raynaud's Phenomenon, Reiter's Syndrome/reactive arthritis, restenosis, rheumatic fever, rheumatic disease, sarcoidosis, Schmidt's syndrome, scleroderma, Sjörgen's Syndrome, stiff-man syndrome, systemic lupus erythematosus (SLE), systemic scleroderma, Takayasu arteritis, temporal arteritis/giant cell arteritis, thyroiditis, Type 1 diabetes, ulcerative colitis, uveitis, vasculitis, vitiligo, interstitial bowel disease or Wegener's Granulomatosis. In some embodiments, the inflammatory or autoimmune disorder is selected from interstitial bowel disease, transplant, Crohn's disease, ulcerative colitis, multiple sclerosis, asthma, rheumatoid arthritis, and psoriasis.

[0413] In some embodiments, the pharmaceutical composition is administered to modulate an autoimmune condition. For example, suppressing an immune response can be beneficial in methods for inhibiting rejection of a tissue, cell, or

organ transplant from a donor by a recipient. Accordingly, in some embodiments, the pharmaceutical compositions described herein are used to limit or prevent graft-related or transplant related diseases or disorders, such as graft versus host disease (GVHD). In some embodiments, the pharmaceutical compositions are used to suppress autoimmune rejection of transplanted or grafted bone marrow, organs, skin, muscle, neurons, islets, or parenchymal cells.

[0414] Pharmaceutical compositions comprising engineered cells and the methods described herein can be used in adoptive cell transfer applications. In some embodiments, cell compositions comprising engineered cells can be used in associated methods to, for example, modulate immunological activity in an immunotherapy approach to the treatment of, for example, a mammalian cancer or, in other embodiments the treatment of autoimmune disorders. The methods employed generally comprise a method of contacting a TIP of the present invention with a mammalian cell under conditions that are permissive to specific binding of the affinity modified IgSF domain and modulation of the immunological activity of the mammalian cell. In some embodiments, immune cells (such as tumor infiltrating lymphocytes (TILs), T-cells (including CD8+ or CD4+ T-cells), or APCs) are engineered to express as a membrane protein and/or as a soluble variant CD155 polypeptide, immunomodulatory protein, or conjugate as described herein. The engineered cells can then be contact a mammalian cell, such as an APC, a second lymphocyte or tumor cell in which modulation of immunological activity is desired under conditions that are permissive of specific binding of the affinity modified IgSF domain to a counter-structure on the mammalian cell such that immunological activity can be modulated in the mammalian cell. Cells can be contacted in vivo or ex vivo.

[0415] In some embodiments, the engineered cells are autologous cells. In other embodiments, the cells are allogeneic. In some embodiments, the cells are autologous engineered cells reinfused into the mammal from which it was isolated. In some embodiments, the cells are allogenic engineered cells infused into the mammal. In some embodiments, the cells are harvested from a patient's blood or tumor, engineered to express a polypeptide (such as the variant CD155 polypeptide, immunomodulatory protein, or conjugate as described herein), expanded in an in vitro culture system (for example, by stimulating the cells), and reinfused into the patient to mediate tumor destruction. In some embodiments, the methods is conducted by adoptive cell transfer wherein cells expressing the TIP (e.g., a T-cell) are infused back into the patient. In some embodiments, the therapeutic compositions and methods of the invention are used in the treatment of a mammalian patient of cancers such as lymphoma, lymphoid leukemia, myeloid leukemia, cervical cancer, neuroblastoma, or multiple myeloma.

VIII. Exemplary Embodiments

[0416] Among the provided embodiments are:

[0417] 1. A variant CD155 polypeptide, comprising an IgV domain or a specific binding fragment thereof, an IgC domain or a specific binding fragment thereof, or both, wherein the variant CD155 polypeptide comprises one or more amino acid modifications in an unmodified CD155 or a specific binding fragment thereof corresponding to position(s) selected from 7, 8, 9, 10, 11, 12, 13, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 67, 68, 69, 70, 72, 73, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 87, 88, 89, 90, 91, 92, 94, 95, 96, 97, 98, 99, 100, 102, 104, 106, 107, 108, 110, 111, 112, 113, 114, 115, or 116 with reference to positions set forth in SEQ ID NO:47.

[0418] 2. The variant CD155 polypeptide of embodiment 1, wherein the amino acid modifications comprise amino acid substitutions, deletions or insertions.

[0419] 3. The variant CD155 polypeptide of embodiment 1 or embodiment 2, wherein the unmodified CD155 is a mammalian CD155 or a specific binding fragment thereof.

[0420] 4. The variant CD155 polypeptide of embodiment 3, wherein the unmodified CD155 is a human CD155 or a specific binding fragment thereof.

[0421] 5. The variant CD155 polypeptide of any one of embodiments 1-4, wherein the unmodified CD155 comprises (i) the sequence of amino acids set forth in SEQ ID NO:47, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:47; or (iii) a portion thereof comprising an IgV domain or specific binding fragment thereof.

[0422] 6. The variant CD155 polypeptide of any one of embodiments 1-5, wherein:

[0423] the specific binding fragment of the IgV domain has a length of at least 50, 60, 70, 80, 90, 100, 110 or more amino acids; or

[0424] the specific binding fragment of the IgV domain comprises a length that is at least 80% of the length of the IgV domain set forth as amino acids 24-139 of SEQ ID NO:20.

[0425] 7. The variant CD155 polypeptide of any one of embodiments 1-6, wherein the variant CD155 comprises up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acid modifications, optionally amino acid substitutions, insertions and/or deletions.

[0426] 8. The variant CD155 of any of embodiments 1-7, wherein the variant CD155 comprises a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:47 or a specific binding fragment thereof.

[0427] 9. The variant CD155 polypeptide of any of embodiments 1-8, wherein the variant CD155 polypeptide exhibits altered binding to the ectodomain of TIGIT, CD226 or CD96 compared to the unmodified CD155.

[0428] 10. The variant CD155 polypeptide of any of embodiments 1-9, wherein the variant CD155 polypeptide exhibits altered binding to the ectodomain of TIGIT or CD226 compared to the unmodified CD155.

[0429] 11. The variant CD155 polypeptide of embodiment 9 or embodiment 10, wherein the altered binding is altered binding affinity and/or altered binding selectivity.

[0430] 12. The variant CD155 polypeptide of any of embodiments 1-11, wherein the one or more amino acid modifications are selected from G7E, D8G, V9A, V9D, V9I, V9L, V10F, V10G, V10I, V11A, V11E, V11M, Q12H, Q12K, Q12L, A13E, A13R, T15I, T15S, Q16H, P18C, P18F, P18H, P18L, P18S, P18T, P18Y, G19D,

F20I, F20S, F20Y, L21S, L21M, G22S, D23A, D23G, D23N, D23Y, S24A, S24P, V25A, V25E, T26M, C29R, Y30C, Y30F, Y30H, Q32L, Q32R, V33M, P34S, N35D, N35F, N35S, M36I, M36R, M36T, E37G, E37P, E37S, E37V, V38A, V38G, T39A, T39S, H40Q, H40R, H40T, V41A, V41M, S42A, S42C, S42G, S42L, S42N, S42P, S42Q, S42T, S42V, S42W, L44P, L44V, T45A, T45G, T45I, T45S, T45Q, T45V, W46C, W46R, A47E, A47G, A47V, R48Q, H49L, H49Q, H49R, G50S, E51G, E51K, E51V, S52A, S52E, S52G, S52K, S52L, S52M, S52P, S52Q, S52R, S52T, S52W, G53R, S54C, S54G, S54H, S54N, S54R, M55I, M55L, M55V, A56V, V57A, V57L, V57T, F58L, F58Y, H59E, H59N, N59R, Q60H, Q60K, Q60P, Q60R, T61A, T61G, T61K, T61M, T61R, T61S, Q62F, Q62H, Q62K, Q62L, Q62M, Q62R, Q62Y, P64S, S65A, S65C, S65G, S65D, S65T, S65Y, S65H, S65N, S65T, S65W, S67A, S67E, S67G, S67H, S67L, S67T, S67V, S67W, E68D, E68G, S69L, S69P, K70E, K70R, K70Q, L72Q, E73D, E73G, E73R, V75A, V75L, A76E, A76G, A76T, A77T, A77V, R78G, R78K, R78S, L79P, L79Q, L79V, G80D, G80S, A81E, A81P, A81T, A81V, E82D, E82G, L83P, L83Q, R84W, N85D, N85Y, N87T, L88P, R89K, M90I, M90L, M90V, F91S, F91T, F91P, G92A, G92E, G92W, R94H, V95A, E96D, D97G, E98D, E98S, G99D, G99Y, N100Y, T102S, L104E, L104M, L104N, L104P, L104Q, L104T, L104Y, V106A, V1061, V106L, T107A, T107L, T107M, T107S, T107V, F108H, F108L, F108Y, Q110R, G111D, G111R, S112I, S112N, S112V, R113G, R113W, S114N, S114T, V115A, V115M, D116G, or D116N, or a conservative amino acid substitution thereof.

[0431] 13. The variant CD155 polypeptide of any of embodiments 1-12, comprising one or more amino acid modifications selected from P18S/P64S/F91S, P18S/F91S/L104P, P18L/L79V/F91S, P18S/F91S, P18T/F91S, P18T/S42P/F91S, G7E/P18T/Y30C/F91S, P18T/F91S/G111D, P18S/F91P, P18T/F91S/F108L, P18S/F91S, P18T/T45A/F91S, P18T/F91S/R94H, P18S/Y30C/F91S, A81V/L83P, A13E/P18S/A56V/F91S, P18T/F91S/V115A, P18T/Q60K, S52M, T45Q/S52L/L104E/G111R, S42G, Q62F, S52Q, S42A/L104Q/G111R, S42A/S52Q/L104Q/G111R, S52W/L104E, S42C, S52W, S52M/L104Q, S42L/S52L/Q62F/L104Q, S42W, S42Q, S52L, S52R, L104E, G111R, S52E, Q62Y, T45Q/S52M/L104E, S42N/L104Q/G111R, S52M/V57L, S42N/S52Q/Q62F, S42A/S52L/L104E/G111R, S42W/S52Q/V57L/Q62Y, L104Q, S42L/S52Q/L104E, S42C/S52L, S42W/S52R/Q62Y/L104Q, T45Q/S52R/L104E, S52R/Q62F/L104Q/G111R, T45Q/S52L/V57L/L104E, S52M/Q62Y, Q62F/L104E/G111R, T45Q/S52Q, S52L/L104E, S42V/S52E, T45Q/S52R/G111R, S42G/S52Q/L104E/G111R, S42N/S52E/V57L/L104E, S42C/S52M/Q62F, S42L, S42A, S42G/S52L/Q62F/L104Q, S42N, P18T/S65A/S67V/F91S, P18F/T39A/T45Q/T61R/S65N/S67L/E73G/R78G, P18T/T45Q/T61R/S65N/S67L, P18F/S65A/S67V/F91S, P18F/T45Q/T61R/S65N/S67L/F91S/L104P, P18S/L79P/L104M, P18S/L104M, L79P/L104M, P18T/T45Q/L79P, P18T/T45Q/T61R/S65H/S67H, P18T/A81E, P18S/D23Y/E37P/S52G/Q62M/G80S/A81P/G99Y/S 112N, A13R/D23Y/E37P/S42P/Q62Y/A81E, A13R/D23Y/E37P/G99Y/S112N, A13R/D23Y/E37P/Q62M/A77V/G80S/A81P/G99Y, P18L/E37S/Q62M/G80S/A81P/G99Y/S112N, P18S/L104T, P18S/Q62H/L79Q/F91S, T45Q/S52K/Q62F/L104Q/G111R, T45Q/S52Q/Q62Y/L104Q/G111R, T45Q/S52Q/Q62Y/L104E/G111R, V57A/T61M/S65W/S67A/E96D/L104T, P18L/V57T/T61S/S65Y/S67A/L104T, P18T/T45Q, P18L/V57A/T61M/S65W/S67A/L104T, T61M/S65W/S67A/L104T, P18S/V41A/S42G/T45G/L104N, P18H/S42G/T45I/S52T/G53R/S54H/V57L/H59E/T61S/S65D/E68G/L104N, P18S/S42G/T45V/F58L/S67W/L104N, P18S/T45I/L104N, P18S/S42G/T45G/L104N/V106A, P18H/H40R/S42G/T45I/S52T/G53R/S54H/V57L/H59E/T61S/S65D/E68G/L104Y/V106L/F108H, E37V/S42G/T45G/L104N, P18S/T45Q/L79P/L104T, P18L/Q62R, A13R/D23Y/E37P/S42L/S52G/Q62Y/A81E, P18L/H49R/L104T/D116N, A13R/D23Y/E37P/Q62M/G80S/A81P/L104T, S65T/L104T, A13R/D23Y/E37P/S52G/V57A/Q62M/K70E/L104T, P18L/A47V/Q62Y/E73D/L104T, H40T/V411M/A47V/S52Q/Q62L/S65T/E73R/D97G/E98S/L104T/D116N, P18L/S42P/T45Q/T61G/S65H/S67E/L104T/D116N, P18S/H40T/V41M/A47V/S52Q/Q62L/S65T/E73R/L104M/V106A, H40T/V41M/A47V/S52Q/Q62L/S65T/E68G/E73R/D97G/E98S/L104T, T45Q/S52E/L104E, T45Q/S52E/Q62F/L104E, P18F/T26M/L44V/Q62K/L79P/F91S/L104M/G111D, P18S/T45S/T61K/S65W/S67A/F91S/G111R, P18S/L79P/L104M/T107M, P18S/S65W/S67A/M90V/V95A/L104Q/G111R, P18S/A47G/L79P/F91S/L104M/T107A/R113W, P18T/D23G/S24A/N35D/H49L/L79P/F91S/L104M/G111R, V9L/P18S/Q60R/V75L/L79P/R89K/F91S/L104E/G111R, P18S/H49R/E73D/L79P/N85D/F91S/V95A/L104M/G111R, V11A/P18S/L79P/F91S/L104M/G111R, V11A/P18S/S54R/Q60P/Q62K/L79P/N85D/F91S/T107M, P18T/S52P/S65A/S67V/L79P/F91S/L104M/G111R, P18T/M36T/L79P/F91S/G111R, D8G/P18S/M36I/V38A/H49Q/A76E/F91S/L104M/T107A/R113W, P18S/S52P/S65A/S67V/L79P/F91S/L104M/T107S/R113W, T15I/P18T/L79P/F91S/L104M/G111R, P18F/T26M/L44V/Q62K/L79P/E82D/F91S/L104M/G111D, P18T/E37G/G53R/Q62K/L79P/F91S/E98D/L104M/T107M, P18L/K70E/L79P/F91S/V95A/G111R, V9I/Q12K/P18F/S65A/S67V/L79P/L104T/G111R/S112I, P18F/S65A/S67V/F91S/L104M/G111R, V9I/V10I/P18S/F20S/T45A/L79P/F91S/L104M/F108Y/G111R/S112V, V9L/P18L/L79P/M90I/F91S/T102S/L104M/G111R, P18C/T26M/L44V/M55I/Q62K/L79P/F91S/L104M/T107M, V9I/P18T/D23G/L79P/F91S/G111R, P18F/L79P/M90L/F91S/V95A/L104M/G111R, P18T/M36T/S65A/S67E/L79Q/A81T/F91S/G111R, V9L/P18T/Q62R/L79P/F91S/L104M/G111R, P18S/S65W/S67A/L104Q/G111R, P18T/G19D/M36T/S54N/L79P/L83Q/F91S/T107M/F108Y, V9L/P18L/M55V/S69L/L79P/A81E/F91S/T107M, P18F/H40Q/T61K/Q62K/L79P/F91S/L104M/T107V, P18S/Q32R/Q62K/R78G/L79P/F91S/T107A/R113W, Q12H/P18T/L21S/G22S/V57A/Q62R/L79P/F91S/T107M, V9I/P18S/S24P/H49Q/F58Y/Q60R/Q62K/L79P/F91S/T107M, P18T/W46C/H49R/S65A/S67V/A76T/L79P/S87T/L104M, P18S/S42T/E51G/L79P/F91S/G92W/T107M, V10F/T15S/P18L/R48Q/L79P/F91S/T107MIV 115M, P18S/L21M/Y30F/N35D/R84W/F91S/T107M/D 116G, P18F/E51V/S54G/Q60R/L79Q/E82G/S87T/M901/

F91S/G92R/T107M, Q16H/P18F/F91S/T107M, P18T/D23G/Q60R/S67L/L79P/F91S/T107M/V115A, D8G/V9I/V11A/P18T/T26M/S52P/L79P/F91S/G92A/T107L/V115A, V9I/P18F/A47E/G50S/E68G/L79P/F91S/T107M, P18S/M55I/Q62K/S69P/L79P/F91S/T107M, P18T/T39S/S52P/S54R/L79P/F91S/T107M, P18S/D23N/L79P/F91S/T107M/S114N, P18S/P34S/E51V/L79P/F91S/G111R, P18S/H59N/V75A/L79P/A81T/F91S/L104M/T107M, P18S/W46R/E68D/L79P/F91S/T107M/R113G, V9L/P18F/T45A/S65A/S67V/R78K/L79V/F91S/T107M/S114T, P18T/M55L/T61R/L79P/F91S/V106I/T107M, T15I/P18S/V33M/N35F/T39S/M55L/R78S/L79P/F91S/T107M, P18S/Q62K/K70E/L79P/F91S/G92E/R113W, P18F/F20I/T26M/A47V/E51K/L79P/F91S, P18T/D23A/Q60H/L79P/M90V/F91S/T107M, P18S/D23G/C29R/N35D/E37G/M55I/Q62K/S65A/S67G/R78G/L79P/F91S/L104M/T107M/Q110R, A13E/P18S/M36R/Q62K/S67T/L79P/N85D/F91S/T107M, V9I/P18T/H49R/L79P/N85D/F91S/L104T/T107M, V9A/P18F/T61S/Q62L/L79P/F91S/G111R, D8E/P18T/T61A/L79P/F91S/T107M, P18S/V41A/H49R/S54C/L79S/N85Y/L88P/F91S/L104M/T107M, V 11E/P18H/F20Y/V25E/N35S/H49R/L79P/F91S/T107M/G111R, V11A/P18F/D23A/L79P/G80D/V95A/T107M, P18S/K70R/L79P/F91S/G111R, V9L/V11M/P18S/N35S/S54G/Q62K/L79P/L104M/T107M/V115M, V9L/P18Y/V25A/V38G/M55V/A77T/L79P/M90I/F91S/L104M, V10G/P18T/L72Q/L79P/F91S/T107M, P18S/H59R/A76G/R78S/L79P, V9A/P18S/M36T/S65G/L79P/F91S/L104T/G111R/S112I, P18T/S52A/V57A/Q60R/Q62K/S65C/L79P/F91T/N100Y/T107M, V11A/P18F/N35D/A47E/Q62K/L79P/F91S/G99D/T107M/S114N, V11A/P18T/N35S/L79P/S87T/F91S, V9D/V11M/Q12L/P18S/E37V/M55I/Q60R/K70Q/L79P/F91S/L104M/T107M, or T15S/P18S/Y30H/Q32L/Q62R/L79P/F91S/T107M.

[0432] 14. The variant CD155 polypeptide of any of embodiments 1-13, wherein the variant CD155 polypeptide comprises one IgC domain or two IgC domains or a specific fragment thereof.

[0433] 15. The variant CD155 polypeptide of any of embodiments 1-13, comprising the sequence of amino acids set forth in any of SEQ ID NOS: 59-80, 178-274, 1230-1252, 1269, and 1610-1655 or a specific binding fragment thereof, or a sequence of amino acids that exhibits at least 95% sequence identity to any of SEQ ID NOS: 59-80, 178-274, 1230-1252, 1269, and 1610-1655 or a specific binding fragment thereof and that contains the one or more of the amino acid modifications.

[0434] 16. The variant CD155 polypeptide of any of embodiments 1-15, wherein the unmodified CD155 comprises an IgV domain or specific binding fragment thereof comprising (i) the sequence of amino acids set forth in SEQ ID NO:58 or 155, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:58 or 155; or (iii) a portion thereof comprising a specific binding fragment thereof.

[0435] 17. The variant CD155 polypeptide of any of embodiments 1-16, wherein the variant CD155 polypeptide comprises the IgV domain or a specific binding fragment thereof.

[0436] 18. The variant CD155 polypeptide of any of embodiments 1-17, wherein the IgV domain or specific binding fragment thereof is the only CD155 portion of the variant CD155 polypeptide.

[0437] 19. The variant CD155 polypeptide of any of embodiments 1-18, comprising the sequence of amino acids set forth in any of SEQ ID NOS: 81-102, 156-177, 275-468, 1184-1229, 1270-1271, 1656-1747 or a specific binding fragment thereof, a sequence of amino acids that exhibits at least 95% sequence identity to any of SEQ ID NOS: 81-102, 156-177, 275-468, 1184-1229, 1270-1271, 1656-1747 or a specific binding fragment thereof and that contains the one or more of the amino acid modifications.

[0438] 20. The variant CD155 polypeptide of any of embodiments 1-19, wherein the variant CD155 polypeptide specifically binds to the ectodomain of one or more of TIGIT, CD226 or CD96 with increased affinity compared to the unmodified CD155.

[0439] 21. The variant CD155 polypeptide of any of embodiments 1-20, wherein the variant CD155 polypeptide specifically binds to the ectodomain of TIGIT or CD226 with increased affinity compared to the unmodified CD155.

[0440] 22. The variant CD155 polypeptide of any of embodiments 1-21, wherein the variant CD155 polypeptide specifically binds to the ectodomain of TIGIT and the ectodomain of CD226 each with increased affinity compared to the unmodified CD155.

[0441] 23. The variant CD155 polypeptide of any of embodiments 1-20, wherein the variant CD155 polypeptide specifically binds to the ectodomain of one or more of TIGIT, CD226 or CD96 with increased affinity and specifically binds to the ectodomain of one or more of the other of TIGIT, CD226 or CD96 with decreased affinity compared to the unmodified CD155.

[0442] 24. The variant CD155 polypeptide of embodiment 23, wherein the variant CD155 polypeptide specifically binds to the ectodomain of TIGIT with increased affinity and specifically binds to the ectodomain of CD226 with decreased affinity compared to the unmodified CD155.

[0443] 25. The variant CD155 polypeptide of any of embodiments 1-24, wherein the variant polypeptide specifically binds to the ectodomain of TIGIT with increased selectivity compared to the unmodified CD155.

[0444] 26. The variant CD155 polypeptide of embodiment 25, wherein the increased selectivity comprises a greater ratio of binding of the variant polypeptide for TIGIT versus CD226 compared to the ratio of binding of the unmodified CD155 polypeptide for TIGIT versus CD226.

[0445] 27. The variant CD155 polypeptide of embodiment 26, wherein the ratio is greater by at least or at least about 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold or more.

[0446] 28. The variant CD155 polypeptide of embodiment 23, wherein the variant CD155 polypeptide specifically binds to the ectodomain of CD226 with increased affinity and specifically binds to the ectodomain of TIGIT with decreased affinity compared to the unmodified CD155.

[0447] 29. The variant CD155 polypeptide of any of embodiments 9-28, wherein the TIGIT is a human TIGIT.

[0448] 30. The variant CD155 polypeptide of any of embodiments 9-29, wherein the CD226 is a human CD226.

[0449] 31. The variant CD155 polypeptide of any of embodiments 9-30, wherein the CD96 is a human CD96.

[0450] 32. The variant CD155 polypeptide of any of embodiments 1-31, wherein the binding activity is altered (increased or decreased) more than 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold 40-fold or 50-fold compared to the unmodified CD155.

[0451] 33. The variant CD155 polypeptide of any of embodiments 1-32 that is a soluble protein.

[0452] 34. The variant CD155 polypeptide of any of embodiments 1-33, wherein the variant CD155 polypeptide is linked to a multimerization domain.

[0453] 35. The variant CD155 polypeptide of embodiment 34, wherein the multimerization domain is an Fe domain or a variant thereof with reduced effector function.

[0454] 36. The variant CD155 polypeptide of any of embodiments 1-35, wherein the variant CD155 polypeptide is linked to a moiety that increases biological half-life of the polypeptide.

[0455] 37. The variant CD155 polypeptide of any of embodiments 1-36, wherein the variant CD155 polypeptide is linked to an Fc domain or a variant thereof with reduced effector function.

[0456] 38. The variant CD155 polypeptide of any of embodiments 37-39, wherein: the Fc domain is mammalian, optionally human; or the variant Fc domain comprises one or more amino acid modifications compared to an unmodified Fc domain that is mammalian, optionally human.

[0457] 39. The variant CD155 polypeptide of any one of embodiments 35, 37 and 38, wherein the Fc domain or variant thereof comprises the sequence of amino acids set forth in SEQ ID NO:56 or SEQ ID NO:57 or a sequence of amino acids that exhibits at least 85% sequence identity to SEQ ID NO:56 or SEQ ID NO:57.

[0458] 40. The variant CD155 polypeptide of any of embodiments 35 and 37-39, wherein the Fc domain comprises one or more amino acid modifications selected from among E233P, L234A, L234V, L235A, L235E, G236del, G237A, S267K, N297G, V302C, and K447del, each by EU numbering.

[0459] 41. The variant CD155 polypeptide of any of embodiments 35 and 37-40, wherein the Fc domain comprises the amino acid modification C220S by EU numbering.

[0460] 42. The variant CD155 polypeptide of any of embodiments 34-41, wherein the variant CD155 polypeptide is linked to the multimerization domain or Fc indirectly via a linker, optionally a G4S linker.

[0461] 43. The variant CD155 polypeptide of any of embodiments 1-17 and 19-32, wherein the variant CD155 polypeptide is a transmembrane immunomodulatory protein further comprising a transmembrane domain linked to the extracellular domain (ECD) or specific binding fragment thereof of the variant CD155 polypeptide.

[0462] 44. The variant CD155 polypeptide of embodiment 3, wherein the transmembrane domain comprises the sequence of amino acids set forth as residues 344-367 of SEQ ID NO:20 or a functional variant thereof that exhibits at least 85% sequence identity to residues 344-367 of SEQ ID NO:20.

[0463] 45. The variant CD155 polypeptide of embodiment 43 or embodiment 44, further comprising a cytoplasmic domain linked to the transmembrane domain.

[0464] 46. The variant CD155 polypeptide of embodiment 47, wherein the cytoplasmic signaling domain comprises the sequence of amino acids set forth as residues 368-417 of SEQ ID NO:20 or a functional variant thereof that exhibits at least 85% sequence identity to residues 368-417 of SEQ ID NO:20.

[0465] 47. The variant CD155 polypeptide of any of embodiments 1-48, wherein the variant CD155 increases IFN-gamma (interferon-gamma) expression relative to the unmodified CD155 in an in vitro primary T-cell assay.

[0466] 48. The variant CD155 polypeptide of any of embodiments 1-48, wherein the variant CD155 decreases IFN-gamma (interferon-gamma) expression relative to the unmodified CD155 in an in vitro primary T-cell assay.

[0467] 49. The variant CD155 polypeptide of any of embodiments 1-50 that is deglycosylated.

[0468] 50. An immunomodulatory protein, comprising the variant CD155 of any of embodiments 1-49 linked, directly or indirectly via a linker, to a second polypeptide comprising an immunoglobulin superfamily (IgSF) domain of an IgSF family member.

[0469] 51. The immunomodulatory protein of embodiment 50, wherein the IgSF domain is an affinity-modified IgSF domain, said affinity-modified IgSF domain comprising one or more amino acid modifications compared to the unmodified or wild-type IgSF domain of the IgSF family member.

[0470] 52. The immunomodulatory protein of embodiment 50 or embodiment 51, wherein the IgSF domain is affinity modified and exhibits altered binding to one or more of its cognate binding partner(s) compared to the unmodified or wild-type IgSF domain of the IgSF family member.

[0471] 53. The immunomodulatory protein of embodiment 52, wherein the IgSF domain exhibits increased binding to one or more of its cognate binding partner(s) compared to the unmodified or wild-type IgSF domain.

[0472] 54. The immunomodulatory protein of any one of embodiments 50-53, wherein the variant CD155 polypeptide is a first variant CD155 polypeptide and the IgSF domain of the second polypeptide is an IgSF domain from a second variant CD155 polypeptide of any of embodiments 1-49, wherein the first and second variant CD155 polypeptides are the same or different.

[0473] 55. The immunomodulatory protein of any one of embodiments 50-54, wherein the variant CD155 polypeptide is capable of specifically binding to TIGIT or CD226 and the IgSF domain is capable of binding to a cognate binding partner other than one specifically bound by the variant CD155 polypeptide.

[0474] 56. The immunomodulatory protein of any of embodiments 50-55, wherein the IgSF domain is from a member of the B7 family.

[0475] 57. The immunomodulatory protein of any of embodiments 50-55, wherein the IgSF domain is a tumor-localizing moiety that binds to a ligand expressed on a tumor or is an inflammatory-localizing moiety that binds to a ligand expressed on a cell or tissue associated with an inflammatory environment.

[0476] 58. The immunomodulatory protein of embodiment 57, wherein the ligand is B7H6.

[0477] 59. The immunomodulatory polypeptide of embodiment 57 or embodiment 58, wherein the IgSF domain is from NKp30.

[0478] 60. The immunomodulatory protein of any embodiments 50-55, wherein the IgSF domain of the second polypeptide is an IgSF domain of a ligand that binds to an inhibitory receptor, or is an affinity-modified IgSF domain thereof.

[0479] 61. The immunomodulatory protein of embodiment 60, wherein the IgSF domain of the second polypeptide is an affinity-modified IgSF domain and the affinity-modified IgSF domain exhibits increased binding affinity and/or binding selectivity for the inhibitory receptor compared to binding of the unmodified IgSF domain to the inhibitory receptor.

[0480] 62. The immunomodulatory protein of embodiment 60 or embodiment 61, wherein: the inhibitory receptor is TIGIT, CD112R, CTLA-4 or PD-1; or the ligand of the inhibitory receptor is CD112, CD80, PD-L1 or PD-L2.

[0481] 63. The immunomodulatory protein of any of embodiments 50-55 and 60-61, wherein the second polypeptide is selected from:

[0482] (i) a variant CD80 polypeptide comprising an IgSF domain set forth in any of SEQ ID NOS: 896-928, 930-968, 970-1002, 1004-1042, 1044-1116;

[0483] (ii) a variant PD-L1 polypeptide comprising an IgSF domain set forth in any of SEQ ID NOS: 470-664, 1753-1755, 1757-2031;

[0484] (iii) a variant PD-L2 polypeptide comprising an IgSF domain set forth in any of SEQ ID NOS: 667-717, 719-725, 727-794, 796-870, 872-895;

[0485] (iv) a variant CD112 polypeptide comprising an IgSF domain set for in any of SEQ ID NOS: 1273-1366, 1368-1609;

[0486] (v) a sequence of amino acids that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 95%, 97%, 98%, 99% or more sequence identity to any of the SEQ ID NOS in (i)-(iv) and that comprises the amino acid modifications, optionally amino acid substitutions, insertions and/or deletions; or

[0487] (vi) a specific binding fragment of any of (i)-(v).

[0488] 64. The immunomodulatory protein of any of embodiments 50-63, further comprising a third polypeptide comprising an IgSF domain of an IgSF family member or an affinity-modified IgSF domain thereof, said affinity-modified IgSF domain comprising one or more amino acid modifications compared to the unmodified or wild-type IgSF domain of the IgSF family member.

[0489] 65. The immunomodulatory protein of embodiment 64, wherein:

[0490] the third polypeptide is the same as the first and/or second polypeptide; or the third polypeptide is different from the first and/or second polypeptide.

[0491] 66. The immunomodulatory protein of embodiment 64 and embodiment 65, wherein the third polypeptide is selected from:

[0492] (i) a variant CD80 polypeptide comprising an IgSF domain set forth in any of SEQ ID NOS: 896-928, 930-968, 970-1002, 1004-1042, 1044-1116;

[0493] (ii) a variant PD-L1 polypeptide comprising an IgSF domain set forth in any of SEQ ID NOS: 470-664, 1753-1755, 1757-2031;

[0494] (iii) a variant PD-L2 polypeptide comprising an IgSF domain set forth in any of SEQ ID NOS: 667-717, 719-725, 727-794, 796-870, 872-895;

[0495] (iv) a variant CD112 polypeptide comprising an IgSF domain set for in any of SEQ ID NOS: 1273-1366, 1368-1609;

[0496] (v) a sequence of amino acids that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 95%, 97%, 98%, 99% or more sequence identity to any of the SEQ ID NOS in (i)-(iv) and that comprises the amino acid modifications, optionally amino acid substitutions, insertions and/or deletions; or

[0497] (vi) a specific binding fragment of any of (i)-(v).

[0498] 67. The immunomodulatory protein of any of embodiments 50-65, wherein the IgSF domain or affinity-modified IgSF domain thereof, optionally of the second or third polypeptide, is or comprises an IgV domain.

[0499] 68. The immunomodulatory protein of any of embodiments 50-67, wherein the variant CD155 polypeptide is or comprises an IgV domain.

[0500] 69. The immunomodulatory protein of any of embodiments 64-68, further comprising at least one additional polypeptide comprising an IgSF domain of an IgSF family member or an affinity-modified IgSF domain thereof, said affinity-modified IgSF domain comprising one or more amino acid modifications compared to the unmodified or wild-type IgSF domain of the IgSF family member.

[0501] 70. The immunomodulatory protein of any of embodiments 50-69, wherein the immunomodulatory protein further comprises a multimerization domain linked to at least one of the variant CD155 polypeptide, or the second polypeptide.

[0502] 71. The immunomodulatory protein of any of embodiments 64-69, wherein the immunomodulatory protein further comprises a multimerization domain linked to at least one of the variant CD155 polypeptide, the second polypeptide and/or the third polypeptide.

[0503] 72. The immunomodulatory protein of any of embodiments 69-71, wherein the multimerization domain is an Fc domain or a variant thereof with reduced effector function.

[0504] 73. The immunomodulatory protein of any of embodiments 69-72, wherein the multimerization domain promotes heterodimer formation.

[0505] 74. An immunomodulatory protein comprising a first variant CD155 polypeptide of any of embodiments 34-42 in which the multimerization domain is a first multimerization domain and a second variant CD155 polypeptide of any of embodiments 34-42 in which the multimerization domain is a second multimerization

domain, wherein the first and second multimerization domains interact to form a multimer containing the first and second variant CD155 polypeptide.

[0506] 75. An immunomodulatory protein comprising the immunomodulatory protein of any of embodiments 70-72, wherein the multimerization domain is a first multimerization domain and interacts with a second multimerization domain to form a multimer comprising the immunomodulatory protein.

[0507] 76. The immunomodulatory protein of embodiment 75, wherein the immunomodulatory protein is a first immunomodulatory protein and a second immunomodulatory protein is linked directly or indirectly via a linker to the second multimerization domain, wherein the multimer comprises the first and second immunomodulatory protein.

[0508] 77. The immunomodulatory protein of embodiment 76, wherein the second immunomodulatory protein is an immunomodulatory protein of any of claims **70-72**.

[0509] 78. The immunomodulatory protein of embodiment 74 or embodiment 75, wherein the multimer is a dimer.

[0510] 79. The immunomodulatory protein of any of embodiments 74-78 that is a homodimer.

[0511] 80. The immunomodulatory protein of any of embodiments 74-79 that is a heterodimer.

[0512] 81. The immunomodulatory protein of any of embodiments 74-80, wherein the first and/or second multimerization domain is an Fc domain or a variant thereof with reduced effector function.

[0513] 82. The immunomodulatory protein of any of embodiments 74-81, wherein the first and second multimerization domain is the same or different.

[0514] 83. A conjugate, comprising a variant CD155 of any of embodiments 1-49 or an immunomodulatory protein of any of embodiments 50-72 linked to a moiety.

[0515] 84. The conjugate of embodiment 83, wherein the moiety is a targeting moiety that specifically binds to a molecule on the surface of a cell.

[0516] 85. The conjugate of embodiment 84, wherein the targeting moiety specifically binds to a molecule on the surface of an immune cell.

[0517] 86. The conjugate of embodiment 85, wherein the immune cell is an antigen presenting cell or a lymphocyte.

[0518] 87. The conjugate of embodiment 86, wherein the targeting moiety is a tumor-localizing moiety that binds to a molecule on the surface of a tumor.

[0519] 88. The conjugate of any of embodiments 83-87, wherein the moiety is a protein, a peptide, nucleic acid, small molecule or nanoparticle.

[0520] 89. The conjugate of any of embodiments 83-88, wherein the moiety is an antibody or antigen-binding fragment.

[0521] 90. The conjugate of any of embodiments 83-89, wherein the conjugate is divalent, tetravalent, hexavalent or octavalent.

[0522] 91. A nucleic acid molecule(s), encoding a variant CD155 polypeptide of any of embodiments 1-49 or an immunomodulatory protein of any of embodiments 50-72.

[0523] 92. The nucleic acid molecule of embodiment 91 that is synthetic nucleic acid.

[0524] 93. The nucleic acid molecule of embodiment 91 or embodiment 92 that is cDNA.

[0525] 94. A vector, comprising the nucleic acid molecule of any of embodiments 91-93.

[0526] 95. The vector of embodiment 94 that is an expression vector.

[0527] 96. The vector of embodiment 94 or embodiment 95, wherein the vector is a mammalian expression vector or a viral vector.

[0528] 97. A cell, comprising the vector of any of embodiments 94-96.

[0529] 98. The cell of embodiment 97 that is a mammalian cell.

[0530] 99. The cell of embodiment 97 or embodiment 98 that is a human cell.

[0531] 100. A method of producing a variant CD155 polypeptide or an immunomodulatory protein, comprising introducing the nucleic acid molecule of any of embodiments 91-93 or vector of any of embodiments 94-96 into a host cell under conditions to express the protein in the cell.

[0532] 101. The method of embodiment 100, further comprising isolating or purifying the variant CD155 polypeptide or immunomodulatory protein from the cell.

[0533] 102. A method of engineering a cell expressing a variant CD155 polypeptide, comprising introducing a nucleic acid molecule encoding the variant CD155 polypeptide of any of embodiments 1-49 or the immunomodulatory protein of any of embodiments 50-82 into a host cell under conditions in which the polypeptide is expressed in the cell.

[0534] 103. An engineered cell, expressing the variant CD155 polypeptide of any of embodiments 1-49 an immunomodulatory protein of any of embodiments 50-82, the nucleic acid molecule of any of embodiments 79-81 or vector of any of embodiments 94-96.

[0535] 104. The engineered cell of embodiment 103, wherein the variant CD155 polypeptide or immunomodulatory protein is encoded by a nucleic acid comprising a sequence of nucleotides encoding a signal peptide.

[0536] 105. The engineered cell of embodiment 103 or embodiment 104, wherein the variant CD155 polypeptide or immunomodulatory protein does not comprise a transmembrane domain and/or is not expressed on the surface of the cell.

[0537] 106. The engineered cell of any of embodiments 103-105, wherein the variant CD155 polypeptide or immunomodulatory protein is secreted from the engineered cell.

[0538] 107. The engineered cell of embodiment 105 or embodiment 106, wherein the engineered cell comprises a variant CD155 polypeptide that comprises a transmembrane domain and/or is the transmembrane immunomodulatory protein of any of embodiments 43-49.

[0539] 108. The engineered cell of any of embodiments 103, 104 and 107, wherein the variant CD155 polypeptide is expressed on the surface of the cell.

[0540] 109. The engineered cell of any of embodiments 103-108, wherein the cell is an immune cell.

[0541] 110. The engineered cell of embodiment 109, wherein the immune cell is an antigen presenting cell (APC) or a lymphocyte.

[0542] 111. The engineered cell of any of embodiments 103-110 that is a primary cell.

[0543] 112. The engineered cell of any of embodiments 103-111, wherein the cell is a mammalian cell.

[0544] 113. The engineered cell of any of embodiments 103-112, wherein the cell is a human cell.

[0545] 114. The engineered cell of any of embodiments 103-113, wherein the lymphocyte is a T cell.

[0546] 115. The engineered cell of embodiment 110, wherein the APC is an artificial APC.

[0547] 116. The engineered cell of any of embodiments 103-115, further comprising a chimeric antigen receptor (CAR) or an engineered T-cell receptor (TCR).

[0548] 117. An infectious agent, comprising a nucleic acid molecule encoding a variant CD155 polypeptide of any of embodiments 1-49 or an immunomodulatory protein of any of embodiments 50-82.

[0549] 118. The infectious agent of embodiment 117, wherein the encoded variant CD155 polypeptide or immunomodulatory protein does not comprise a transmembrane domain and/or is not expressed on the surface of a cell in which it is expressed.

[0550] 119. The infectious agent of embodiment 117 or embodiment 118, wherein the encoded variant CD155 polypeptide or immunomodulatory protein is secreted from a cell in which it is expressed.

[0551] 120. The infectious agent of embodiment 119, wherein the encoded variant CD155 polypeptide comprises a transmembrane domain.

[0552] 121. The infectious agent of embodiment 119 or embodiment 120, wherein the encoded variant CD155 polypeptide is expressed on the surface of a cell in which it is expressed.

[0553] 122. The infectious agent of any of embodiments 117-121, wherein the infectious agent is a bacterium or a virus.

[0554] 123. The infectious agent of embodiment 122, wherein the infectious agent is a virus and the virus is an oncolytic virus.

[0555] 124. The infectious agent of embodiment 123, wherein the oncolytic virus is an adenovirus, adeno-associated virus, herpes virus, Herpes Simplex Virus, Vesticular Stomatic virus, Reovirus, Newcastle Disease virus, parvovirus, measles virus, vesticular stomatitis virus (VSV), Coxsackie virus or a Vaccinia virus.

[0556] 125. The infectious agent of embodiment 123, wherein the virus specifically targets dendritic cells (DCs) and/or is dendritic cell-tropic.

[0557] 126. The infectious agent of embodiment 25, wherein the virus is a lentiviral vector that is pseudotyped with a modified Sindbis virus envelope product.

[0558] 127. The infectious agent of any of embodiments 117-126, further comprising a nucleic acid molecule encoding a further gene product that results in death of a target cell or that can augment or boost an immune response.

[0559] 128. The infectious agent of embodiment 127, wherein the further gene product is selected from an anticancer agent, an anti-metastatic agent, an antiangiogenic agent, an immunomodulatory molecule, an immune checkpoint inhibitor, an antibody, a cytokine, a growth factor, an antigen, a cytotoxic gene product, a pro-apoptotic gene product, an anti-apoptotic gene product, a cell matrix degradative gene, genes for tissue regeneration or reprogramming human somatic cells to pluripotency.

[0560] 129. A pharmaceutical composition, comprising the variant CD155 polypeptide of any of embodiments 1-49, an immunomodulatory protein of any of embodiments 0-82, a conjugate of any of embodiments 83-90, an engineered cell of any of embodiments 103-116 or an infectious agent of any of embodiments 117-128.

[0561] 130. The pharmaceutical composition of embodiment 129, comprising a pharmaceutically acceptable excipient.

[0562] 131. The pharmaceutical composition of embodiment 129 or embodiment 130, wherein the pharmaceutical composition is sterile.

[0563] 132. An article of manufacture comprising the pharmaceutical composition of any of embodiments 129-131 in a vial or container.

[0564] 133. The article of manufacture of embodiment 132, wherein the vial or container is sealed.

[0565] 134. A kit comprising the pharmaceutical composition of any of embodiments 129-131, and instructions for use.

[0566] 135. A kit comprising the article of manufacture of embodiment 133 or embodiment 134, and instructions for use.

[0567] 136. A method of modulating an immune response in a subject, comprising administering the pharmaceutical composition of any of embodiments 129-131 to the subject.

[0568] 137. A method of modulating an immune response in a subject, comprising administering the engineered cells of any of embodiments 103-116.

[0569] 138. The method of embodiment 137, wherein the engineered cells are autologous to the subject.

[0570] 139. The method of embodiment 138, wherein the engineered cells are allogenic to the subject.

[0571] 140. The method of any of embodiments 136-139, wherein modulating the immune response treats a disease or condition in the subject.

[0572] 141. The method of any of embodiments 136-140, wherein the immune response is increased.

[0573] 142. The method of any of embodiments 136, 140 and 141, wherein a variant CD155 polypeptide or immunomodulatory protein that is soluble is administered to the subject.

[0574] 143. The method of embodiment 142, wherein the soluble immunomodulatory protein is an immunomodulatory Fc fusion protein.

[0575] 144. The method of any of embodiments 136 and 140-143, wherein a variant CD155 polypeptide of any of embodiments 1-42 and 47-49, or the immunomodulatory protein of any of embodiments 50-82 is administered to the subject.

[0576] 145. The method of any of embodiments 136-144, wherein an engineered cell comprising a secretable variant CD155 polypeptide is administered to the subject.

[0577] 146. The method of any of embodiments 136-141 and 145, wherein an engineered cell of any of embodiments 103-106 and 109-116 is administered to the subject.

[0578] 147. The method of any of embodiments 136, 140, and 141, wherein an infectious agent encoding a variant CD155 polypeptide that is a secretable immunomodulatory protein is administered to the subject, optionally under conditions in which the infectious agent infects a tumor cell or immune cell and the secretable immunomodulatory protein is secreted from the infected cell.

[0579] 148. The method of any of embodiments 140-147, wherein the disease or condition is a tumor or cancer.

[0580] 149. The method of any one of embodiments 140-148, wherein the disease or condition is selected from melanoma, lung cancer, bladder cancer, a hematological malignancy, liver cancer, brain cancer, renal cancer, breast cancer, pancreatic cancer, colorectal cancer, spleen cancer, prostate cancer, testicular cancer, ovarian cancer, uterine cancer, gastric carcinoma, a musculoskeletal cancer, a head and neck cancer, a gastrointestinal cancer, a germ cell cancer, or an endocrine and neuroendocrine cancer.

[0581] 150. The method of any of embodiments 136-139, wherein the immune response is decreased.

[0582] 151. The method of any of embodiments 136, 140 and 150, wherein an immunomodulatory protein or conjugate comprising a variant CD155 polypeptide linked to a moiety that localizes to a cell or tissue of an inflammatory environment is administered to the subject.

[0583] 152. The method of embodiment 151, wherein the moiety comprises an antibody or an antigen-binding fragment thereof or comprises a second polypeptide comprising a wild-type IgSF domain or variant thereof.

[0584] 153. The method of any of embodiments 136, 140 and 150-152, wherein the immunomodulatory protein of any of embodiments 57-59 or the conjugate of any of embodiments 83-90 is administered to the subject.

[0585] 154. The method of any of embodiments 136-140 and 150, wherein a variant CD155 polypeptide that is a transmembrane immunomodulatory protein is administered to the subject.

[0586] 155. The method of any of embodiments 136-140, 150, and 154, wherein the engineered cell comprising a variant CD155 polypeptide that is a transmembrane immunomodulatory protein of any of embodiments 43-49 is administered to the subject.

[0587] 156. The method of any of embodiments 136, 140 and 150, wherein an infectious agent encoding a variant CD155 polypeptide that is a transmembrane immunomodulatory protein is administered to the subject, optionally under conditions in which the infectious agent infects a cell in the subject and the transmembrane immunomodulatory protein is expressed on the surface of the infected cell.

[0588] 157. The method of any of embodiments 136, 140 and 150-156, wherein the disease or condition is an inflammatory or autoimmune disease or condition.

[0589] 158. The method of any of embodiments 136-140 and 150-157, wherein the disease or condition is an antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, a vasculitis, an autoimmune skin disease, transplantation, a Rheumatic disease, an inflammatory gastrointestinal disease, an inflammatory eye disease, an inflammatory neurological disease, an inflammatory pulmonary disease, an inflammatory endocrine disease, or an autoimmune hematological disease.

[0590] 159. The method of any of embodiments 136-140 and 150-158, wherein the disease or condition is selected from inflammatory bowel disease, transplant, Crohn's disease, ulcerative colitis, multiple sclerosis, asthma, rheumatoid arthritis, or psoriasis.

IX. Examples

[0591] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1

Generation of Mutant DNA Constructs of IgSF Domains

[0592] Example 1 describes the generation of mutant DNA constructs of human CD155 IgSF domains for translation and expression on the surface of yeast as yeast display libraries.

[0593] A. Degenerate Libraries

[0594] Mutant DNA constructs encoding a variant of the IgV domain of CD155 were generated. Constructs were generated based on a wildtype human CD155 sequence set forth in SEQ ID NO:155 (containing the immunoglobulin-like V-type (IgV) domain as follows:

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WPPPGTGDVVVQAPTQVPGFLGDSVTLPCYLQVPNMEVTHVSQLTWARHG

ESGSMAVFHQTQGPSYSESKRLEFVAARLGAELRNASLRMFGLRVEDEGN

YTCLFVTFPQGSRSVDIWLRVL
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[0595] For libraries that target specific residues for complete or partial randomization with degenerate codons, the DNA encoding SEQ ID NO:155 were ordered from Integrated DNA Technologies (Coralville, IA) as a set of overlapping oligonucleotides of up to 80 base pairs (bp) in length. To generate a library of diverse variants of the IgV domain, the oligonucleotides contained desired degenerate codons, such as specific mixed base sets to code for various amino acid substitutions, at desired amino acid positions. Degenerate codons were generated using an algorithm at the URL: rosettadesign.med.unc.edu/SwiftLib/.

[0596] In general, positions to mutate and degenerate codons were chosen from crystal structure information (PDB: 3UDW) or homology models built from this structure containing the target-ligand pairs of interest to identify ligand contact residues, such as target side chain residues that interact with the ligand, as well as residues that are at the protein interaction interface. This analysis was performed using a structure viewer available at the URL: spdbv.vital-it.ch.

[0597] The next step in library design was the alignment of human, mouse, rat, and monkey CD155 sequences to identify conserved residues. Based on this analysis, conserved target residues were mutated with degenerate codons that only specified conservative amino acid changes plus the wild-type residue. Residues that were not conserved, were mutated more aggressively, but also included the wild-type residue. Degenerate codons that also encoded the wild-type

residue were deployed to avoid excessive mutagenesis of target protein. For the same reason, only up to 20 positions were targeted for mutagenesis for each library. Mutational analysis was focused on contact and non-contact interfacial residues that were within 6 Å of the binding surface with their side chains directed toward the ligand/counter structure.

[0598] The oligonucleotides were dissolved in sterile water, mixed in equimolar ratios, heated to 95° C. for five minutes and slowly cooled to room temperature for annealing. IgV domain-specific oligonucleotide primers that anneal to the start and end of the IgV domain gene sequence were then used to generate PCR product. IgV domain-specific oligonucleotides which overlap by 40 bp with a modified version of pBYDS03 cloning vector (Life Technologies USA), beyond and including the BamHI and KpnI cloning sites, were then used to amplify 100 ng of PCR product from the prior step to generate a total of at least 12 μg of DNA for every electroporation. Both PCRs used OneTaq 2×PCR Master Mix (New England Biolabs, USA). The second PCR products were purified using a PCR purification kit (Qiagen, Germany) and resuspended in sterile deionized water. Alternatively, Ultramers (Integrated DNA Technologies) of up to 200 bp in length were used in conjunction with megaprimer PCR (URL: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC146891/pdf/253371.pdf) to generate larger stretches of degenerate codons that could not be as easily incorporated using multiple small overlapping primers. Following the generation of full length product using megaprimer PCR, the mutant IgV domain library was PCR amplified again using DNA primers containing 40 bp overlap region with the modified pBYDSO3 cloning variant for homologous recombination into yeast.

[0599] To prepare for library insertion, the modified yeast display version of vector pBYDSO3 was digested with BamHI and KpnI restriction enzymes (New England Biolabs, USA) and the large vector fragment was gel-purified and dissolved in sterile, deionized water. Electroporation-ready DNA for the next step was generated by mixing 12 μg of library DNA insert with 4 μg of linearized vector in a total volume of 50 μL deionized and sterile water.

[0600] B. Random Libraries

[0601] Random libraries were also constructed to identify variants of the IgV domain of CD155 set forth in SEQ ID NO:155 containing the IgV domain. DNA encoding the wild-type IgV domain was cloned between the BamHI and KpnI restriction sites of the modified yeast display vector pBYDSO3. The DNA was then mutagenized with the Genemorph II Kit (Agilent, USA) to generate an average of three to five amino acid changes per library variant. Mutagenized DNA was then amplified by the two-step PCR and further processed as described above for targeted libraries.

Example 2

Introduction of DNA Libraries into Yeast

[0602] Example 2 describes the introduction of CD155 DNA libraries into yeast.

[0603] To introduce degenerate and random library DNA into yeast, electroporation-competent cells of yeast strain BJ5464 (ATCC.org; ATCC number 208288) were prepared and electroporated on a Gene Pulser II (Biorad, USA) with the electroporation-ready DNA from the steps above essentially as described (Colby, D. W. et al. 2004 Methods Enzymology 388, 348-358). The only exception is that transformed cells were grown in non-inducing minimal selective SCD-Leu medium to accommodate the LEU2 selectable marker carried by modified plasmid pBYDSO3. One liter of SCD-Leu media consists of 14.7 grams sodium citrate, 4.29 grams citric acid monohydrate, 20 grams dextrose, 6.7 grams yeast nitrogen base, and 1.6 grams yeast synthetic drop-out media supplement without leucine. The Medium was filter sterilized before use using a 0.22 μm vacuum filter device.

[0604] Library size was determined by plating serial dilutions of freshly recovered cells on SCD-Leu agar plates and then extrapolating library size from the number of single colonies from plating that generated at least 50 colonies per plate. In general, library sizes ranged from 10E8 to 10E9 transformants based on this dilution assay. The remainder of the electroporated culture was grown to saturation in SCD-Leu and cells from this culture were subcultured (e.g. 1/100) into fresh SCD-Leu once more to minimize the fraction of untransformed cells, and grown overnight. To maintain library diversity, this subculturing step was carried out using an inoculum that contained at least 10× more cells than the calculated library size. Cells from the second saturated culture were resuspended in fresh medium containing sterile 25% (weight/volume) glycerol to a density of 10E10/mL and frozen and stored at −80° C. (frozen library stock).

Example 3

Yeast Selection

[0605] Example 3 describes the selection of yeast expressing affinity modified variants of CD155.

[0606] A number of cells equal to at least 10 times the estimated library size were thawed from individual library stocks, suspended to 0.1×10E6 cells/mL in non-inducing SCD-Leu medium, and grown overnight. The next day, a number of cells equal to 10 times the library size were centrifuged at 2000 RPM for two minutes and resuspended to 0.5×10E6 cells/mL in inducing SCDG-Leu media. One liter of the SCDG-Leu induction media consists of 5.4 grams Na_2HPO_4, 8.56 grams of $NaH_2PO_4 \cdot H_2O$, 20 grams galactose, 2.0 grams dextrose, 6.7 grams yeast nitrogen base, and 1.6 grams of yeast synthetic drop out media supplement without leucine dissolved in water and sterilized through a 0.22 μm membrane filter device. The culture was grown in induction medium for 1 day at room temperature to induce expression of library proteins on the yeast cell surface.

[0607] Cells were sorted two to three times using magnetic beads loaded with cognate ligand to reduce non-binders and enrich for all variant CD155 with the ability to bind their exogenous recombinant counter-structure proteins. This was then followed by one to two rounds of fluorescence activated cell sorting (FACS) using exogenous counter-structure protein staining to enrich the fraction of yeast cells that displays improved binders. Magnetic bead enrichment and selections by flow cytometry were carried out essentially as described in Miller, K. D. Current Protocols in Cytometry 4.7.1-4.7.30, July 2008.

[0608] With CD155 libraries, target ligand proteins were employed as follows: human rTIGIT.Fc (i.e., recombinant TIGIT-Fc fusion protein) and rCD226.Fc was purchased from R & D Systems, USA. Magnetic Protein A beads were obtained from New England Biolabs, USA. For two-color, flow cytometric sorting, a Bio-Rad S3e sorter was used.

CD155 display levels were monitored with an anti-hemagglutinin antibody labeled with Alexafluor 488 (Life Technologies, USA). Ligand binding of Fc fusion proteins, rTIGIT.Fc or rCD226.Fc, were detected with PE conjugated human Ig specific goat Fab (Jackson ImmunoResearch, USA). Doublet yeast were gated out using forward scatter (FSC)/side scatter (SSC) parameters, and sort gates were based upon higher ligand binding detected in FL2 that possessed more limited tag expression binding in FL1.

[0609] Yeast outputs from the flow cytometric sorts were assayed for higher specific binding affinity. Sort output yeast were expanded and re-induced to express the particular IgSF affinity modified domain variants they encode. This population then can be compared to the parental, wild-type yeast strain, or any other selected outputs, such as the bead output yeast population, by flow cytometry.

[0610] For CD155, the second FACS outputs (F2) were compared to parental CD155 yeast for binding rTIGIT.Fc or rCD226.Fc by double staining each population with anti-HA (hemagglutinin) tag expression and the anti-human Fc secondary to detect ligand binding.

[0611] Selected variant CD155 IgV domains were further formatted as fusion proteins and tested for binding and functional activity as described below.

Example 4

Reformatting Selection Outputs as Fc-Fusions and in Various Immunomodulatory Protein Types

[0612] Example 4 describes reformatting of selection outputs identified in Example 3 as immunomodulatory proteins containing an affinity modified (variant) immunoglobulin-like V-type (IgV) domain of CD155 fused to an Fc molecule (variant IgV domain-Fc fusion molecules).

[0613] Output cells from final flow cytometric CD155 sorts were grown to terminal density in SCD-Leu medium. Plasmid DNA from each output was isolated using a yeast plasmid DNA isolation kit (Zymo Research, USA). For Fc fusions, PCR primers with added restriction sites suitable for cloning into the Fc fusion vector of choice were used to batch-amplify from the plasmid DNA preps the coding DNA for the mutant target IgV domains. After restriction digestion, the PCR products were ligated into an appropriate Fc fusion vector followed by heat shock transformation into *E. coli* strain XL1 Blue (Agilent, USA) or NEB5alpha (New England Biolabs) as directed by supplier. Alternatively, the outputs were PCR amplified with primers containing 40 bp overlap regions on either end with a modified Fc fusion vector to carry out in vitro recombination using Gibson Assembly Mastermix (New England Biolabs, USA), which was subsequently used for heat shock transformation into *E. coli* strain NEB5alpha. Exemplary of an Fc fusion vector is pFUSE-hIgG1-Fc2 (InvivoGen, USA).

[0614] Dilutions of transformation reactions were plated on LB-agar containing 100 μg/mL carbenicillin (Teknova, USA) to isolate single colonies for selection. Up to 96 colonies from each transformation were then grown in 96 well plates to saturation overnight at 37° C. in LB-broth (Teknova cat #L8112) and a small aliquot from each well was submitted for DNA sequencing of the IgV domain insert in order to identify mutation(s) in all clones. Sample preparation for DNA sequencing was carried out using protocols provided by the service provider (Genewiz; South Plainfield, NJ). After removal of sample for DNA sequencing, glycerol was then added to the remaining cultures for a final glycerol content of 25% and plates were stored at −20° C. for future use as master plates (see below). Alternatively, samples for DNA sequencing were generated by replica plating from grown liquid cultures onto solid agar plates using a disposable 96 well replicator (VWR, USA). These plates were incubated overnight to generate growth patches and the plates were submitted to Genewiz for DNA sequencing following their specifications.

[0615] After analysis of the Genewiz-generated DNA sequencing data, clones of interest were recovered from master plates and individually grown to saturation in 5 mL liquid LB-broth containing 100 μg/mL carbenicillin (Teknova, USA) and 2 mL of each culture were then used for preparation of approximately 10 μg of miniprep plasmid DNA of each clone using a standard kit such as the PureYield Plasmid Miniprep System (Promega, USA). Identification of clones of interest generally involved the following steps. First, DNA sequence data files were downloaded from the Genewiz website. All sequences were then manually curated so that they start at the beginning of the IgV domain coding region. The curated sequences were then batch-translated using a suitable program available at the URL: www.ebi.ac.uk/Tools/st/emboss_transeq/. The translated sequences were then aligned using a suitable program available at the URL: multalin.toulouse.inra.fr/multalin/multalin.html. Alternatively, Genewiz sequences were processed to generate alignments using Ugene software (http://ugene.net).

[0616] Clones of interest were then identified using the following criteria: 1.) identical clone occurs at least two times in the alignment and 2.) a mutation occurs at least two times in the alignment and preferably in distinct clones. Clones that meet at least one of these criteria were enriched by the sorting process most likely due to improved binding.

[0617] To generate recombinant immunomodulatory proteins that are Fc fusion proteins containing an IgV domain of CD155 with at least one affinity-modified domain (e.g. variant CD155 IgV-Fc), the encoding DNA was generated to encode a protein as follows: variant (mutant) CD155 IgV domain followed by a linker of three alanines (AAA) followed by a human IgG1 Fc set forth in SEQ ID NO:1135 containing the mutations R292C, N297G and V302C by EU numbering (corresponding to R77C, N82G, and V87C with reference to wild-type human IgG1 Fc set forth in SEQ ID NO: 56). Since the construct does not include any antibody light chains that can form a covalent bond with a cysteine, the human IgG1 Fc also contains replacement of the cysteine residues to a serine residue at position 220 (C220S) by EU numbering (corresponding to position 5 (C5S) with reference to the wild-type or unmodified Fc set forth in SEQ ID NO: 56).

Example 5

Expression and Purification of Fc-Fusions

[0618] Example 5 describes the high throughput expression and purification of Fc-fusion proteins containing variant IgV CD155 as described in the above Examples.

[0619] Recombinant variant Fc fusion proteins were produced from suspension-adapted human embryonic kidney (HEK) 293 cells using the Expi293 expression system (Invitrogen, USA). 4 μg of each plasmid DNA from the previous step was added to 200 μL Opti-MEM (Invitrogen,

USA) at the same time as 10.8 μL ExpiFectamine was separately added to another 200 μL Opti-MEM. After 5 minutes, the 200 μL of plasmid DNA was mixed with the 200 μL of ExpiFectamine and was further incubated for an additional 20 minutes before adding this mixture to cells. Ten million Expi293 cells were dispensed into separate wells of a sterile 10 ml, conical bottom, deep 24 well growth plate (Thomson Instrument Company, USA) in a volume 4 mL Expi293 media (Invitrogen, USA). Plates were shaken for 5 days at 120 RPM in a mammalian cell culture incubator set to 95% humidity and 8% CO_2. Following a 5 day incubation, cells were pelleted and culture supernatants were retained.

[0620] Proteins were purified from supernatants using a high throughput 96 well Protein A purification kit using the manufacturer's protocol (Catalog number 45202, Life Technologies, USA). Resulting elution fractions were buffer exchanged into PBS using Zeba 96 well spin desalting plate (Catalog number 89807, Life Technologies, USA) using the manufacturer's protocol. Purified protein was quantitated using 280 nm absorbance measured by Nanodrop instrument (Thermo Fisher Scientific, USA), and protein purity was assessed by loading 5 μg of protein on NUPAGE pre-cast, polyacrylamide gels (Life Technologies, USA) under denaturing and reducing conditions and subsequent gel electrophoresis. Proteins were visualized in gel using standard Coomassie staining.

Example 6

Assessment of Binding and Activity of Affinity-Matured IgSF Domain-Containing Molecules

[0621] A. Binding to Cell-Expressed Counter Structures

[0622] This Example describes Fc-fusion binding studies of purified proteins from the above Examples to assess specificity and affinity of CD155 domain variant immunomodulatory proteins for cognate binding partners.

[0623] To produce cells expressing cognate binding partners, full-length mammalian surface expression constructs for each of human CD226, TIGIT and CD96 were designed in pcDNA3.1 expression vector (Life Technologies) and sourced from Genscript, USA. Binding studies were carried out on transfected HEK293 cells generated to express the full-length mammalian surface ligands using the transient transfection system (Life Technologies, USA) described above. As a control, binding to mock (non-transfected) cells also was assessed. The number of cells needed for the experiment was determined, and the appropriate 30 mL scale of transfection was performed using the manufacturer's suggested protocol. For each CD226, TIGIT, CD96 or mock 30 mL transfection, 75 million Expi293F cells were incubated with 30 μg expression construct DNA and 1.5 mL diluted ExpiFectamine 293 reagent for 48 hours, at which point cells were harvested for staining.

[0624] For staining by flow cytometry, 200,000 cells of appropriate transient transfection or negative control (mock) were plated in 96 well round bottom plates. Cells were spun down and resuspended in staining buffer (PBS (phosphate buffered saline), 1% BSA (bovine serum albumin), and 0.1% sodium azide) for 20 minutes to block non-specific binding. Afterwards, cells were centrifuged again and resuspended in staining buffer containing 100 nM to 1 nM variant CD155 Fc fusion protein in 50 μL. Primary staining was performed on ice for 45 minutes, before washing cells in staining buffer twice. PE-conjugated anti-human Fc (Jackson ImmunoResearch, USA) was diluted 1:150 in 50 μL staining buffer and added to cells and incubated another 30 minutes on ice. Secondary antibody was washed out twice, cells were fixed in 4% formaldehyde/PBS, and samples were analyzed on FACScan flow cytometer (Becton Dickinson, USA).

[0625] Mean Fluorescence Intensity (MFI) was calculated for each transfectant and negative parental line with Cell Quest Pro software (Becton Dickinson, USA). A ratio of the MFI value of the tested variant compared to the parental (WT) MFI value was also calculated. Exemplary results for binding to CD226, TIGIT, CD96 or mock cells is set forth in Table 10. Table 10 also indicates the amino acid substitutions in the IgV of the variant CD155 selected as described in the above Examples.

[0626] B. Bioactivity Characterization

[0627] This Example further describes Fc-fusion variant protein bioactivity characterization in human primary T cell in vitro assays. Costimulatory bioactivity of CD155 fusion variants was determined in anti-CD3 coimmobilization assays. 1 nM or 4 nM mouse anti-human CD3 (OKT3, Biolegends, USA) was diluted in PBS with 1 nM to 80 nM rCD155.Fc variant proteins. This mixture was added to tissue culture treated flat bottom 96 well plates (Corning, USA) overnight to facilitate adherence of the stimulatory proteins to the wells of the plate. The next day, unbound protein was washed off the plates and 100,000 purified human pan T cells (BenTech Bio, US) or human T cell clone BC3 (Astarte Biologics, USA) were added to each well in a final volume of 200 μL of Ex-Vivo 15 media (Lonza, Switzerland). Cells were cultured 3 days before harvesting culture supernatants and measuring human IFN-gamma levels with Duoset ELISA kit (R&D Systems, USA).

[0628] Exemplary results from an anti-CD3 coimmobilization assay are set forth in Table 10 for selected exemplary variants.

[0629] Soluble rCD155.Fc bioactivity is also tested in a human Mixed Lymphocyte Reaction (MLR). Human primary dendritic cells (DC) are generated by culturing monocytes isolated from PBMC (BenTech Bio, USA) in vitro for 7 days with 500 U/mL rIL-4 (R&D Systems, USA) and 250 U/mL rGM-CSF (R&D Systems, USA) in Ex-Vivo 15 media (Lonza, Switzerland). 10,000 matured DC and 100,000 purified allogeneic CD4+ T cells (BenTech Bio, USA) are co-cultured with variant CD155 Fc fusion proteins and controls in 96 well round bottom plates in 200 μL final volume of Ex-Vivo 15 media. On day 5, IFN-gamma secretion in culture supernatants is analyzed using the Human IFN-gamma Duoset ELISA kit (R&D Systems, USA). Optical density is measured by VMax ELISA Microplate Reader (Molecular Devices, USA) and quantitated against titrated rIFN-gamma standard included in the IFN-gamma Duo-set kit (R&D Systems, USA).

TABLE 10

Variant CD155 selected against cognate binding partners. Molecule sequences, binding data, and costimulatory bioactivity data.

CD155 mutations	SEQ ID NO (IgV)	CD226 tfxn MFI (CD226 MFI parental ratio)	TIGIT tfxn MFI (TIGIT MFI parental ratio)	CD96 MFI (CD96 MFI parental ratio)	Mock Expi293 MFI (Mock MFI parental ratio)	Anti-CD3 IFN-gamma (pg/ml) (Anti-CD3 IFN-gamma parental ratio)
P18S, P64S, F91S	156	497825 (133.7)	247219 (91.1)	140065 (45.4)	3528 (1.2)	270.1 (0.7)
P18S, F91S, L104P	157	26210 (7.0)	75176 (27.7)	10867 (3.5)	2130 (0.7)	364.2 (0.9)
L44P	158	581289 (156.1)	261931 (96.5)	152252 (49.4)	3414 (1.2)	277.6 (0.7)
A56V	159	455297 (122.3)	280265 (103.2)	161162 (52.2)	2601 (0.9)	548.2 (1.4)
P18L, L79V, F91S	160	5135 (1.4)	4073 (1.5)	3279 (1.1)	2719 (0.9)	1241.5 (3.2)
P18S, F91S	161	408623 (109.8)	284190 (104.7)	147463 (47.8)	3348 (1.1)	760.6 (2.0)
P18T, F91S	162	401283 (107.8)	223985 (82.5)	157644 (51.1)	3065 (1.1)	814.7 (2.1)
P18T, S42P, F91S	163	554105 (148.8)	223887 (82.5)	135395 (43.9)	3796 (1.3)	539.7 (1.4)
G7E, P18T, Y30C, F91S	164	12903 (3.5)	12984 (4.8)	7906 (2.6)	2671 (0.9)	275.9 (0.7)
P18T, F91S, G111D	165	438327 (117.7)	287315 (105.8)	167583 (54.3)	4012 (1.4)	307.2 (0.8)
P18S, F91P	166	4154 (1.1)	3220 (1.2)	2678 (0.9)	2816 (1.0)	365.7 (0.9)
P18T, F91S, F108L	167	394546 (106.0)	298680 (110.0)	193122 (62.6)	2926 (1.0)	775.4 (2.0)
P18T, T45A, F91S	169	435847 (117.1)	222044 (81.8)	191026 (61.9)	2948 (1.0)	1546.8 (4.0)
P18T, F91S, R94H	170	3589 (1.0)	2942 (1.1)	2509 (0.8)	2390 (0.8)	1273.2 (3.3)
P18S, Y30C, F91S	171	382352 (102.7)	276358 (101.8)	56934 (18.5)	3540 (1.2)	426.5 (1.1)
A81V, L83P	172	4169 (1.1)	2912 (1.1)	2616 (0.8)	2993 (1.0)	339.7 (0.9)
L88P	173	65120 (17.5)	74845 (27.6)	35280 (11.4)	2140 (0.7)	969.2 (2.5)
Wild type	174	3723 (1.0)	2715 (1.0)	3085 (1.0)	2913 (1.0)	389.6 (1.0)
R94H	175	18905 (5.1)	104013 (38.3)	11727 (3.8)	1663 (0.6)	372.6 (1.0)
A13E, P18S, A56V, F91S	175	357808 (96.1)	179060 (66.0)	118570 (38.4)	2844 (1.0)	349.2 (0.9)
P18T, F91S, V115A	176	38487 (10.3)	46313 (17.1)	22718 (7.4)	2070 (0.7)	1574.5 (4.0)
P18T, Q60K	177	238266 (64.0)	173730 (64.0)	154448 (50.1)	4778 (1.6)	427.2 (1.1)

Example 7

Yeast Selection for Generation of Additional Variant Fc-Fusion Molecules

[0630] Example 7 describes the selection of yeast expressing affinity modified variants of CD155 and reformatting selected outputs as Fc fusion for the generation of additional Fc-fusion proteins containing IgV CD155 variants.

[0631] Following the affinity maturation selection described in Example 3, plasmid DNA with the target gene was extracted from the yeast cells using yeast miniprep, and the target gene was further mutated using the GeneMorph II Random Mutagenesis Kit. To lower the polymerase fidelity and therefore increase its error rate, several PCR reactions were setup with varying concentrations of MnCl2 from 0.0 to 1.0 mM. The resultant gene was amplified to achieve sufficient quantities for electroporation using OneTaq 2×PCR master mix. These gene products were electroporated into yeast cells, then grown and induced before an additional round of selection was performed.

[0632] The additional selection consisted of two positive selections with the desired counter structures TIGIT (FACS1 and FACS2) followed by one negative selection with the counter structure CD226 (FACS3) to select away from CD226 and improve binding specificity of the variant CD155. FACS selection were performed essentially as described in Example 3 above except the concentrations of the counter structures (TIGIT) and selection stringency of the positive sorts were varied to optimize lead identification. The concentration of CD226 for the negative selection was kept at 100 nM.

[0633] After selections were performed, outputs were plated onto SCD agar plates to isolate individual clones for

analysis via flow cytometry or were subjected to yeast miniprep to extract plasmid DNA to use as template for mutagenesis for an additional round of selections as described above. To isolate individual yeast clones, colonies were picked from SCD agar plates and grown in starter cultures of 500 μL of SCD in deep 96 well plates (VWR, USA), for overnight growth at 300 Celsius and 300 rpm. The following day, the yeast cells were passaged into fresh SCD medium to an optical density (OD_{600}) of ~0.5, and were allowed to shake at 30° Celsius and 300 rpm for 5-6 hours until the OD_{600} reached ~2.0. Cells then were collected by centrifugation and resuspended in SCDG medium at an OD_{600} of ~1.0 for 16 induction at room temperature.

[0634] Following induction, cells were labeled with counter structures used in the positive (TIGIT) and negative (CD226) selections to identify leads containing the desired phenotype. The plasmid DNA from these leads was extracted using yeast miniprep, and the target gene was PCR amplified using OneTaq 2×PCR master mix and cloned into an Fc vector using Gibson Assembly Master Mix (New England Biolabs, USA). The intact plasmid DNA was transformed into chemically competent DH5a cells (New England Biolabs, USA, USA), which were plated on rectangular LB-agar plates containing carbenicillin in a 12×8 grid format to isolate single colonies for sequencing and selection. These plates were incubated overnight to generate growth patches and the plates were submitted to Genewiz for DNA sequencing of the IgV domain variant insert in order to identify the mutation(s) in all clones.

[0635] Identification of clones of interest generally involved the following steps. First, DNA sequence data files were downloaded from the Genewiz website. All sequences were then manually curated so that they started at the beginning of the IgV domain coding region. The curated sequences were then batch-translated using a suitable program available at the URL: www.ebi.ac.uk/Tools/st/emboss_transeq/. The translated sequences were then aligned using a suitable program available at the URL: multalin.toulouse.inra.fr/multalin/multalin.html.

[0636] Unique clones were transfected into HEK293 cells for expression and purification of recombinant human Fc tagged protein as described in Example 5. The additional Fc variants were formatted as Fc fusions as follows. For variants set forth in Tables 11A-11C, the selected variant IgV domains were generated as Fc-fusion proteins containing: variant (mutant) IgV domain followed by a linker of three alanines (AAA) followed by a human IgG1 Fc containing the mutations C220S, R292C, N297G and V302C (SEQ ID NO:1135). For variants set forth in Tables 11D and 11E, the selected variant IgV domains were generated as Fc-fusion proteins containing: variant (mutant) IgV domain followed by a GSGGGGS linker followed by a human IgG1 Fc containing the mutations L234A, L235E, G237A, E356D and M358L by EU numbering (SEQ ID NO:1119).

Example 8

Assessment of Binding to Cell-Expressed Counter Structures

[0637] This Example describes Fc-fusion binding studies of purified variant IgV Fc fusion proteins identified and generated in Example 7 to assess specificity and affinity of additional CD155 domain variant immunomodulatory proteins for cognate binding partners.

[0638] HEK293 cells expressing the full-length mammalian surface expression constructs for human TIGIT, CD226 or CD96 were generated and binding was assessed as described in Example 6. In this experiment, HEK293 cells also were transfected with CD112R. Mean Fluorescence Intensity (MFI) value for binding of 100 nM of each variant Fc-fusion molecule was calculated and compared to the binding of the corresponding unmodified (wildtype) ECD-Fc fusion molecule not containing the amino acid substitution(s). Tables 11A-11B sets forth the binding results for exemplary variant Fc-fusion molecules and also indicates amino acid substitutions in the IgV of the variant CD155 selected as described in Example 7. Binding to wildtype CD155 IgV-Fc molecule and an antibody targeting human Fc (anti-hFc PE) were also tested as controls.

[0639] As shown in Tables 11 Å and 111B, the selections resulted in the identification of additional variant CD155 IgV-Fc fusion molecules that exhibited altered binding to at least one cognate binding partner.

TABLE 11A

Additional CD155 Variants and Binding Data.

		TIGIT		CD226		CD112R		CD96	
CD155 Mutation(s)	SEQ ID NO (IgV)	MFI at 100 nM	Fold ↑ to WT ECD	MFI at 100 nM	Fold ↑ to WT ECD	MFI at 100 nM	Fold ↑ to WT ECD	MFI at 100 nM	Fold ↑ to WT ECD
S52M	372	1865.3	0.00	1901.0	0.01	1553.4	0.87	1609.8	0.02
T45Q, S52L, L104E, G111R	373	2287.0	0.01	2390.4	0.01	1735.1	0.97	1575.1	0.02
S42G	374	4837.5	0.01	2448.1	0.01	1815.4	1.02	1699.6	0.02
Q62F	375	2209.5	0.01	2572.1	0.01	2706.5	1.52	2760.7	0.03
S52Q	376	2288.1	0.01	2022.3	0.01	1790.1	1.00	1822.3	0.02
S42A, L104Q, G111R	377	1923.7	0.00	1901.7	0.01	1815.1	1.02	1703.8	0.02
S42A, S52Q, L104Q, G111R	378	1807.5	0.00	2157.2	0.01	1894.4	1.06	1644.0	0.02
S52W, L104E	379	1938.2	0.00	1905.6	0.01	2070.6	1.16	1629.5	0.02
S42C	380	1914.0	0.00	2096.1	0.01	1685.0	0.95	1592.4	0.02
S52W	381	1991.6	0.00	2037.3	0.01	1612.8	0.90	1712.9	0.02
S52M, L104Q	382	2666.6	0.01	2252.2	0.01	1706.0	0.96	1633.1	0.02
S42L, S52L, Q62F, L104Q	383	2021.4	0.00	2643.8	0.02	1730.1	0.97	2318.7	0.02

TABLE 11A-continued

Additional CD155 Variants and Binding Data.

CD155 Mutation(s)	SEQ ID NO (IgV)	TIGIT MFI at 100 nM	TIGIT Fold ↑ to WT ECD	CD226 MFI at 100 nM	CD226 Fold ↑ to WT ECD	CD112R MFI at 100 nM	CD112R Fold ↑ to WT ECD	CD96 MFI at 100 nM	CD96 Fold ↑ to WT ECD
S42W	384	2434.5	0.01	2133.4	0.01	2325.7	1.30	2555.4	0.03
S42Q	385	2073.5	0.00	2225.9	0.01	1905.1	1.07	2143.1	0.02
S52L	386	2224.8	0.01	2676.3	0.02	2038.6	1.14	2043.2	0.02
S52R	387	4395.4	0.01	3964.4	0.02	2741.7	1.54	4846.9	0.05
L104E	388	3135.4	0.01	2264.2	0.01	1803.5	1.01	1556.7	0.02
G111R	389	2082.7	0.00	2791.3	0.02	2470.9	1.39	3317.1	0.03
S52E	390	2655.4	0.01	2599.8	0.02	1904.9	1.07	1799.0	0.02
Q62Y	391	2528.6	0.01	2621.4	0.02	1918.4	1.08	1827.5	0.02
T45Q, S52M, L104E	392	79498.2	0.19	143238.5	0.83	2600.6	1.46	6310.4	0.06
S42N, L104Q, G111R	393	2432.1	0.01	2311.3	0.01	1847.4	1.04	1958.3	0.02
S52M, V57L	394	1760.7	0.00	2431.6	0.01	2006.9	1.13	1858.7	0.02
S42N, S52Q, Q62F	395	2402.7	0.01	2152.0	0.01	1855.0	1.04	1737.6	0.02
S42A, S52L, L104E, G111R	396	2262.7	0.01	1889.4	0.01	1783.2	1.00	1606.2	0.02
S42W, S52Q, V57L, Q62Y	397	1961.4	0.00	2138.3	0.01	1844.9	1.03	1699.6	0.02
L104Q	398	10314.4	0.02	3791.4	0.02	2119.9	1.19	1542.6	0.02
S42L, S52Q, L104E	399	1946.9	0.00	6474.3	0.04	1749.0	0.98	1702.2	0.02
S42C, S52L	400	1762.5	0.00	2147.3	0.01	1663.4	0.93	1484.7	0.01
S42W, S52R, Q62Y, L104Q	401	1918.8	0.00	2300.1	0.01	1824.6	1.02	1756.0	0.02
T45Q, S52R, L104E	402	121636.9	0.29	142381.2	0.82	2617.9	1.47	3748.2	0.04
S52R, Q62F, L104Q, G111R	403	2969.2	0.01	3171.6	0.02	1725.4	0.97	2362.3	0.02
T45Q, S52L, V57L, L104E	404	2857.7	0.01	5943.5	0.03	1496.8	0.84	1533.3	0.02
S52M, Q62Y	405	1926.6	0.00	2000.3	0.01	1771.6	0.99	1651.1	0.02
Q62F, L104E, G111R	406	1966.4	0.00	2043.5	0.01	1701.9	0.95	1524.8	0.02
T45Q, S52Q	407	4812.8	0.01	5787.5	0.03	1765.6	0.99	2451.3	0.02
S52L, L104E	408	4317.8	0.01	2213.9	0.01	1756.9	0.99	1829.3	0.02
S42V, S52E	409	2055.0	0.00	2272.6	0.01	1808.0	1.01	2530.2	0.03
T45Q, S52R, G111R	410	4092.3	0.01	2075.2	0.01	1793.6	1.01	2336.6	0.02
S42G, S52Q, L104E, G111R	411	2010.1	0.00	2019.2	0.01	1706.4	0.96	1707.6	0.02
S42N, S52E, V57L, L104E	412	1784.2	0.00	1743.6	0.01	1690.1	0.95	1538.7	0.02
Wildtype	155	1964.7	0.00	2317.1	0.01	2169.6	1.22	1893.4	0.02
S42C, S52M, Q62F	413	1861.0	0.00	2084.2	0.01	1592.3	0.89	1481.3	0.01
S42L	414	1930.4	0.00	2187.2	0.01	1743.2	0.98	1618.4	0.02
Wildtype	155	2182.6	0.01	2374.5	0.01	1743.1	0.98	1680.4	0.02
S42A	415	1929.2	0.00	2188.6	0.01	1733.7	0.97	1623.6	0.02
S42G, S52L, Q62F, L104Q	416	1924.3	0.00	2157.6	0.01	1661.3	0.93	1642.1	0.02
S42N	417	1817.4	0.00	1910.9	0.01	1699.7	0.95	1691.5	0.02
CD155 IgV Fc	H1020	4690	0.01	4690	0.03	2941	1.65	3272	0.03
Wildtype CD155 ECD-Fc	47 (ECD)	423797	1.00	172839	1.00	1783	1.00	99037	1.00
Anti-human Fc PE	—	1506.3	0.00	3774	0.02	1587	0.89	1618	0.02

TABLE 11B

Additional CD155 Variants and Binding Data.

CD155 Mutation(s)	SEQ ID NO (IgV)	TIGIT MFI at 100 nM	TIGIT Fold Increase to WT ECD	CD226 MFI at 100 nM	CD226 Fold Increase to WT ECD	CD96 MFI at 100 nM	CD96 Fold Increase to WT ECD
P18T, S65A, S67V, F91S	418	297843	1.99	351195	3.22	128180	1.68
P18F, T39A, T45Q, T61R, S65N, S67L, E73G, R78G	419	Little to no protein produced					

TABLE 11B-continued

Additional CD155 Variants and Binding Data.

CD155 Mutation(s)	SEQ ID NO (IgV)	TIGIT MFI at 100 nM	TIGIT Fold Increase to WT ECD	CD226 MFI at 100 nM	CD226 Fold Increase to WT ECD	CD96 MFI at 100 nM	CD96 Fold Increase to WT ECD
P18T, T45Q, T61R, S65N, S67L	420	224682	1.50	270175	2.48	22820	0.30
P18F, S65A, S67V, F91S	421	534106	3.57	350410	3.21	144069	1.89
P18F, T45Q, T61R, S65N, S67L, F91S, L104P	422	Little to no protein produced					
P18S, L79P, L104M	423	342549	2.29	320823	2.94	107532	1.41
P18S, L104M	424	449066	3.00	295126	2.70	121266	1.59
L79P, L104M	425	3210	0.02	8323	0.08	2894	0.04
P18T, T45Q, L79P	426	542878	3.63	371498	3.40	193719	2.55
P18T, T45Q, T61R, S65H, S67H	427	312337	2.09	225439	2.07	152903	2.01
P18T, A81E	428	Little to no protein produced					
P18S, D23Y, E37P, S52G, Q62M, G80S, A81P, G99Y, S112N	429	Little to no protein produced					
A13R, D23Y, E37P, S42P, Q62Y, A81E	430	4161	0.03	11673	0.11	5762	0.08
A13R, D23Y, E37P, G99Y, S112N	431	Little to no protein produced					
A13R, D23Y, E37P, Q62M, A77V, G80S, A81P, G99Y	432	Little to no protein produced					
P18L, E37S, Q62M, G80S, A81P, G99Y, S112N	433	5900	0.04	14642	0.13	3345	0.04
P18S, L104T	434	321741	2.15	367470	3.37	108569	1.43
P18S, Q62H, L79Q, F91S	435	283357	1.89	324877	2.98	125541	1.65
P18S, F91S	161	222780	1.49	300049	2.75	48542	0.64
T45Q, S52K, Q62F, L104Q, G111R	436	Little to no protein produced					
T45Q, S52Q, Q62Y, L104Q, G111R	437	Little to no protein produced					
T45Q, S52Q, Q62Y, L104E, G111R	438	Little to no protein produced					
V57A, T61M, S65W, S67A, E96D, L104T	439	Little to no protein produced					
P18L, V57T, T61S, S65Y, S67A, L104T	440	278178	1.86	276870	2.54	121499	1.60
P18T, T45Q	441	326769	2.18	357515	3.28	92389	1.21
P18L, V57A, T61M, S65W, S67A, L104T	442	Little to no protein produced					
T61M, S65W, S67A, L104T	443	360915	2.41	417897	3.83	148954	1.96
P18S, V41A, S42G, T45G, L104N	444	3821	0.03	11449	0.10	3087	0.04
P18H, S42G, T45I, S52T, G53R, S54H, V57L, H59E, T61S, S65D, E68G, L104N	445	5066	0.03	177351	1.63	3700	0.05
P18S, S42G, T45V, F58L, S67W, L104N	446	14137	0.09	15175	0.14	15324	0.20
P18S, T45I, L104N	447	141745	0.95	298011	2.73	97246	1.28
P18S, S42G, T45G, L104N, V106A	448	29387	0.20	117965	1.08	15884	0.21
P18H, H40R, S42G, T45I, S52T, G53R, S54H, V57L, H59E, T61S, S65D, E68G, L104Y, V106L, F108H	449	12335	0.08	14657	0.13	15779	0.21
E37V, S42G, T45G, L104N	450	Little to no protein produced					
P18S, T45Q, L79P, L104T	451	206674	1.38	285512	2.62	87790	1.15
P18L, Q62R	452	66939	0.45	25063	0.23	10928	0.14
A13R, D23Y, E37P, S42L, S52G, Q62Y, A81E	453	Little to no protein produced					
P18L, H49R, L104T, D116N	454	167980	1.12	214677	1.97	62451	0.82
A13R, D23Y, E37P, Q62M, G80S, A81P, L104T	455	Little to no protein produced					
S65T, L104T	456	205942	1.38	187147	1.71	65207	0.86
A13R, D23Y, E37P, S52G, V57A, Q62M, K70E, L104T	457	Little to no protein produced					
P18L, A47V, Q62Y, E73D, L104T	458	146142	0.98	248926	2.28	73956	0.97
H40T, V41M, A47V, S52Q, Q62L, S65T, E73R, D97G, E98S, L104T, D116N	459	Little to no protein produced					
P18L, S42P, T45Q, T61G, S65H, S67E, L104T, D116N	460	153536	1.03	402503	3.69	53044	0.70
P18S, H40T, V41M, A47V, S52Q, Q62L, S65T, E73R, L104M, V106A	461	Little to no protein produced					
H40T, V41M, A47V, S52Q, Q62L, S65T, E68G, E73R, D97G, E98S, L104T	462	Little to no protein produced					

TABLE 11B-continued

Additional CD155 Variants and Binding Data.

CD155 Mutation(s)	SEQ ID NO (IgV)	TIGIT MFI at 100 nM	TIGIT Fold Increase to WT ECD	CD226 MFI at 100 nM	CD226 Fold Increase to WT ECD	CD96 MFI at 100 nM	CD96 Fold Increase to WT ECD
T45Q, S52E, L104E	463	Little to no protein produced					
T45Q, S52E, Q62F, L104E	464	132850	0.89	276434	2.53	14558	0.19
Wildtype CD155 ECD-Fc	47 (ECD)	149692	1.00	109137	1.00	76083	1.00
Anti-human Fc PE	—	2287	0.02	4799	0.04	2061	0.03

[0640] Mean Fluorescence Intensity (MFI) was calculated for cognate binding partners TIGIT and CD226 for additional selected variant CD155 IgV-Fc fusion molecules in two separate experiments. A ratio of the TIGIT MFI value compared to the CD226 MIFI value was also calculated. Tables 11C-11E set forth the binding results for exemplary variant Fc-fusion molecules and also indicate amino acid substitutions in the IgV of the variant CD155 selected as described in Example 7. As shown in Table 10C-10E, the indicated variant CD155 IgV-Fc fusion molecules exhibited increased binding specifically for TIGIT but not CD226.

TABLE 11C

Additional CD155 Variants and Binding Data.

CD155 Mutations	SEQ ID NO (IgV)	TIGIT MFI at 100 nM	TIGIT Fold Increase to WT IgV	CD226 MFI at 100 nM	CD226 Fold Increase to WT IgV	CD96 MFI at 100 nM	CD96 Fold Increase to WT IgV
P18F, T26M, L44V, Q62K, L79P, F91S, L104M, G111D	465	117327	1.2	1613	0.1	1629	0.1
P18S, T45S, T61K, S65W, S67A, F91S, G111R	466	124936	1.3	2114	0.1	2223	0.1
P18S, L79P, L104M, T107M	467	110512	1.1	18337	0.9	22793	1.3
P18S, S65W, S67A, M90V, V95A, L104Q, G111R	468	101726	1.0	1605	0.1	2571	0.1
Wildtype CD155-ECD	47 (ECD)	98935	1.0	20029	1.0	17410	1.0

TABLE 11D

Additional CD155 Variants and Binding Data.

CD155 Mutations	SEQ ID NO (IgV)	TIGIT MFI at 11.1 nM	TIGIT Fold Change from CD155-ECD	CD226 MFI at 11.1 nM	CD226 Fold Change from CD155-ECD	CD96 MFI at 11.1 nM	CD96 Fold Change from CD155-ECD
P18S, A47G, L79P, F91S, L104M, T107A, R113W	1207	56,409	1.19	1,191	0.08	25,362	1.49
P18T, D23G, S24A, N35D, H49L, L79P, F91S, L104M, G111R	1208	128,536	2.72	987	0.06	3,497	0.20
V9L, P18S, Q60R, V75L, L79P, R89K, F91S, L104E, G111R	1209	125,329	2.65	986	0.06	959	0.06
P18S, H49R, E73D, L79P, N85D, F91S, V95A, L104M, G111R	1210	Little to no protein produced					
V11A, P18S, L79P, F91S, L104M, G111R	1211	48,246	1.02	974	0.06	923	0.05
V11A, P18S, S54R, Q60P, Q62K, L79P, N85D, F91S, T107M	1212	190,392	4.02	1,019	0.07	1,129	0.07
P18T, S52P, S65A, S67V, L79P, F91S, L104M, G111R	1213	121,611	2.57	986	0.06	16,507	0.97
P18T, M36T, L79P, F91S, G111R	1214	150,015	3.17	1,029	0.07	2,514	0.15

TABLE 11D-continued

Additional CD155 Variants and Binding Data.

CD155 Mutations	SEQ ID NO (IgV)	TIGIT MFI at 11.1 nM	TIGIT Fold Change from CD155-ECD	CD226 MFI at 11.1 nM	CD226 Fold Change from CD155-ECD	CD96 MFI at 11.1 nM	CD96 Fold Change from CD155-ECD
D8G, P18S, M36I, V38A, H49Q, A76E, F91S, L104M, T107A, R113W	1215	79,333	1.68	1,026	0.07	2,313	0.14
P18S, S52P, S65A, S67V, L79P, F91S, L104M, T107S, R113W	1216	23,766	0.50	1,004	0.07	1,080	0.06
T15I, P18T, L79P, F91S, L104M, G111R	1217	55,498	1.17	1,516	0.10	1,030	0.06
P18F, T26M, L44V, Q62K, L79P, E82D, F91S, L104M, G111D	1218	213,640	4.51	991	0.06	1,276	0.07
P18T, E37G, G53R, Q62K, L79P, F91S, E98D, L104M, T107M	1219	251,288	5.31	2,001	0.13	45,878	2.69
P18L, K70E, L79P, F91S, V95A, G111R	1220	62,608	1.32	1,117	0.07	973	0.06
V9I, Q12K, P18F, S65A, S67V, L79P, L104T, G111R, S112I	1221	81,932	1.73	803	0.05	68,295	4.00
P18F, S65A, S67V, F91S, L104M, G111R	1222	30,661	0.65	901	0.06	3,193	0.19
V9I, V10I, P18S, F20S, T45A, L79P, F91S, L104M, F108Y, G111R, S112V	1223	151,489	3.20	973	0.06	974	0.06
V9L, P18L, L79P, M90I, F91S, T102S, L104M, G111R	1224	155,279	3.28	910	0.06	10,568	0.62
P18C, T26M, L44V, M55I, Q62K, L79P, F91S, L104M, T107M	1225	137,521	2.91	973	0.06	111,085	6.51
V9I, P18T, D23G, L79P, F91S, G111R	1226	151,426	3.20	897	0.06	2,725	0.16
P18F, L79P, M90L, F91S, V95A, L104M, G111R	1227	125,639	2.66	917	0.06	3,939	0.23
P18F, L79P, M90L, F91S, V95A, L104M, G111R	1227	115,156	2.43	1,073	0.07	2,464	0.14
P18T, M36T, S65A, S67E, L79Q, A81T, F91S, G111R	1228	10,616	0.22	1,130	0.07	963	0.06
V9L, P18T, Q62R, L79P, F91S, L104M, G111R	1229	195,111	4.12	835	0.05	1,497	0.09
CD155-ECD-Fc	—	47,319	1.00	15,421	1.00	17,067	1.00
Fc Control	—	2,298	0.05	1,133	0.07	996	0.06

TABLE 11E

Additional CD155 Variants and Binding Data.

CD155 Mutations	SEQ ID NO (IgV)	TIGIT MFI at 25 nM	TIGIT Fold Change from CD155-ECD	CD226 MFI at 25 nM	CD226 Fold Change from CD155-ECD	CD112R MFI at 25 nM	CD112R Fold Change from CD155-ECD	CD96 MFI at 25 nM	CD96 Fold Change from CD155-ECD
P18T, G19D, M36T, S54N, L79P, L83Q, F91S, T107M, F108Y	1702	905	0.02	748	0.02	1276	1.56	726	0.01
V9L, P18L, M55V, S69L, L79P, A81E, F91S, T107M	1703	58656	1.34	11166	0.29	920	1.13	67364	1.39
P18F, H40Q, T61K, Q62K, L79P, F91S, L104M, T107V	1704	108441	2.48	853	0.02	918	1.13	8035	0.17
P18S, Q32R, Q62K, R78G, L79P, F91S, T107A, R113W	1705	5772	0.13	701	0.02	843	1.03	831	0.02
Q12H, P18T, L21S, G22S, V57A, Q62R, L79P, F91S, T107M	1706	1084	0.02	687	0.02	876	1.07	818	0.02

TABLE 11E-continued

Additional CD155 Variants and Binding Data.

CD155 Mutations	SEQ ID NO (IgV)	TIGIT MFI at 25 nM	TIGIT Fold Change from CD155-ECD	CD226 MFI at 25 nM	CD226 Fold Change from CD155-ECD	CD112R MFI at 25 nM	CD112R Fold Change from CD155-ECD	CD96 MFI at 25 nM	CD96 Fold Change from CD155-ECD
V9I, P18S, S24P, H49Q, F58Y, Q60R, Q62K, L79P, F91S, T107M	1707	69926	1.60	1089	0.03	1026	1.26	43856	0.90
P18T, W46C, H49R, S65A, S67V, A76T, L79P, S87T, L104M	1708	918	0.02	640	0.02	803	0.98	717	0.01
P18S, S42T, E51G, L79P, F91S, G92W, T107M	1709	12630	0.29	707	0.02	857	1.05	1050	0.02
P18S, S42T, E51G, L79P, F91S, G92W, T107M	1709	7476	0.17	851	0.02	935	1.15	924	0.02
V10F, T15S, P18L, R48Q, L79P, F91S, T107M, V115M	1710	1168	0.03	792	0.02	901	1.10	998	0.02
P18S, L21M, Y30F, N35D, R84W, F91S, T107M, D116G	1711	1377	0.03	743	0.02	946	1.16	1033	0.02
P18F, E51V, S54G, Q60R, L79Q, E82G, S87T, M90I, F91S, G92R, T107M	1712	46090	1.05	15701	0.41	1012	1.24	61814	1.27
Q16H, P18F, F91S, T107M	1713	Little to no protein produced							
P18T, D23G, Q60R, S67L, L79P, F91S, T107M, V115A	1714	64091	1.47	30931	0.81	874	1.07	108875	2.24
D8G, V9I, V11A, P18T, T26M, S52P, L79P, F91S, G92A, T107L, V115A	1715	52508	1.20	9483	0.25	817	1.00	97770	2.01
V9I, P18F, A47E, G50S, E68G, L79P, F91S, T107M	1716	55167	1.26	54341	1.43	752	0.92	102115	2.10
P18S, M55I, Q62K, S69P, L79P, F91S, T107M	1717	Little to no protein produced							
P18T, T39S, S52P, S54R, L79P, F91S, T107M	1718	45927	1.05	744	0.02	1038	1.27	1225	0.03
P18S, D23N, L79P, F91S, T107M, S114N	1719	Little to no protein produced							
P18S, P34S, E51V, L79P, F91S, G111R	1720	7917	0.18	769	0.02	853	1.04	892	0.02
P18S, H59N, V75A, L79P, A81T, F91S, L104M, T107M	1721	800	0.02	676	0.02	915	1.12	759	0.02
P18S, W46R, E68D, L79P, F91S, T107M, R113G	1722	1359	0.03	717	0.02	798	0.98	737	0.02
V9L, P18F, T45A, S65A, S67V, R78K, L79V, F91S, T107M, S114T	1723	130274	2.98	153569	4.04	812	1.00	85605	1.76
P18T, M55L, T61R, L79P, F91S, V106I, T107M	1724	133399	3.05	1906	0.05	827	1.01	57927	1.19
T15I, P18S, V33M, N35F, T39S, M55L, R78S, L79P, F91S, T107M	1725	7550	0.17	1015	0.03	789	0.97	2709	0.06
P18S, Q62K, K70E, L79P, F91S, G92E, R113W	1726	11173	0.26	691	0.02	735	0.90	1951	0.04
P18F, F20I, T26M, A47V, E51K, L79P, F91S	1727	136088	3.11	54026	1.42	1401	1.72	96629	1.99
P18T, D23A, Q60H, L79P, M90V, F91S, T107M	1728	43795	1.00	98241	2.58	888	1.09	70891	1.46
P18S, D23G, C29R, N35D, E37G, M55I, Q62K, S65A, S67G, R78G, L79P, F91S, L104M, T107M, Q110R	1729	1599	0.04	1030	0.03	1115	1.37	1944	0.04
A13E, P18S, M36R, Q62K, S67T, L79P, N85D, F91S, T107M	1730	Little to no protein produced							
V9I, P18T, H49R, L79P, N85D, F91S, L104T, T107M	1731	46375	1.06	76851	2.02	794	0.97	80210	1.65
V9A, P18F, T61S, Q62L, L79P, F91S, G111R	1732	26109	0.60	891	0.02	825	1.01	2633	0.05
D8E, P18T, T61A, L79P, F91S, T107M	1733	Little to no protein produced							
P18S, V41A, H49R, S54C, L79S, N85Y, L88P, F91S, L104M, T107M	1734	1098	0.03	830	0.02	876	1.07	1678	0.03
V11E, P18H, F20Y, V25E, N35S, H49R, L79P, F91S, T107M, G111R	1735	979	0.02	846	0.02	844	1.03	928	0.02

TABLE 11E-continued

Additional CD155 Variants and Binding Data.

CD155 Mutations	SEQ ID NO (IgV)	TIGIT MFI at 25 nM	TIGIT Fold Change from CD155-ECD	CD226 MFI at 25 nM	CD226 Fold Change from CD155-ECD	CD112R MFI at 25 nM	CD112R Fold Change from CD155-ECD	CD96 MFI at 25 nM	CD96 Fold Change from CD155-ECD
V11A, P18F, D23A, L79P, G80D, V95A, T107M	1736	45249	1.04	913	0.02	830	1.02	33883	0.70
P18S, K70R, L79P, F91S, G111R	1737	16180	0.37	793	0.02	854	1.05	1182	0.02
P18T, D23A, Q60H, L79P, M90V, F91S, T107M	1728	175673	4.02	161958	4.26	879	1.08	50981	1.05
V9L, V11M, P18S, N35S, S54G, Q62K, L79P, L104M, T107M, V115M	1738	2999	0.07	2315	0.06	893	1.09	925	0.02
V9L, P18Y, V25A, V38G, M55V, A77T, L79P, M90I, F91S, L104M	1739	138011	3.16	26015	0.68	919	1.13	17970	0.37
V10G, P18T, L72Q, L79P, F91S, T107M	1740	4253	0.10	1584	0.04	863	1.06	3643	0.07
P18S, H59R, A76G, R78S, L79P	1741	130622	2.99	79435	2.09	1009	1.24	44493	0.91
V9A, P18S, M36T, S65G, L79P, F91S, L104T, G111R, S112I	1742	92503	2.12	989	0.03	886	1.09	7850	0.16
P18T, S52A, V57A, Q60R, Q62K, S65C, L79P, F91T, N100Y, T107M	1743	187338	4.29	10579	0.28	908	1.11	3791	0.08
V11A, P18F, N35D, A47E, Q62K, L79P, F91S, G99D, T107M, S114N	1744	Little to no protein produced							
V11A, P18T, N35S, L79P, S87T, F91S	1745	218660	5.00	273825	7.20	1269	1.56	69871	1.44
V9D, V11M, Q12L, P18S, E37V, M55I, Q60R, K70Q, L79P, F91S, L104M, T107M	1746	8693	0.20	790	0.02	852	1.04	1991	0.04
T15S, P18S, Y30H, Q32L, Q62R, L79P, F91S, T107M	1747	16213	0.37	2092	0.06	1056	1.29	6994	0.14
CD155-ECD-Fc	—	43704	1.00	38032	1.00	816	1.00	48638	1.00
CD112-IgV	—	1289		824		17819		1172	0.02

Example 9

Assessment of Bioactivity of Affinity-Matured IgSF Domain-Containing Molecules Using Jurkat/IL2/TIGIT Reporter Assay

[0641] This Example describes a Jurkat/IL2/TIGIT reporter assay to assess bioactivity of CD155 domain variant immunomodulatory proteins.

[0642] Jurkat effector cells expressing IL-2-luciferase reporter and TIGIT on its surface were suspended in Jurkat Assay buffer (RPMI1640+5% FBS) at 2×10^6 cells/mL and 3 μg/mL anti-CD28 was added. Jurkat cells were then plated at 50 μL/well for a total of 100,000 cells per well.

[0643] To each well with Jurkat cells plated, 25 μL of each variant CD155 IgV-Fc fusion molecule or control proteins (wild type CD155 ECD-Fc or an Fc control) was added. All proteins were added at four concentrations: 200 nM, 66.6, 22.2 nM and 7.4 nM. The Jurkat cells with CD155 variant IgV-Fc fusion molecules or control proteins were incubated for 15 minutes at room temperature. K562 derived artificial antigen presenting cells (aAPC) cells displaying cell surface anti-CD3 single chain Fv (OKT3) and endogenously expressing CD155 and CD112 were brought to 0.67×10^6 cells/mL and 25 μL of cells was added to each well bringing the final volume of each well to 100 μL. Each well had a final ratio of 6:1 Jurkat:K562 cells and protein concentration of 50, 16.7, 5.6 or 1.9 nM and 1.5 μg/mL anti-CD28 per well. Jurkat cells and K562 cells were incubated for 5-6 hours at 37 degrees Celsius in a humidified 5% C02 incubation chamber. Plates were then removed from the incubator and acclimated to room temperature for 15 minutes. 100 μL of a cell lysis and luciferase substrate solution (BioGlo luciferase reagent, Promega) was added to each well and the plates were incubated on an orbital shaker for 10 minutes. Luminescence was measured with a 1 second per well integration time using a BioTek Cytation luminometer.

[0644] An average relative luminescence value was determined for each variant CD155 IgV Fc and a fold increase in IL-2 reporter signal was calculated for each variant compared to wildtype CD155 ECD-Fc protein.

[0645] As shown in Table 12A, luciferase activity of Jurkat effector cells expressing TIGIT and IL-2-luciferase reporter co-cultured with anti-CD3/CD155 aAPC and variant CD155 IgV-Fc molecules were altered (increased) for some molecules tested. The differences in luminescence signals demonstrate the differences in binding of the variant CD155 IgV-Fc molecules to TIGIT and the resulting blockade of inhibitory activity. In the Table, Column 2 sets forth the SEQ ID NO identifier for each variant IgV domain contained in the tested variant IgV-Fc fusion molecule.

TABLE 12A

Jurkat/IL-2/TIGIT Reporter Assay

CD155 Mutation(s)	SEQ ID NO (IgV)	Proteins added at 5.6 nM: Average Relative Light Units (RLU)	Proteins added at 5.6 nM: Fold Increase in IL2 Reporter Signal
P18F, T26M, L44V, Q62K, L79P, F91S, L104M, G111D	465	502	1.0
P18S, T45S, T61K, S65W, S67A, F91S, G111R	466	864	1.7
P18S, L79P, L104M, T107M	467	538	1.1
P18S, S65W, S67A, M90V, V95A, L104Q, G111R	468	649	1.3
Wildtype CD155-ECD	47 (ECD)	511	1.0
Fc control		511	1.0

[0646] Additional bioactivity of CD155 domain variant immunomodulatory proteins were assessed using the Jurkat/IL2/TIGIT reporter assay to assess bioactivity of CD155 domain variant immunomodulatory proteins. The assay was carried out substantially as described above, except that all proteins were added at two concentrations: 200 nM or 20 nM. After incubation at room temperature as described above, K562 derived artificial antigen presenting cells (aAPC) cells displaying cell surface anti-CD3 single chain Fv (OKT3) and endogenously expressing CD155 and CD112 were brought to 0.8×10^6 cells/mL and 25 μL of cells was added to each well bringing the final volume of each well to 100 μL. Each well had a final ratio of 5:1 Jurkat: K562 cells and protein concentration of 50 nM, or 5 nM and 1.5 μg/mL anti-CD28 per well. Jurkat cells and K562 cells were incubated, cells were lysed and luminescence was measured as described above. A background adjusted, relative luminescence value was determined for each variant CD155 IgV Fc and a fold increase (or decrease) in IL-2 reporter signal was calculated for each variant compared to wildtype CD155 ECD-Fc protein.

[0647] As shown in Table 12B-12E, luciferase activity of Jurkat effector cells expressing TIGIT and IL-2-luciferase reporter co-cultured with anti-CD3 expressing aAPC and 50 or 5 nM variant CD155 IgV-Fc molecules were altered for some molecules tested. The differences in luminescence signals demonstrate the differences in binding of the variant CD155 IgV-Fc molecules to TIGIT and the resulting blockade or stimulation of inhibitory activity. In the Table 111B-11C, Column 2 sets forth the SEQ ID NO identifier for each variant IgV domain contained in the tested variant IgV-Fc fusion molecule.

TABLE 12B

Bioactivity in Jurkat/IL2/TIGIT + K562/OKT3 Reporter Assay

CD155 Mutation(s)	SEQ ID NO NO.	Proteins added at 5.0 nM: Background Subtracted RLU	Proteins added at 5.0 nM: Fold Change Compared to CD155-ECD
S52M	372	879	2.6
T45Q, S52L, L104E, G111R	373	546	1.6
S42G	374	336	1.0
Q62F	375	324	1.0

TABLE 12B-continued

Bioactivity in Jurkat/IL2/TIGIT + K562/OKT3 Reporter Assay

CD155 Mutation(s)	SEQ ID NO NO.	Proteins added at 5.0 nM: Background Subtracted RLU	Proteins added at 5.0 nM: Fold Change Compared to CD155-ECD
S52Q	376	241	0.7
S42A, L104Q, G111R	377	328	1.0
S42A, S52Q, L104Q, G111R	378	179	0.5
S52W, L104E	379	444	1.3
S42C	380	110	0.3
S52W	381	Not tested	
S52M, L104Q	382	71	0.2
S42L, S52L, Q62F, L104Q	383	10	0.0
S42W	384	567	1.7
S42Q	385	762	2.3
S52L	386	803	2.4
S52R	387	671	2.0
L104E	388	448	1.3
G111R	389	286	0.9
S52E	390	205	0.6
Q62Y	391	385	1.2
T45Q, S52M, L104E	392	377	1.1
S42N, L104Q, G111R	393	587	1.8
S52M, V57L	394	Not tested	
S42N, S52Q, Q62F	395	890	2.7
S42A, S52L, L104E, G111R	396	508	1.5
S42W, S52Q, V57L, Q62Y	397	Not tested	
L104Q	398	953	2.9
S42L, S52Q, L104E	399	518	1.6
S42C, S52L	400	219	0.7
S42W, S52R, Q62Y, L104Q	401	681	2.0
T45Q, S52R, L104E	402	135	0.4
S52R, Q62F, L104Q, G111R	403	Not tested	
T45Q, S52L, V57L, L104E	404	266	0.8
S52M, Q62Y	405	Not tested	
Q62F, L104E, G111R	406	58	0.2
T45Q, S52Q	407	0	0.0
S52L, L104E	408	322	1.0
S42V, S52E	409	224	0.7
T45Q, S52R, G111R	410	264	0.8
S42G, S52Q, L104E, G111R	411	190	0.6
S42N, S52E, V57L, L104E	412	324	1.0
Wildtype	155	1048	3.1
S42C, S52M, Q62F	413	969	2.9
S42L	414	926	2.8
Wildtype	155	958	2.9
S42A	415	862	2.6
S42G, S52L, Q62F, L104Q	416	674	2.0
S42N	417	737	2.2
CD155-ECD Fc	—	334	1.0
Fc Control	—	533	1.6

TABLE 12C

Bioactivity in Jurkat/IL2/TIGIT + K562/OKT3 Reporter Assay

CD155 Mutation(s)	SEQ ID NO (IgV)	Proteins added at 5.0 nM: RLU	Proteins added at 5.0 nM: Fold Increase Compared to CD155-FL ECD
P18T, S65A, S67V, F91S	418	541	1.62
P18T, T45Q, T61R, S65N, S67L	420	Not tested	
P18F, S65A, S67V, F91S	421	Not tested	
P18S, L79P, L104M	423	Not tested	
P18S, L104M	424	805	2.41
L79P, L104M	425	Not tested	

TABLE 12C-continued

Bioactivity in Jurkat/IL2/TIGIT + K562/OKT3 Reporter Assay

CD155 Mutation(s)	SEQ ID NO (IgV)	Proteins added at 5.0 nM RLU	Proteins added at 5.0 nM Fold Increase Compared to CD155-FL ECD
P18T, T45Q, L79P	426	519	1.55
P18T, T45Q, T61R, S65H, S67H	427	Not tested	
A13R, D23Y, E37P, S42P, Q62Y, A81E	430	Not tested	
P18L, E37S, Q62M, G80S, A81P, G99Y, S112N	433	Not tested	
P18S, L104T	434	754	2.26
P18S, Q62H, L79Q, F91S	435	910	2.72
P18S, F91S	161	599	1.79
P18L, V57T, T61S, S65Y, S67A, L104T	440	821	2.46
P18T, T45Q	441	623	1.87
T61M, S65W, S67A, L104T	443	893	2.67
P18S, V41A, S42G, T45G, L104N	444	647	1.94
P18H, S42G, T45I, S52T, G53R, S54H, V57L, H59E, T61S, S65D, E68G, L104N	445	Not tested	
P18S, S42G, T45V, F58L, S67W, L104N	446	Not tested	
P18S, T45I, L104N	447	664	1.99

TABLE 12C-continued

Bioactivity in Jurkat/IL2/TIGIT + K562/OKT3 Reporter Assay

CD155 Mutation(s)	SEQ ID NO (IgV)	Proteins added at 5.0 nM RLU	Proteins added at 5.0 nM Fold Increase Compared to CD155-FL ECD
P18S, S42G, T45G, L104N, V106A	448	488	1.46
P18H, H40R, S42G, T45I, S52T, G53R, S54H, V57L, H59E, T61S, S65D, E68G, L104Y, V106L, F108H	449	442	1.32
P18S, T45Q, L79P, L104T	451	538	1.61
P18L, Q62R	452	Not tested	
P18L, H49R, L104T, D116N	454	703	2.10
S65T, L104T	456	816	2.44
P18L, A47V, Q62Y, E73D, L104T	458	776	2.32
P18L, S42P, T45Q, T61G, S65H, S67E, L104T, D116N	460	327	0.98
T45Q, S52E, Q62F, L104E	464	514	1.54
CD155-ECD Fc	47 (ECD)	334	1.00
Fc Control	—	533	1.60

TABLE 12D

Jurkat/IL2/TIGIT + K562/OKT3 Reporter Assay Results

CD155 Mutation(s)	SEQ ID NO (IgV)	Proteins added at 50 nM RLU	Proteins added at 50 nM Normalized Fold Change from CD155-ECD
P18S, A47G, L79P, F91S, L104M, T107A, R113W	1207	3096	5.0
P18T, D23G, S24A, N35D, H49L, L79P, F91S, L104M, G111R	1208	1981	3.2
V9L, P18S, Q60R, V75L, L79P, R89K, F91S, L104E, G111R	1209	1113	1.8
P18S, H49R, E73D, L79P, N85D, F91S, V95A, L104M, G111R	1210	Little to no protein produced	
V11A, P18S, L79P, F91S, L104M, G111R	1211	280	0.4
V11A, P18S, S54R, Q60P, Q62K, L79P, N85D, F91S, T107M	1212	1644	2.6
P18T, S52P, S65A, S67V, L79P, F91S, L104M, G111R	1213	2216	3.6
P18T, M36T, L79P, F91S, G111R	1214	2586	4.1
D8G, P18S, M36I, V38A, H49Q, A76E, F91S, L104M, T107A, R113W	1215	2502	4.0
P18S, S52P, S65A, S67V, L79P, F91S, L104M, T107S, R113W	1216	−52	−0.1
T15I, P18T, L79P, F91S, L104M, G111R	1217	225	0.4
P18F, T26M, L44V, Q62K, L79P, E82D, F91S, L104M, G111D	1218	2487	4.0
P18T, E37G, G53R, Q62K, L79P, F91S, E98D, L104M, T107M	1219	5382	8.6
P18L, K70E, L79P, F91S, V95A, G111R	1220	1480	2.4
V9I, Q12K, P18F, S65A, S67V, L79P, L104T, G111R, S112I	1221	1428	2.3
P18F, S65A, S67V, F91S, L104M, G111R	1222	351	0.6
V9I, V10I, P18S, F20S, T45A, L79P, F91S, L104M, F108Y, G111R, S112V	1223	−1183	−1.9
V9L, P18L, L79P, M90I, F91S, T102S, L104M, G111R	1224	1493	2.4
P18C, T26M, L44V, M55I, Q62K, L79P, F91S, L104M, T107M	1225	979	1.6
V9I, P18T, D23G, L79P, F91S, G111R	1226	1228	2.0
P18F, L79P, M90L, F91S, V95A, L104M, G111R	1227	1085	1.7
P18F, L79P, M90L, F91S, V95A, L104M, G111R	1227	1455	2.3
P18T, M36T, S65A, S67E, L79Q, A81T, F91S, G111R	1228	−551	−0.9
V9L, P18T, Q62R, L79P, F91S, L104M, G111R	1229	4588	7.4
CD155-ECD-Fc	—	624	1.0
Fc Control	—	0	0.0

TABLE 12E

Bioactivity in Jurkat/IL2 + K562/OKT3 Reporter Assay

CD155 Mutation(s)	SEQ ID NO (IgV)	RLU	Normalized Fold Change from CD155-ECD
P18T, G19D, M36T, S54N, L79P, L83Q, F91S, T107M, F108Y	1702	215	0.3
V9L, P18L, M55V, S69L, L79P, A81E, F91S, T107M	1703	1940	3.1
P18F, H40Q, T61K, Q62K, L79P, F91S, L104M, T107V	1704	691	1.1
P18S, Q32R, Q62K, R78G, L79P, F91S, T107A, R113W	1705	−1361	−2.2
Q12H, P18T, L21S, G22S, V57A, Q62R, L79P, F91S, T107M	1706	−2551	−4.1
V91, P18S, S24P, H49Q, F58Y, Q60R, Q62K, L79P, F91S, T107M	1707	658	1.1
P18T, W46C, H49R, S65A, S67V, A76T, L79P, S87T, L104M	1708	−2131	−3.4
P18S, S42T, E51G, L79P, F91S, G92W, T107M	1709	−1780	−2.9
P18S, S42T, E51G, L79P, F91S, G92W, T107M	1709	−1502	−2.4
V10F, T15S, P18L, R48Q, L79P, F91S, T107M, V115M	1710	−2144	−3.4
P18S, L21M, Y30F, N35D, R84W, F91S, T107M, D116G	1711	−1385	−2.2
P18F, E51V, S54G, Q60R, L79Q, E82G, S87T, M90I, F91S, G92R, T107M	1712	3620	5.8
Q16H, P18F, F91S, T107M	1713	Little to no protein produced	
P18T, D23G, Q60R, S67L, L79P, F91S, T107M, V115A	1714	5374	8.6
D8G, V9I, V11A, P18T, T26M, S52P, L79P, F91S, G92A, T107L, V115A	1715	5235	8.4
V9I, P18F, A47E, G50S, E68G, L79P, F91S, T107M	1716	1870	3.0
P18S, M55I, Q62K, S69P, L79P, F91S, T107M	1717	Little to no protein produced	
P18T, T39S, S52P, S54R, L79P, F91S, T107M	1718	−51	−0.1
P18S, D23N, L79P, F91S, T107M, S114N	1719	Little to no protein produced	
P18S, P34S, E51V, L79P, F91S, G111R	1720	2222	2.1
P18S, H59N, V75A, L79P, A81T, F91S, L104M, T107M	1721	3635	3.5
P18S, W46R, E68D, L79P, F91S, T107M, R113G	1722	958	0.9
V9L, P18F, T45A, S65A, S67V, R78K, L79V, F91S, T107M, S114T	1723	62 03	6.0
P18T, M55L, T61R, L79P, F91S, V106I, T107M	1724	4661	4.5
T15I, P18S, V33M, N35F, T39S, M55L, R78S, L79P, F91S, T107M	1725	1350	1.3
P18S, Q62K, K70E, L79P, F91S, G92E, R113W	1726	2077	2.0
P18F, F20I, T26M, A47V, E51K, L79P, F91S	1727	3188	3.1
P18T, D23A, Q60H, L79P, M90V, F91S, T107M	1728	6959	6.7
P18S, D23G, C29R, N35D, E37G, M55I, Q62K, S65A, S67G, R78G, L79P, F91S, L104M, T107M, Q110R	1729	1199	1.2
A13E, P18S, M36R, Q62K, S67T, L79P, N85D, F91S, T107M	1730	Little to no protein produced	
V9I, P18T, H49R, L79P, N85D, F91S, L104T, T107M	1731	4165	4.0
V9A, P18F, T61S, Q62L, L79P, F91S, G111R	1732	1369	1.3
D8E, P18T, T61A, L79P, F91S, T107M	1733	Little to no protein produced	
P18S, V41A, H49R, S54C, L79S, N85Y, L88P, F91S, L104M, T107M	1734	−1076	−1.0
V11E, P18H, F20Y, V25E, N35S, H49R, L79P, F91S, T107M, G111R	1735	−624	−0.6
V11A, P18F, D23A, L79P, G80D, V95A, T107M	1736	573	0.6
P18S, K70R, L79P, F91S, G111R	1737	−770	−0.7
P18T, D23A, Q60H, L79P, M90V, F91S, T107M	1728	2504	2.4
V9L, V11M, P18S, N35S, S54G, Q62K, L79P, L104M, T107M, V115M	1738	−696	−0.7
V9L, P18Y, V25A, V38G, M55V, A77T, L79P, M90I, F91S, L104M	1739	1198	1.2
V10G, P18T, L72Q, L79P, F91S, T107M	1740	210	0.2
P18S, H59R, A76G, R78S, L79P	1741	2721	2.6
V9A, P18S, M36T, S65G, L79P, F91S, L104T, G111R, S112I	1742	1090	1.1
P18T, S52A, V57A, Q60R, Q62K, S65C, L79P, F91T, N100Y, T107M	1743	2129	2.1
V11A, P18F, N35D, A47E, Q62K, L79P, F91S, G99D, T107M, S114N	1744	Little to no protein produced	
V11A, P18T, N35S, L79P, S87T, F91S	1745	2193	2.1
V9D, V11M, Q12L, P18S, E37V, M55I, Q60R, K70Q, L79P, F91S, L104M, T107M	1746	−394	−0.4
T15S, P18S, Y30H, Q32L, Q62R, L79P, F91S, T107M	1747	189	0.2
CD155-ECD-Fc	—	1034	1.0
Fc Control	—	0	0.0

Example 10

Additional Affinity Modified IgSF Domains

[0648] This examples describe the design, creation, and screening of additional affinity modified CD80 (B7-1), CD112, PD-L1, PD-L2 and CD86 (B37-2) immunomodulatory proteins, which are other components of the immune synapse (IS) that have a demonstrated dual role in both immune activation and inhibition. Affinity-modified NK(p30 variants also were generated and screened. These examples demonstrate that affinity modification of IgSF domains yields proteins that can act to both increase and decrease immunological activity. Various combinations of those domains fused in pairs (i.e., stacked) with a variant affinity modified CD155 to form a Type II immunomodulatory protein to achieve immunomodulatory activity.

[0649] Mutant DNA constructs of encoding a variant of the ECD domain of human CD80, or IgV domains of CD112, PD-L1 and PD-L2 for translation and expression as yeast display libraries were generated substantially as described in Example 1. For target libraries that target specific residues for complete or partial randomization with degenerate codons and/or random libraries were constructed to identify variants of the IgV of CD80 (SEQ ID NO:1043), variants of the IgV of CD112 (SEQ ID NO: 1367), variants of the IgV of PD-L1 (SEQ ID NO:665), and variants of the IgV of PD-L2 (SEQ ID NO:726) substantially as described in Example 1. Similar methods also were used to generate libraries of the IgC-like domain of NKp30 (SEQ ID NO:1168).

[0650] The degenerate or random library DNA was introduced into yeast substantially as described in Example 2 to generate yeast libraries. The libraries were used to select yeast expressing affinity modified variants of CD80, CD112, PD-L1, PD-L2, CD86 (B7-2), and NKp30 substantially as described in Example 3. Cells were processed to reduce non-binders and to enrich for CD80, CD112, PD-L1 or PD-L2 variants with the ability to bind their exogenous recombinant counter-structure proteins substantially as described in Example 3.

[0651] With CD80, CD86 and NKp30 libraries, target ligand proteins were sourced from R&D Systems (USA) as follows: human rCD28.Fc (i.e., recombinant CD28-Fc fusion protein), rPD-L1.Fc, rCTLA4.Fc, and rB7H6.Fc. Two-color flow cytometry was performed substantially as described in Example 3. Yeast outputs from the flow cytometric sorts were assayed for higher specific binding affinity. Sort output yeast were expanded and re-induced to express the particular IgSF affinity modified domain variants they encode. This population then can be compared to the parental, wild-type yeast strain, or any other selected outputs, such as the bead output yeast population, by flow cytometry.

[0652] In the case of NKp30 yeast variants selected for binding to B7-H6, the F2 sort outputs gave MFI values of 533 when stained with 16.6 nM rB7H6.Fc, whereas the parental NKp30 strain MFI was measured at 90 when stained with the same concentration of rB7H6.Fc (6-fold improvement).

[0653] Among the NKp30 variants that were identified, was a variant that contained mutations L30V/A60V/S64P/S86G with reference to positions in the NKp30 extracellular domain corresponding to positions set forth in SEQ ID NO:54.

[0654] For CD80 variants provided in Tables 13A-B, CD80 libraries consisted of positive selection with the desired counter structure CTLA4 and negative selection with the counter structure CD28. For PD-L1 and PD-L2, yeast display targeted or random PD-L1 or PD-L2 libraries were selected against PD-1. Alternatively, for PD-L1, selections were performed with human rCD80.Fc (i.e., human recombinant CD80 Fc fusion protein from R&D Systems, USA). Selections were carried out largely as described for PD-1 . . . . This was then followed by two to three rounds of flow cytometry sorting using exogenous counter-structure protein staining to enrich the fraction of yeast cells that displays improved binders. Magnetic bead enrichment and selections by flow cytometry are essentially as described in Miller, K. D. Current Protocols in Cytometry 4.7.1-4.7.30, July 2008. PD-L1 variants in Table 14A-B were assessed for binding to cell-expressed counter structures. Additional PD-L1 variants identified in the screen as described above are set forth in Table 14C.

[0655] For CD112 variants provided in Table 16A, CD112 libraries were selected against each of TIGIT, CD112R, and CD226, separately. For additional CD112 variants provided in Table 16B-C, selection involved two positive selections with the desired counter structures TIGIT and CD112R followed by one negative selection with the counter structure CD226 to select away from CD226 and improve binding specificity of the variant CD112. Selection was performed essentially as described in Example 3 above except the concentrations of the counter structures (TIGIT/CD112R) and selection stringency of the positive sorts were varied to optimize lead identification. The concentration of CD226 for the negative selection was kept at 100 nM.

[0656] Exemplary selection outputs were reformatted as immunomodulatory proteins containing an affinity modified (variant) IgV of CD80, variant IgV of CD112, variant IgV or ECD of PD-L1, variant IgV of PD-L2, each fused to an Fc molecule (variant IgV-Fc fusion molecules) substantially as described in Example 4 and the Fc-fusion protein was expressed and purified substantially as described in Example 5.

[0657] Binding of exemplary IgSF domain variants to cell-expressed counter structures was then assessed substantially as described in Example 6. Cells expressing cognate binding partners were produced and binding studies and flow cytometry were carried out substantially as described in Example 6. In addition, the bioactivity of the Fc-fusion variant protein was characterized by either mixed lymphocyte reaction (MLR) or anti-CD3 coimmobilization assay substantially as described in Example 6.

[0658] As above, for each Table, the exemplary amino acid substitutions are designated by amino acid position number corresponding to the respective reference unmodified ECD sequence (Table 2). The amino acid position is indicated in the middle, with the corresponding unmodified (e.g. wild-type) amino acid listed before the number and the identified variant amino acid substitution listed (or inserted designated by a) after the number.

[0659] Also shown is the binding activity as measured by the Mean Fluorescence Intensity (MFI) value for binding of each variant Fc-fusion molecule to cells engineered to express the cognate counter structure ligand the ratio of the MFI compared to the binding of the corresponding unmodified Fc fusion molecule not containing the amino acid substitution(s) to the same cell-expressed counter structure

ligand. The functional activity of the PD-L2 variant Fc-fusion molecules to modulate the activity of T cells also is shown based on the calculated levels of IFN-gamma in culture supernatants (pg/ml) generated with the indicated variant Fc fusion molecule in an MLR assay. Table 15B also depicts the ratio of IFN-gamma produced by each variant IgV-Fc compared to the corresponding unmodified IgV-Fc in an MLR assay.

[0660] As shown in Tables 13A-16C, the selections resulted in the identification of a number of CD112, PD-L1, PD-L2, and CD80 IgSF domain variants that were affinity-modified to exhibit increased binding for at least one, and in some cases more than one, cognate counter structure ligand. In addition, the results showed that affinity modification of the variant molecules also exhibited improved activities to both increase and decrease immunological activity.

TABLE 13A

Variant CD80 Binding to HEK293 Cells Transfected with CTLA4, CD28 or PD-L1

		CTLA4		CD28		PD-L1		
CD80 mutation(s)	SEQ ID NO (IgV)	MFI at 66.6 nM	Fold change to WT	MFI at 66.6 nM	Fold change to WT	MFI at 22.2 nM	Fold change to WT	Ratio of CTLA4:CD28
L70P	1044				Not tested			
I30F/L70P	1045				Not tested			
Q27H/T41S/A71D	1046	368176	2.3	25051	1.01	24181	N/A	14.7
I30T/L70R	1047	2234	0.0	2596	0.10	5163	N/A	0.9
T13R/C16R/L70Q/A71D	1048	197357	1.2	16082	0.65	9516	N/A	12.3
T57I	1049	393810	2.4	23569	0.95	3375	N/A	16.7
M43I/C82R	1050	3638	0.0	3078	0.12	7405	N/A	1.2
V22L/M38V/M47T/A71D/L85M	1051	175235	1.1	3027	0.12	6144	N/A	57.9
I30V/T57I/L70P/A71D/A91T	1052	116085	0.7	10129	0.41	5886	N/A	11.5
V22I/L70M/A71D	1053	163825	1.0	22843	0.92	33404	N/A	7.2
N55D/L70P/E77G	1054				Not tested			
T57A/I69T	1055				Not tested			
N55D/K86M	1056	3539	0.0	3119	0.13	5091	N/A	1.1
L72P/T79I	1057	50176	0.3	3397	0.14	6023	N/A	14.8
L70P/F92S	1058	4035	0.0	2948	0.12	6173	N/A	1.4
T79P	1059	2005	0.0	2665	0.11	4412	N/A	0.8
E35D/M47I/L65P/D90N	1060	4411	0.0	2526	0.10	4034	N/A	1.7
L25S/E35D/M47I/D90N	1061	61265	0.4	4845	0.20	20902	N/A	12.6
Q27X*/S44P/I67T/P74S/E81G/E95D	1062	195637	1.2	17524	0.71	17509	N/A	11.2
A71D	1063	220090	1.4	16785	0.68	29642	N/A	13.1
T13A/Q27X*/I61N/A71D	1064	195061	1.2	17519	0.71	21717	N/A	11.1
E81K/A91S	1065	98467	0.6	3309	0.13	44557	N/A	29.8
A12V/M47V/L70M	1066	81616	0.5	7400	0.30	31077	N/A	11.0
K34E/T41A/L72V	1067	88982	0.6	3755	0.15	35293	N/A	23.7
T41S/A71D/V84A	1068	103010	0.6	5573	0.22	83541	N/A	18.5
E35D/A71D	1069	106069	0.7	18206	0.73	40151	N/A	5.8
E35D/M47I	1070	353590	2.2	14350	0.58	149916	N/A	24.6
K36R/G78A	1071	11937	0.1	2611	0.11	5715	N/A	4.6
Q33E/T41A	1072	8292	0.1	2442	0.10	3958	N/A	3.4
M47V/N48H	1073	207012	1.3	14623	0.59	145529	N/A	14.2
M47L/V68A	1074	74238	0.5	13259	0.53	11223	N/A	5.6
S44P/A71D	1075	8839	0.1	2744	0.11	6309	N/A	3.2
Q27H/M43I/A71D/R73S	1076	136251	0.8	12391	0.50	8242	N/A	11.0
E35D/T57I/L70Q/A71D	1078	121901	0.8	21284	0.86	2419	N/A	5.7
M47I/E88D	1079	105192	0.7	7337	0.30	97695	N/A	14.3
M42I/I61V/A71D	1080	54478	0.3	6074	0.24	4226	N/A	9.0
P51A/A71D	1081	67256	0.4	4262	0.17	5532	N/A	15.8
H18Y/M47I/T57I/A71G	1082	136455	0.8	20081	0.81	13749	N/A	6.8
V20I/M47V/T57I/V84I	1083	183516	1.1	26922	1.08	3583	N/A	6.8
WT	1043	161423	1.0	24836	1.00	Not tested	N/A	6.5

*Stop codon at indicated position

TABLE 13B

Variant CD80 Binding to HEK293 Cells Transfected with CTLA4, CD28 or PD-L1

		CTLA4		CD28		PD-L1		
CD80 mutation(s)	SEQ ID NO (IgV)	MFI at 66.6 nM	Fold change to WT	MFI at 66.6 nM	Fold change to WT	MFI at 22.2 nM	Fold change to WT	Ratio of CTLA4:CD28
V20I/M47V/A71D	1084	149937	7.23	15090	9.33	9710	5.48	9.9
A71D/L72V/E95K	1085	140306	6.77	6314	3.90	8417	4.75	22.2
V22L/E35G/A71D/L72P	1086	152588	7.36	8150	5.04	1403	0.79	18.7
E35D/A71D	1087	150330	7.25	14982	9.26	13781	7.77	10.0
E35D/I67L/A71D	1088	146087	7.04	11175	6.91	9354	5.28	13.1
T13R/M42V/M47I/A71D	1090	108900	5.25	16713	10.33	1869	1.05	6.5
E35D	1091	116494	5.62	3453	2.13	25492	14.38	33.7
E35D/M47I/L70M	1092	116531	5.62	14395	8.90	49131	27.71	8.1
E35D/A71/L72V	1093	134252	6.47	11634	7.19	13125	7.40	11.5
E35D/M43L/L70M	1094	102499	4.94	3112	1.92	40632	22.92	32.9
A26P/E35D/M43I/L85Q/ E88D	1095	83139	4.01	5406	3.34	9506	5.36	15.4
E35D/D46V/L85Q	1096	85989	4.15	7510	4.64	38133	21.51	11.4
Q27L/E35D/M47I/T57I/ L70Q/E88D	1097	59793	2.88	14011	8.66	1050	0.59	4.3
Q27H/E35G/A71D/L72P/ T79I	1089	85117	4.10	10317	6.38	1452	0.82	8.3
M47V/I69F/A71D/V83I	1098	76944	3.71	15906	9.83	3399	1.92	4.8
E35D/T57A/A71D/L85Q	1099	85724	4.13	3383	2.09	1764	0.99	25.3
H18Y/A26T/E35D/A71D/ L85Q	1100	70878	3.42	6487	4.01	8026	4.53	10.9
E35D/M47L	1101	82410	3.97	11508	7.11	58645	33.08	7.2
E23D/M42V/M43I/I58V/ L70R	1102	37331	1.80	10910	6.74	2251	1.27	3.4
V68M/L70M/A71D/E95K	1103	56479	2.72	10541	6.51	38182	21.53	5.4
N55I/T57I/I69F	1104	2855	0.14	1901	1.17	14759	8.32	1.5
E35D/M43I/A71D	1105	63789	3.08	6369	3.94	27290	15.39	10.0
T41S/T57I/L70R	1106	59844	2.89	4902	3.03	19527	11.01	12.2
H18Y/A71D/L72P/E88V	1107	68391	3.30	8862	5.48	1085	0.61	7.7
V20I/A71D	1108	60323	2.91	10500	6.49	3551	2.00	5.7
E23G/A26S/E35D/T62N/ A71D/L72V/L85M	1109	59025	2.85	5484	3.39	10662	6.01	10.8
A12T/E24D/E35D/D46V/ I61V/L72P/E95V	1110	63738	3.07	7411	4.58	1221	0.69	8.6
V22L/E35D/M43L/A71G/ D76H	1111	2970	0.14	1498	0.93	1851	1.04	2.0
E35G/K54E/A71D/L72P	1112	71899	3.47	3697	2.29	1575	0.89	19.4
L70Q/A71D	1113	45012	2.17	18615	11.50	1692	0.95	2.4
A26E/E35D/M47L/L85Q	1114	40325	1.94	2266	1.40	55548	31.33	17.8
D46E/A71D	1115	69674	3.36	16770	10.36	22777	12.85	4.2
Y31H/E35D/T41S/V68L/ K93R/R94W	1116	3379	0.16	2446	1.51	18863	10.64	1.4
WT CD80 IgV-Fc	1063) IgV)	20739	1.00	1618	1.00	1773	1.00	12.8
WT CD80 IgV/IgC-Fc	—	72506	3.50	3072	1.90	4418	2.49	23.6

TABLE 14A

Selected PD-L1 variants and binding data.

		Binding to Jurkat/PD-1 Cells	
PD-L1 Mutation(s)	SEQ ID NO (IgV)	MFI at 50 nM	Fold increase over wildtype PD-L1 IgV-Fc
K28N, M41V, N45T, H51N, K57E	600	12585	2.4
I20L, I36T, N45D, I47T	601	3119	0.6
I20L, M41K, K44E	602	9206	1.8
P6S, N45T, N78I, I83T	603	419	0.1
N78I	604	2249	0.4
M41K, N78I	605	Little or no protein produced	
N17D, N45T, V50A, D72G	611	Little or no protein produced	
I20L, F49S	612	Little or no protein produced	
N45T, V50A	613	23887	4.6
I20L, N45T, N78I	614	29104	5.6

TABLE 14A-continued

Selected PD-L1 variants and binding data.

		Binding to Jurkat/PD-1 Cells	
PD-L1 Mutation(s)	SEQ ID NO (IgV)	MFI at 50 nM	Fold increase over wildtype PD-L1 IgV-Fc
N45T, N78I	606	24865	4.7
I20L, N45T	607	24279	4.6
I20L, N45T, V50A	615	34158	6.5
N45T	608	6687	1.3
M41K	609	5079	1.0
M41V, N45T	616	Little or no protein produced	
M41K, N45T	617	Little or no protein produced	
A33D, S75P, D85E	618	685	0.1
M18I, M41K, D43G, H51R, N78I	619	20731	4.0

TABLE 14A-continued

Selected PD-L1 variants and binding data.

PD-L1 Mutation(s)	SEQ ID NO (IgV)	Binding to Jurkat/PD-1 Cells: MFI at 50 nM	Binding to Jurkat/PD-1 Cells: Fold increase over wildtype PD-L1 IgV-Fc
V11E, I20L, I36T, N45D, H60R, S75P	620	3313	0.6
A33D, V50A	621	Little or no protein produced	
S16G, A33D, K71E, S75P	622	Little or no protein produced	
E27G, N45T, M97I	623	881	0.2
E27G, N45T, K57R	624	5022	1.0
A33D, E53V	625	650	0.1
D43G, N45D, V58A	626	63960	12.2
E40G, D43V, N45T, V50A	627	809	0.2
Y14S, K28E, N45T	628	16232	3.1
A33D, N78S	629	1725	0.3
A33D, N78I	630	8482	1.6
A33D, N45T	631	17220	3.3
A33D/N45T/N78I	632	Little or no protein produced	
E27G, N45T, V50A	633	25267	4.8
N45T, V50A, N78S	634	28572	5.4
N45T, V50A	613	18717	3.6
I20L, N45T, V110M	635	464	0.1
I20L, I36T, N45T, V50A	636	7658	1.5
N45T, L74P, S75P	637	5251	1.0
N45T, S75P	638	12200	2.3
S75P, K106R	639	388	0.1
S75P	640	1230	0.2
A33D, S75P	643	306	0.1
A33D, S75P, D104G	642	251	0.0
A33D, S75P	641	1786	0.3
I20L, E27G, N45T, V50A	644	29843	5.7
I20L, E27G, D43G, N45D, V58A, N78I	645	69486	13.3
I20L, D43G, N45D, V58A, N78I	646	72738	13.9
I20L, A33D, D43G, N45D, V58A, N78I	647	80205	15.3

TABLE 14A-continued

Selected PD-L1 variants and binding data.

PD-L1 Mutation(s)	SEQ ID NO (IgV)	Binding to Jurkat/PD-1 Cells: MFI at 50 nM	Binding to Jurkat/PD-1 Cells: Fold increase over wildtype PD-L1 IgV-Fc
I20L, D43G, N45D, N78I	648	67018	12.8
E27G, N45T, V50A, N78I	649	30677	5.9
N45T, V50A, N78I	650	32165	6.1
V11A, I20L, E27G, D43G, N45D, H51Y, S99G	651	73727	14.1
I20L, E27G, D43G, N45T, V50A	652	36739	7.0
I20L, K28E, D43G, N45D, V58A, Q89R, G101G-ins (G101GG)	1755	80549	15.4
I20L, I36T, N45D	610	16870	3.2
I20L, K28E, D43G, N45D, E53G, V58A, N78I	655	139	0.0
A33D, D43G, N45D, V58A, S75P	656	58484	11.2
K23R, D43G, N45D	657	67559	12.9
I20L, D43G, N45D, V58A, N78I, D90G, G101D	658	259	0.0
D43G, N45D, L56Q, V58A, G101G-ins (G101GG)	1755	88277	16.8
I20L, K23E, D43G, N45D, V58A, N78I	660	89608	17.1
I20L, K23E, D43G, N45D, V50A, N78I	661	88829	16.9
T19I, E27G, N45I, V50A, N78I, M97K	662	25496	4.9
I20L, M41K, D43G, N45D	663	599	0.1
K23R, N45T, N78I	664	84980	16.2
Full length PD-L1 Fc	—	18465	3.5
Wild type PD-L1 IgV	665 (IgV)	5243	1.0
Anti-PD-1 monoclonal antibody (nivolumab)	—	79787	15.2
Human IgG	—	198	0.0

TABLE 14B

Flow Binding to Cells Expressing PD-1 or CD80

PD-L1 Mutation(s)	SEQ ID NO (ECD)	PD-1: MFI at 20 nM	PD-1: Fold Change Compared to WT PD-L1	CD80: MFI at 20 nM	CD80: Fold Change Compared to WT PD-L1
K57R, S99G	1757	2953	0.9	16253	121.3
K57R, S99G, F189L	1758	1930	0.6	12906	96.3
M18V, M97L, F193S, R195G, E200K, H202Q	1759	69	0.0	241	1.8
I36S, M41K, M97L, K144Q, R195G, E200K, H202Q, L206F	1760	3498	1.1	68715	512.8
C22R, Q65L, L124S, K144Q, R195G, E200N, H202Q, T221L	1761	Little or no protein produced			
M18V, I98L, L124S, P198T, L206F	1762	2187	0.7	143	1.1
S99G, N117S, I148V, K171R, R180S	1763	Little or no protein produced			
I36T, M97L, A103V, Q155H	1764	120	0.0	128	1.0
K28I, S99G	1765	830	0.3	693	5.2
R195S	1766	3191	1.0	138	1.0
A79T, S99G, T185A, R195G, E200K, H202Q, L206F	1767	1963	0.6	643	4.8
K57R, S99G, L124S, K144Q	1768	2081	0.7	14106	105.3
K57R, S99G, R195G	1769	2479	0.8	10955	81.8
D55V, M97L, S99G	1770	11907	3.8	71242	531.7

TABLE 14B-continued

Flow Binding to Cells Expressing PD-1 or CD80					
		PD-1		CD80	
PD-L1 Mutation(s)	SEQ ID NO (ECD)	MFI at 20 nM	Fold Change Compared to WT PD-L1	MFI at 20 nM	Fold Change Compared to WT PD-L1
E27G, I36T, D55N, M97L, K111E	1771	1904	0.6	88724	662.1
E54G, M97L, S99G	1772	8414	2.7	51905	387.4
G15A, I36T, M97L, K111E, H202Q	1773	112	0.0	13530	101.0
G15A, I36T, V129D	1774	114	0.0	136	1.0
G15A, I36T, V129D, R195G	1775	125	0.0	134	1.0
G15A, V129D	1776	2075	0.7	128	1.0
I36S, M97L	1777	3459	1.1	44551	332.5
I36T, D55N, M97L, K111E, A204T	1778	265	0.1	62697	467.9
I36T, D55N, M97L, K111E, V129A, F173L	1779	393	0.1	72641	542.1
I36T, D55S, M97L, K111E, I148V, R180S	1780	94	0.0	30704	229.1
I36T, G52R, M97L, V112A, K144E, V175A, P198T	1781	81	0.0	149	1.1
I36T, I46V, D55G, M97L, K106E, K144E, T185A, R195G	1782	69	0.0	190	1.4
I36T, I83T, M97L, K144E, P198T	1783	62	0.0	6216	46.4
I36T, M97L, K111E	1784	Little or no protein produced			
I36T, M97L, K144E, P198T	1785	197	0.1	40989	305.9
I36T, M97L, Q155H, F193S, N201Y	1786	69	0.0	1251	9.3
I36T, M97L, V129D	1787	523	0.2	50905	379.9
L35P, I36S, M97L, K111E	1788	190	0.1	155	1.2
M18I, I36T, E53G, M97L, K144E, E199G, V207A	1789	104	0.0	47358	353.4
M18T, I36T, D55N, M97L, K111E	1790	138	0.0	71440	533.1
M18V, M97L, T176N, R195G	1791	1301	0.4	45300	338.1
M97L, S99G	1792	12906	4.1	81630	609.2
N17D, M97L, S99G	1793	10079	3.2	73249	546.6
S99G, T185A, R195G, P198T	1794	2606	0.8	22062	164.6
V129D, H202Q	1795	2001	0.6	219	1.6
V129D, P198T	1796	3245	1.0	152	1.1
V129D, T150A	1797	1941	0.6	142	1.1
V93E, V129D	1798	1221	0.4	150	1.1
Y10F, M18V, S99G, Q138R, T203A	1799	70	0.0	412	3.1
WT PD-L1 (IgV + IgC) Fc	—	3121	1.0	134	1.0
CTLA4-Fc	—	59	N/A	199670	N/A
Anti-PD1 mAb	—	31482	N/A	134	N/A
Fc Control	1155	59	N/A	132	N/A

TABLE 14C

Additional Affinity-Matured IgSF Domain-Containing Molecules	
PD-L1 Mutation(s)	SEQ ID NO (ECD)
N45D	1800
K160M, R195G	1801
N45D, K144E	1802
N45D, P198S	1803
N45D, P198T	1804
N45D, R195G	1805
N45D, R195S	1806
N45D, S131F	1807
N45D, V58D	1808
V129D, R195S	1809
I98T, F173Y, L196S	1810
N45D, E134G, L213P	1811
N45D, F173I, S177C	1812

TABLE 14C-continued

Additional Affinity-Matured IgSF Domain-Containing Molecules	
PD-L1 Mutation(s)	SEQ ID NO (ECD)
N45D, I148V, R195G	1813
N45D, K111T, R195G	1814
N45D, N113Y, R195S	1815
N45D, N165Y, E170G	1816
N45D, Q89R, I98V	1817
N45D, S131F, P198S	1818
N45D, S75P, P198S	1819
N45D, V50A, R195T	1820
E27D, N45D, T183A, I188V	1821
F173Y, T183I, L196S, T203A	1822
K23N, N45D, S75P, N120S	1823
N45D, G102D, R194W, R195G	1824
N45D, G52V, Q121L, P198S	1825

TABLE 14C-continued

Additional Affinity-Matured IgSF Domain-Containing Molecules

PD-L1 Mutation(s)	SEQ ID NO (ECD)
N45D, I148V, R195G, N201D	1826
N45D, K111T, T183A, I188V	1827
N45D, Q89R, F189S, P198S	1828
N45D, S99G, C137R, V207A	1829
N45D, T163I, K167R, R195G	1830
N45D, T183A, T192S, R194G	1831
N45D, V50A, I119T, K144E	1832
T19A, N45D, K144E, R195G	1833
V11E, N45D, T130A, P198T	1834
V26A, N45D, T163I, T185A	1835
K23N, N45D, L124S, K167T, R195G	1836
K23N, N45D, Q73R, T163I	1837

TABLE 14C-continued

Additional Affinity-Matured IgSF Domain-Containing Molecules

PD-L1 Mutation(s)	SEQ ID NO (ECD)
K28E, N45D, W149R, S158G, P198T	1838
K28R, N45D, K57E, I98V, R195S	1839
K28R, N45D, V129D, T163N, R195T	1840
M41K, D43G, N45D, R64S, R195G	1841
M41K, D43G, N45D, R64S, S99G	1842
N45D, R68L, F173L, D197G, P198S	1843
N45D, V50A, I148V, R195G, N201D	1844
M41K, D43G, K44E, N45D, R195G, N201D	1845
N45D, V50A, L124S, K144E, L179P, R195G	1846

TABLE 15A

Variant PD-L2 selected against PD-1. Molecule sequence and binding data.

PD-L2 mutation(s)	SEQ ID NO (IgV)	Binding to Jurkat/PD-1 Cells: MFI at 50 nM	Binding to Jurkat/PD-1 Cells: Fold increase over wildtype PD-L2 IgV-Fc	Fortebio binding to PD-1-Fc Response Units
H15Q	667	15998	1.63	0.007
N24D	668	1414	0.14	−0.039
E44D	669	2928	0.3	−0.006
V89D	670	3361	0.34	0.005
Q82R, V89D	671	44977	4.57	1.111
E59G, Q82R	672	12667	1.29	−0.028
S39I, V89D	673	26130	2.65	0.26
S67L, V89D	674	15991	1.62	0.608
S67L, I85F	675	529	0.05	−0.005
S67L, I86T	676	6833	0.69	0.141
H15Q, K65R	677	13497	1.37	−0.001
H15Q, Q72H, V89D	678	12629	1.28	0.718
H15Q, S67L, R76G	679	47201	4.8	0.418
H15Q, R76G, I85F	680	2941	0.3	−0.038
H15Q, T47A, Q82R	681	65174	6.62	0.194
H15Q, Q82R, V89D	682	49652	5.04	1.198
H15Q, C23S, I86T	683	830	0.08	−0.026
H15Q, S39I, I86T	684	1027	0.1	0.309
H15Q, R76G, I85F	685	1894	0.19	−0.006
E44D, V89D, W91R	686	614	0.06	−0.048
I13V, S67L, V89D	687	26200	2.66	1.42
H15Q, S67L, I86T	688	15952	1.62	0.988
I13V, H15Q, S67L, I86T	689	21570	2.19	1.391
I13V, H15Q, E44D, V89D	690	23958	2.43	1.399
I13V, S39I, E44D, Q82R, V89D	691	71423	7.26	0.697
I13V, E44D, Q82R, V89D	692	45191	4.59	1.283
I13V, Q72H, R76G, I86T	693	10429	1.06	0.733
I13V, H15Q, R76G, I85F	694	4736	0.48	−0.04
H15Q, S67L, R76G, I85F	696	2869	0.29	0.025
H15Q, S39I, R76G, V89D	695	Little or no protein produced		
H15Q, T47A, Q72H, R76G, I86T	697	32103	3.26	0.512
H15Q, T47A, Q72H, R76G	698	16500	1.68	0.327
I13V, H15Q, T47A, Q72H, R76G	699	73412	7.46	0.896
H15Q, E44D, R76G, I85F	700	2885	0.29	−0.013
H15Q, S39I, S67L, V89D	701	45502	4.62	1.174
H15Q, N32D, S67L, V89D	702	25880	2.63	1.407
N32D, S67L, V89D	703	31753	3.23	1.155
H15Q, S67L, Q72H, R76G, V89D	704	40180	4.08	1.464
H15Q, Q72H, Q74R, R76G, I86T	705	4049	0.41	0.093
G28V, Q72H, R76G, I86T	706	5563	0.57	0.003
I13V, H15Q, S39I, E44D, S67L	707	63508	6.45	0.889
E44D, S67L, Q72H, Q82R, V89D	708	51467	5.23	1.061
H15Q, V89D	709	17672	1.8	0.31
H15Q, T47A	710	26578	2.7	0.016

TABLE 15A-continued

Variant PD-L2 selected against PD-1. Molecule sequence and binding data.

PD-L2 mutation(s)	SEQ ID NO (IgV)	Binding to Jurkat/PD-1 Cells: MFI at 50 nM	Binding to Jurkat/PD-1 Cells: Fold increase over wildtype PD-L2 IgV-Fc	Fortebio binding to PD-1-Fc Response Units
I13V, H15Q, Q82R	711	76146	7.74	0.655
I13V, H15Q, V89D	712	28745	2.92	1.331
I13V, S67L, Q82R, V89D	713	58992	5.99	1.391
I13V, H15Q, Q82R, V89D	714	49523	5.03	1.419
H15Q, V31M, S67L, Q82R, V89D	715	67401	6.85	1.37
I13V, H15Q, T47A, Q82R	716	89126	9.05	0.652
I13V, H15Q, V31A, N45S, Q82R, V89D	717	68016	6.91	1.327
H15Q, T47A, H69L, Q82R, V89D	719	65598	6.66	1.44
I13V, H15Q, T47A, H69L, R76G, V89D	720	54340	5.52	1.719
I12V, I13V, H15Q, T47A, Q82R, V89D	721	61207	6.22	1.453
I13V, H15Q, R76G, D77N, Q82R, V89D	722	33079	3.36	0.065
I13V, H15Q, T47A, R76G, V89D	723	53668	5.45	1.596
I13V, H15Q, T47A, Q82R, V89D	724	63320	6.43	1.418
I13V, H15Q, T47A, Q82R, V89D	725	60980	6.2	1.448
I13V, H15Q, I36V, T47A, S67L, V89D	727	52835	5.37	1.627
H15Q, T47A, K65R, S67L, Q82R, V89D	728	79692	8.1	1.453
H15Q, L33P, T47A, S67L, P71S, V89D	729	45726	4.65	1.467
I13V, H15Q, Q72H, R76G, I86T	730	24450	2.48	1.355
H15Q, T47A, S67L, Q82R, V89D	731	67962	6.9	1.479
F2L, H15Q, D46E, T47A, Q72H, R76G, Q82R, V89D	732	23039	2.34	1.045
I13V, H15Q, L33F, T47A, Q82R, V89D	733	62254	6.32	1.379
H15Q, N24S, T47A, Q72H, R76G, V89D	735	32077	3.26	0.4
I13V, H15Q, E44V, T47A, Q82R, V89D	736	61005	6.2	1.329
H15Q, N18D, T47A, Q72H, V73A, R76G, I86T, V89D	737	48317	4.91	0.475
I13V, H15Q, T37A, E44D, S48C, S67L, Q82R, V89D	738	47605	4.84	1.255
H15Q, L33H, S67L, R76G, Q82R, V89D	739	62326	6.33	1.507
I13V, H15Q, T47A, Q72H, R76G, I86T	740	49016	4.98	1.477
H15Q, S39I, E44D, Q72H, V75G, R76G, Q82R, V89D	741	43713	4.44	0.646
H15Q, T47A, S67L, R76G, Q82R, V89D	742	71897	7.3	1.539
I13V, H15Q, T47A, S67L, Q72H, R76G, Q82R, V89D	743	71755	7.29	1.536
Wild Type PD-L2 IgV	726	9843	1	−0.024
Full length ECD of PD-L2	31	2145	0.22	0.071
Full length ECD of PD-L1 (R&D Systems)	30	23769	2.41	1.263
Anti-PD-1 monoclonal antibody (nivolumab)	—	87002	8.84	0.899

TABLE 15B

Bioactivity Data of PD-L2 variants selected against PD-1 in MLR.

PD-L2 mutation(s)	SEQ ID NO (IgV)	IFN gamma levels pg/ml	Fold increase over wildtype PD-L2 IgV-Fc
H15Q	667	1817.1	1.32
N24D	668	1976.3	1.44
E44D	669	1499.4	1.09
V89D	670	1168.1	0.85
Q82R, V89D	671	1617	1.17
E59G, Q82R	672	1511.3	1.1
S39I, V89D	673	1314.5	0.95
S67L, V89D	674	1230.1	0.89
S67L, I85F	675	1281.9	0.93
S67L, I86T	676	1020.4	0.74
H15Q, K65R	677	1510.8	1.1
H15Q, Q72H, V89D	678	1272.2	0.92
H15Q, S67L, R76G	679	1426.2	1.04
H15Q, R76G, I85F	680	1725.7	1.25
H15Q, T47A, Q82R	681	1317.9	0.96
H15Q, Q82R, V89D	682	1081.2	0.79
H15Q, C23S, I86T	683	1847.2	1.34
H15Q, S39I, I86T	684	1415.2	1.03

TABLE 15B-continued

Bioactivity Data of PD-L2 variants selected against PD-1 in MLR.

PD-L2 mutation(s)	SEQ ID NO (IgV)	IFN gamma levels pg/ml	Fold increase over wildtype PD-L2 IgV-Fc
H15Q, R76G, I85F	685	1437.8	1.04
E44D, V89D, W91R	686	1560.1	1.13
I13V, S67L, V89D	687	867.5	0.63
H15Q, S67L, I86T	688	1034.2	0.75
I13V, H15Q, S67L, I86T	689	1014.4	0.74
I13V, H15Q, E44D, V89D	690	1384.2	1.01
I13V, S39I, E44D, Q82R, V89D	691	935.6	0.68
I13V, E44D, Q82R, V89D	692	1009.5	0.73
I13V, Q72H, R76G, I86T	693	1953	1.42
I13V, H15Q, R76G, I85F	694	1528.5	1.11
H15Q, S67L, R76G, I85F	696	1318.7	0.96
H15Q, T47A, Q72H, R76G, I86T	697	1599.6	1.16
H15Q, T47A, Q72H, R76G	698	1462.5	1.06
I13V, H15Q, T47A, Q72H, R76G	699	1469.8	1.07
H15Q, E44D, R76G, I85F	700	1391.6	1.01
H15Q, S39I, S67L, V89D	701	1227	0.89
H15Q, N32D, S67L, V89D	702	1285.7	0.93
N32D, S67L, V89D	703	1194	0.87

TABLE 15B-continued

Bioactivity Data of PD-L2 variants selected against PD-1 in MLR.

PD-L2 mutation(s)	SEQ ID NO (IgV)	IFN gamma levels pg/ml	Fold increase over wildtype PD-L2 IgV-Fc
H15Q, S67L, Q72H, R76G, V89D	704	1061.2	0.77
H15Q, Q72H, Q74R, R76G, I86T	705	933.8	0.68
G28V, Q72H, R76G, I86T	706	1781.6	1.29
I13V, H15Q, S39I, E44D, S67L	707	1256.9	0.91
E44D, S67L, Q72H, Q82R, V89D	708	1281.4	0.93
H15Q, V89D	709	1495.4	1.09
H15Q, T47A	710	1637.2	1.19
I13V, H15Q, Q82R	711	1432.9	1.04
I13V, H15Q, V89D	712	1123	0.82
I13V, S67L, Q82R, V89D	713	1372.8	1
I13V, H15Q, Q82R, V89D	714	1596.6	1.16
H15Q, V31M, S67L, Q82R, V89D	715	1206.5	0.88
I13V, H15Q, T47A, Q82R	716	1703.3	1.24
I13V, H15Q, V31A, N45S, Q82R, V89D	717	1723.1	1.25
H15Q, T47A, H69L, Q82R, V89D	719	1732.5	1.26
I13V, H15Q, T47A, H69L, R76G, V89D	720	1075.5	0.78
I12V, I13V, H15Q, T47A, Q82R, V89D	721	1533.2	1.11
I13V, H15Q, R76G, D77N, Q82R, V89D	722	1187.9	0.86
I13V, H15Q, T47A, R76G, V89D	723	1253.7	0.91
I13V, H15Q, T47A, Q82R, V89D	724	1445.5	1.05
I13V, H15Q, T47A, Q82R, V89D	725	1737	1.26
I13V, H15Q, I36V, T47A, S67L, V89D	727	1357.4	0.99
H15Q, T47A, K65R, S67L, Q82R, V89D	728	1335.3	0.97
H15Q, L33P, T47A, S67L, P71S, V89D	729	1289.1	0.94
I13V, H15Q, Q72H, R76G, I86T	730	1221	0.89

TABLE 15B-continued

Bioactivity Data of PD-L2 variants selected against PD-1 in MLR.

PD-L2 mutation(s)	SEQ ID NO (IgV)	IFN gamma levels pg/ml	Fold increase over wildtype PD-L2 IgV-Fc
H15Q, T47A, S67L, Q82R, V89D	731	1197.1	0.87
F2L, H15Q, D46E, T47A, Q72H, R76G, Q82R, V89D	737	1170.7	0.85
I13V, H15Q, L33F, T47A, Q82R, V89D	733	1468.4	1.07
I13V, H15Q, T47A, E58G, S67L, Q82R, V89D	734	836.1	0.61
H15Q, N24S, T47A, Q72H, R76G, V89D	735	1091.8	0.79
I13V, H15Q, E44V, T47A, Q82R, V89D	736	1270.5	0.92
H15Q, N18D, T47A, Q72H, V73A, R76G, I86T, V89D	737	1065.8	0.77
I13V, H15Q, T37A, E44D, S48C, S67L, Q82R, V89D	738	1751.7	1.27
H15Q, L33H, S67L, R76G, Q82R, V89D	739	1502	1.09
I13V, H15Q, T47A, Q72H, R76G, I86T	740	1088.1	0.79
H15Q, S39I, E44D, Q72H, V75G, R76G, Q82R, V89D	741	940.9	0.68
H15Q, T47A, S67L, R76G, Q82R, V89D	742	1097.8	0.8
I13V, H15Q, T47A, S67L, Q72H, R76G, Q82R, V89D	743	1559.6	1.13
Wild Type PD-L2 IgV	726	1376.8	1
Full length ECD of PD-L2	31	1173.2	0.85
Full length ECD of PD-L1	30	2190.9	1.59
Nivolumab (anti-PD-1)	—	418.9	0.3

TABLE 16A

Variant CD112 selected against cognate binding partners. Molecule sequences, binding data, and costimulatory bioactivity data.

CD112 mutation(s)	SEQ ID NO (IgV)	TIGIT tfxn MFI (TIGIT MFI parental ratio)	CD112R tfxn MFI (CD112R MFI parental ratio)	CD226 MFI (CD226 MFI parental ratio)	Mock Expi293 MFI (Mock MFI parental ratio)	Anti-CD3 IFN-gamma (pg/ml) (Anti-CD3 IFN-gamma parental ratio)
WT CD112	1367	210829 (1.00)	1452 (1.00)	265392 (1.00)	1112 (1.00)	676.6 (1.00)
Y33H, A112V, G117D	1368	12948 (0.06)	1552 (1.07)	1368 (0.01)	1241 (1.12)	164.8 (0.24)
V19A, Y33H, S64G, S80G, G98S, N106Y, A112V	1369	48356 (0.23)	1709 (1.18)	2831 (0.01)	1098 (0.99)	
L32P, A112V	1370	191432 (0.91)	1557 (1.07)	11095 (0.04)	1259 (1.13)	390.4 (0.58)
A95V, A112I	1371	238418 (1.13)	1706 (1.17)	51944 (0.20)	1215 (1.09)	282.5 (0.42)
P28S, A112V	1372	251116 (1.19)	1985 (1.37)	153382 (0.58)	1189 (1.07)	503.4 (0.74)
P27A, T38N, V101A, A112V	1373	255803 (1.21)	2138 (1.47)	222822 (0.84)	1399 (1.26)	240.7 (0.36)
S118F	1374	11356 (0.05)	5857 (4.03)	6938 (0.03)	1270 (1.14)	271.7 (0.40)
R12W, H48Y, F54S, S118F	1375	10940 (0.05)	3474 (2.39)	5161 (0.02)	1069 (0.96)	
R12W, Q79R, S118F	1376	2339 (0.01)	7370 (5.08)	1880 (0.01)	1338 (1.20)	447.4 (0.66)
T113S, S118Y	1377	6212 (0.03)	6823 (4.70)	1554 (0.01)	1214 (1.09)	225.1 (0.33)
S118Y	1378	2921 (0.01)	6535 (4.50)	2003 (0.01)	1463 (1.32)	190.4 (0.28)
N106I, S118Y	1379	2750 (0.01)	7729 (5.32)	1815 (0.01)	1222 (1.10)	265.8 (0.39)

TABLE 16A-continued

Variant CD112 selected against cognate binding partners. Molecule sequences, binding data, and costimulatory bioactivity data.

CD112 mutation(s)	SEQ ID NO (IgV)	TIGIT tfxn MFI (TIGIT MFI parental ratio)	CD112R tfxn MFI (CD112R MFI parental ratio)	CD226 MFI (CD226 MFI parental ratio)	Mock Expi293 MFI (Mock MFI parental ratio)	Anti-CD3 IFN-gamma (pg/ml) (Anti-CD3 IFN-gamma parental ratio)
N106I, S118F	1380	1841 (0.01)	9944 (6.85)	1529 (0.01)	1308 (1.18)	437.9 (0.65)
A95T, L96P, S118Y	1381	2352 (0.01)	4493 (3.09)	1412 (0.01)	1329 (1.19)	292.4 (0.43)
Y33H, P67S, N106Y, A112V	1382	225015 (1.07)	3259 (2.24)	204434 (0.77)	1296 (1.17)	618.8 (0.91)
N106Y, A112V	1383	6036 (0.03)	1974 (1.36)	15334 (0.06)	1108 (1.00)	409.9 (0.61)
T18S, Y33H, A112V	1384	252647 (1.20)	1347 (0.93)	183181 (0.69)	1412 (1.27)	601.8 (0.89)
P9S, Y33H, N47S, A112V	1385	240467 (1.14)	1418 (0.98)	203608 (0.77)	1361 (1.22)	449.1 (0.66)
P42S, P67H, A112V	1386	204484 (0.97)	1610 (1.11)	188647 (0.71)	1174 (1.06)	530.6 (0.78)
P27L, L32P, P42S, A112V	1387	219883 (1.04)	1963 (1.35)	84319 (0.32)	1900 (1.71)	251.6 (0.37)
G98D, A112V	1388	4879 (0.02)	2369 (1.63)	6100 (0.02)	1729 (1.55)	387.0 (0.57)
Y33H, S35P, N106Y, A112V	1389	250724 (1.19)	1715 (1.18)	94373 (0.36)	1495 (1.34)	516.2 (0.76)
L32P, P42S, T100A, A112V	1390	242675 (1.15)	1742 (1.20)	202567 (0.76)	1748 (1.57)	435.3 (0.64)
P27S, P45S, N106I, A112V	1391	223557 (1.06)	1799 (1.24)	84836 (0.32)	1574 (1.42)	277.5 (0.41)
Y33H, N47K, A112V	1392	251339 (1.19)	1525 (1.05)	199601 (0.75)	1325 (1.19)	483.2 (0.71)
Y33H, N106Y, A112V	1393	297169 (1.41)	1782 (1.23)	258315 (0.97)	1440 (1.30)	485.4 (0.72)
K78R, D84G, A112V, F114S	1394	236662 (1.12)	1638 (1.13)	24850 (0.09)	1345 (1.21)	142.5 (0.21)
Y33H, N47K, F54L, A112V	1395	14483 (0.07)	1617 (1.11)	2371 (0.01)	1353 (1.22)	352.8 (0.52)
Y33H, A112V	1396	98954 (0.47)	1216 (0.84)	1726 (0.01)	1298 (1.17)	
A95V, A112V	1397	168521 (0.80)	2021 (1.39)	200789 (0.76)	1459 (1.31)	412.9 (0.61)
R12W, A112V	1398	135635 (0.64)	1582 (1.09)	23378 (0.09)	1412 (1.27)	165.8 (0.24)
A112V	1404	213576 (1.01)	1986 (1.37)	151900 (0.57)	1409 (1.27)	211.4 (0.31)
Y33H, A112V	1396	250667 (1.19)	1628 (1.12)	230578 (0.87)	1216 (1.09)	612.7 (0.91)
R12W, P27S, A112V	1399	3653 (0.02)	1308 (0.90)	9105 (0.03)	1051 (0.94)	
Y33H, V51M, A112V	1400	218698 (1.04)	1384 (0.95)	195450 (0.74)	1170 (1.05)	709.4 (1.05)
Y33H, A112V, S118T	1401	219384 (1.04)	1566 (1.08)	192645 (0.73)	1313 (1.18)	396.3 (0.59)
Y33H, V101A, A112V, P115S	1402	5605 (0.03)	1582 (1.09)	5079 (0.02)	1197 (1.08)	
H24R, T38N, D43G, A112V	1403	227095 (1.08)	1537 (1.06)	229311 (0.86)	1336 (1.20)	858.6 (1.27)
A112V	1404	4056 (0.02)	1356 (0.93)	10365 (0.04)	986 (0.89)	
P27A, A112V	1405	193537 (0.92)	1531 (1.05)	230708 (0.87)	3084 (2.77)	355.1 (0.52)
A112V, S118T	1406	233173 (1.11)	1659 (1.14)	121817 (0.46)	845 (0.76)	533.3 (0.79)
R12W, A112V, M122I	1407	235935 (1.12)	1463 (1.01)	217748 (0.82)	1350 (1.21)	528.0 (0.78)
Q83K, N106Y, A112V	1408	205948 (0.98)	2042 (1.41)	234958 (0.89)	1551 (1.39)	481.4 (0.71)
R12W, P27S, A112V, S118T	1409	11985 (0.06)	2667 (1.84)	12756 (0.05)	1257 (1.13)	334.4 (0.49)
P28S, Y33H, A112V	1410	4711 (0.02)	1412 (0.97)	3968 (0.01)	955 (0.86)	

TABLE 16A-continued

Variant CD112 selected against cognate binding partners. Molecule sequences, binding data, and costimulatory bioactivity data.

CD112 mutation(s)	SEQ ID NO (IgV)	TIGIT tfxn MFI (TIGIT MFI parental ratio)	CD112R tfxn MFI (CD112R MFI parental ratio)	CD226 MFI (CD226 MFI parental ratio)	Mock Expi293 MFI (Mock MFI parental ratio)	Anti-CD3 IFN-gamma (pg/ml) (Anti-CD3 IFN-gamma parental ratio)
P27S, Q90R, A112V	1411	3295 (0.02)	1338 (0.92)	6755 (0.03)	1048 (0.94)	
L15V, P27A, A112V, S118T	1412	209888 (1.00)	1489 (1.03)	84224 (0.32)	1251 (1.13)	512.3 0.76)
Y33H, N106Y, T108I, A112V	1413			Not tested		
Y33H, P56L, V75M, V101M, A112V	1414			Not tested		

TABLE 16B

Additional CD112 Variants and Binding Data.

		TIGIT		CD226		CD112R		CD96	
CD112 Mutation(s)	SEQ ID NO (IgV)	MFI 100 nM	Fold Increase to WT IgV	MFI at 100 nM	Fold Increase to WT IgV	MFI at 100 nM	Fold Increase to WT IgV	MFI at 100 nM	Fold Increase to WT IgV
S118F	1374	1763	0.02	1645	0.08	2974	0.61	1659	0.19
N47K, Q79R, S118F	1497	1738	0.02	1689	0.09	2637	0.54	1647	0.19
Q40R, P60T, A112V, S118T	1498	4980	0.06	1608	0.08	2399	0.50	2724	0.32
F114Y, S118F	1499	110506	1.34	7325	0.37	1502	0.31	1553	0.18
N106I, S118Y	1379	1981	0.02	1700	0.09	2394	0.49	1582	0.19
S118Y	1378	101296	1.23	9990	0.50	1429	0.30	1551	0.18
Y33H, K78R, S118Y	1500	2276	0.03	2115	0.11	3429	0.71	2082	0.24
N106I, S118F	1380	1875	0.02	1675	0.08	2365	0.49	1662	0.19
R12W, A46T, K66M, Q79R, N106I, T113A, S118F	1501	3357	0.04	1808	0.09	1664	0.34	4057	0.48
Y33H, A112V, S118F	1502	3376	0.04	2886	0.15	3574	0.74	3685	0.43
R12W, Y33H, N106I, S118F	1503	100624	1.22	24513	1.24	1490	0.31	2060	0.24
L15V, Q90R, S118F	1504	5791	0.07	4169	0.21	2752	0.57	4458	0.52
N47K, D84G, N106I, S118Y	1505	3334	0.04	2819	0.14	2528	0.52	3498	0.41
L32P, S118F	1506	3881	0.05	2506	0.13	2659	0.55	2518	0.29
Y33H, Q79R, A112V, S118Y	1507	Low to no protein produced							
T18A, N106I, S118T	1508	84035	1.02	10208	0.52	1585	0.33	1590	0.19
L15V, Y33H, N106Y, A112V, S118F	1509	Low to no protein produced							
V37M, S118F	1510	96986	1.18	2523	0.13	1985	0.41	1849	0.22
N47K, A112V, S118Y	1511	1980	0.02	1859	0.09	2733	0.56	1825	0.21
A46T, A112V	1512	4224	0.05	4685	0.24	3288	0.68	4273	0.50
P28S, Y33H, N106I, S118Y	1513	6094	0.07	2181	0.11	1891	0.39	3021	0.35
P30S, Y33H, N47K, V75M, Q79R, N106I, S118Y	1514	2247	0.03	2044	0.10	1796	0.37	2658	0.31
V19A, N47K, N106Y, K116E, S118Y	1515	2504	0.03	2395	0.12	2174	0.45	2852	0.33
Q79R, T85A, A112V, S118Y	1516	2192	0.03	1741	0.09	2367	0.49	1620	0.19
Y33H, A112V	1396	20646	0.25	1465	0.07	1794	0.37	2589	0.30
V101M, N106I, S118Y	1517	55274	0.67	6625	0.33	1357	0.28	1494	0.17
Y33H, Q79R, N106I, A112V, S118T	1518	6095	0.07	1760	0.09	2393	0.49	3033	0.36
Q79R, A112V	1519	1571	0.02	1490	0.08	2284	0.47	1326	0.16
Y33H, A46T, Q79R, N106I, S118F	1520	90813	1.10	15626	0.79	1298	0.27	3571	0.42
A112V, G121S	1521	95674	1.16	19992	1.01	1252	0.26	4005	0.47
Y33H, Q79R, N106I, S118Y	1522	36246	0.44	2118	0.11	1970	0.41	3250	0.38

TABLE 16B-continued

Additional CD112 Variants and Binding Data.

CD112 Mutation(s)	SEQ ID NO (IgV)	TIGIT MFI 100 nM	TIGIT Fold Increase to WT IgV	CD226 MFI at 100 nM	CD226 Fold Increase to WT IgV	CD112R MFI at 100 nM	CD112R Fold Increase to WT IgV	CD96 MFI at 100 nM	CD96 Fold Increase to WT IgV
Y33H, N106I, A112V	1523	47352	0.57	4217	0.21	2641	0.55	1488	0.17
Y33H, A46T, V101M, A112V, S118T	1524	14413	0.17	1596	0.08	2335	0.48	1441	0.17
L32P, L99M, N106I, S118F	1525	3056	0.04	1791	0.09	2210	0.46	2000	0.23
L32P, T108A, S118F	1526	104685	1.27	4531	0.23	2308	0.48	1518	0.18
A112V	1404	4937	0.06	1903	0.10	1646	0.34	3011	0.35
R12W, Q79R, A112V	1527	55539	0.67	6918	0.35	1386	0.29	1740	0.20
Y33H, N106Y, E110G, A112V	1528	2786	0.03	2517	0.13	1787	0.37	2023	0.24
Y33H, N106I, S118Y	1529	1967	0.02	1579	0.08	2601	0.54	1517	0.18
Q79R, S118F	1530	82055	1.00	7582	0.38	1298	0.27	1970	0.23
Y33H, Q79R, G98D, V101M, A112V	1531	21940	0.27	1632	0.08	1141	0.24	18423	2.16
N47K, T81S, V101M, A112V, S118F	1532	6889	0.08	1311	0.07	1303	0.27	1145	0.13
G82S, S118Y	1533	4267	0.05	1938	0.10	2140	0.44	2812	0.33
Y33H, A112V, S118Y	1534	14450	0.18	1532	0.08	2353	0.49	3004	0.35
Y33H, N47K, Q79R, N106Y, A112V	1535	70440	0.85	3557	0.18	1447	0.30	1679	0.20
Y33H, S118T	1536	113896	1.38	17724	0.89	1252	0.26	5001	0.59
R12W, Y33H, Q79R, V101M, A112V	1537	3376	0.04	2727	0.14	2047	0.42	2339	0.27
S118F	1374	2685	0.03	1864	0.09	2520	0.52	1566	0.18
Wildtype CD112-IgV Fc	1367 (IgV)	82414	1.00	19803	1.00	4842	1.00	8541	1.00
CD112 ECD-Fc	48 (ECD)	29157	0.35	8755	0.44	1107	0.23	1103	0.13
Anti-hFc PE	—	1383	0.02	1461	0.07	1358	0.28	1468	0.17

TABLE 16C

Additional CD112 Variants and Binding Data.

CD112 Mutation(s)	SEQ ID NO (IgV)	TIGIT MFI 20 nM	TIGIT Fold Increase to WT IgV	CD226 MFI at 20 nM	CD226 Fold Increase to WT IgV	CD112R MFI at 20 nM	CD112R Fold Increase to WT IgV	CD96 MFI at 20 nM	CD96 Fold Increase to WT IgV
N106I, S118Y	1379	1288	0.04	1334	0.12	6920	4.16	1102	0.44
Y33H, Q83K, A112V, S118T	1586	115690	3.31	10046	0.93	1128	0.68	2053	0.82
R12W, Q79R, S118F	1376	1436	0.04	1296	0.12	6546	3.93	1046	0.42
V29M, Y33H, N106I, S118F	1587	Not tested							
Y33H, A46T, A112V	1588	111256	3.18	14974	1.39	1148	0.69	3333	1.34
Y33H, Q79R, S118F	1589	1483	0.04	1326	0.12	7425	4.46	1138	0.46
Y33H, N47K, F74L, S118F	1590	1338	0.04	1159	0.11	1516	0.91	1140	0.46
R12W, V101M, N106I, S118Y	1591	1378	0.04	1249	0.12	5980	3.59	1182	0.47
A46T, V101A, N106I, S118Y	1592	1359	0.04	1199	0.11	6729	4.04	1173	0.47
Y33H, N106Y, A112V	1393	113580	3.25	17771	1.65	1207	0.72	2476	0.99
N106Y, A112V, S118T	1593	Not tested							
S76P, T81I, V101M, N106Y, A112V, S118F	1594	Not tested							
N106Y, A112V	1383	29015	0.83	2760	0.26	1159	0.70	1639	0.66
P9R, L21V, P22L, I34M, S69F, F74L, A87V, A112V, L125A	1595	1920	0.05	1218	0.11	1107	0.66	1074	0.43
Y33H, V101M, A112V	1596	126266	3.61	24408	2.27	1150	0.69	4535	1.82
N106I, S118F	1380	1776	0.05	1385	0.13	9058	5.44	1370	0.55
V29A, L32P, S118F	1597	1265	0.04	1148	0.11	5057	3.04	1194	0.48
A112V	1404	69673	1.99	6387	0.59	1140	0.68	1214	0.49
Y33H, V101M, A112V	1596	133815	3.83	24992	2.32	1184	0.71	6338	2.54

TABLE 16C-continued

Additional CD112 Variants and Binding Data.

CD112 Mutation(s)	SEQ ID NO (IgV)	TIGIT MFI 20 nM	TIGIT Fold Increase to WT IgV	CD226 MFI at 20 nM	CD226 Fold Increase to WT IgV	CD112R MFI at 20 nM	CD112R Fold Increase to WT IgV	CD96 MFI at 20 nM	CD96 Fold Increase to WT IgV
P28S, Y33H, N106I, S118Y	1513	2745	0.08	1689	0.16	6625	3.98	1978	0.79
Y33H, V101M, N106I, A112V	1598	118654	3.40	21828	2.03	1253	0.75	3871	1.55
R12W, Y33H, N47K, Q79R, S118Y	1599	171390	4.91	5077	0.47	1124	0.68	2636	1.06
A112V, S118T	1406	103203	2.95	15076	1.40	1155	0.69	1426	0.57
Y33H, A46T, A112V, S118T	1600	141859	4.06	29436	2.74	1184	0.71	5760	2.31
Y33H, A112V, F114L, S118T	1601	5161	0.15	1734	0.16	1184	0.71	1249	0.50
A112V	1404	78902	2.26	6224	0.58	1114	0.67	1181	0.47
Y33H, T38A, A46T, V101M, A112V	1602	111293	3.19	25702	2.39	1192	0.72	99015	39.69
Q79R, A112V	1519	96674	2.77	7264	0.67	1130	0.68	1216	0.49
Y33H, N106I, S118Y	1529	5720	0.16	1453	0.14	6543	3.93	1248	0.50
P28S, Y33H, S69P, N106I, A112V, S118Y	1603	22393	0.64	1378	0.13	1550	0.93	19174	7.68
Y33H, P42L, N47K, V101M, A112V	1604	214116	6.13	13878	1.29	1315	0.79	4753	1.91
Y33H, N47K, F74S, Q83K, N106I, F111L, A112V, S118T	1605	6719	0.19	1319	0.12	1305	0.78	1278	0.51
Y33H, A112V, S118T, V119A	1606	184794	5.29	10204	0.95	1269	0.76	4321	1.73
Y33H, N106I, A112V, S118F	1607	6872	0.20	1591	0.15	2308	1.39	2796	1.12
Y33H, K66M, S118F, W124L	1608	1724	0.05	1259	0.12	6782	4.07	1197	0.48
S118F	1374	1325	0.04	1213	0.11	7029	4.22	1135	0.46
N106I, A112V	1609	111342	3.19	4241	0.39	1546	0.93	1178	0.47
Y33H, A112V	1396	177926	5.09	13761	1.28	1152	0.69	3117	1.25
WT CD112 IgV	1367	34932	1.00	10762	1.00	1665	1.00	2495	1.00
WT CD112-Fc ECD	48 (ECD)	28277	0.81	8023	0.75	1253	0.75	1064	0.43
Anti-huFc PE	—	1138	0.03	1006	0.09	1010	0.61	1062	0.43

Example 11

Generation of Stacked Molecules Containing Different Affinity-Modified Domains

[0661] This Example describes further immunomodulatory proteins that were generated as multi-domain stack constructs containing at least two different affinity modified IgV domains from identified variant PD-L2 polypeptides and identified variant CD155 polypeptides described above. Specifically, the exemplary variant PD-L2 IgV H15Q/T47A/K65R/S67L/Q82R/V89D (SEQ ID NO:880) and the exemplary variant CD155 IgV molecule P18S/S65W/S67A/L104Q/G111R (SEQ ID NO:1271) were linked together and fused to an Fc in various configurations. Stack constructs were obtained as geneblocks (Integrated DNA Technologies, Coralville, IA) that either encoded the entire chain or they were generated by obtaining geneblocks that encoded PDL2-CD155 in its various configurations for subsequent Gibson assembly into Fc fusion vector using a Gibson assembly kit (New England Biolabs). Homodimeric and heterodimeric stacks were generated in various configurations as summarized in FIG. **5**A and as follows.

[0662] Homodimeric stack constructs were generated containing identical Fc subunits in which the variant PD-L2 IgV and variant CD155 IgV were variously linked to the N- or C-terminus of a human IgG1 Fc region via a 2×GGGS (SEQ ID NO:1182) or 3×GGGGS (SEQ ID NO: 1181) peptide linker. In this study, the exemplary IgG1 Fc region is set forth in SEQ ID NO:1119 and contained the mutations L234A, L235E, G237A, E356D and M358L by EU numbering (corresponding to L19A, L20E, G22A, E141D and M143L with reference to wild-type human IgG1 Fc set forth in SEQ ID NO:56). Further, the Fc region contained replacement of the cysteine residues to a serine residue at position 5 (C5S) compared to the wild-type or unmodified Fc set forth in SEQ ID NO: 56 (corresponding to C220S by EU numbering). In some examples, the exemplary IgG1 Fc set forth in SEQ ID NO:1253 was used, which contained the above mutations and additionally lacked the C-terminal lysine corresponding to position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 56 (corresponding to K447del by EU numbering). Other Fc regions also are suitable for generation of stack molecules. Exemplary generated stacks are set forth below.

[0663] The homodimeric variant IgV-stacked-Fc fusion molecules containing various configurations of variant IgV domains from PD-L2 (SEQ ID NO: 880) and CD155 (SEQ ID NO:1271) were expressed and purified substantially as described in Example 5. The encoding nucleic acid molecule was designed to produce homodimeric stacks in various configurations of sequences in the order shown:

[0664] PD-L2/CD155 Stack 1 (SEQ ID NO: 1121): CD155 variant (SEQ ID NO: 1271)—2×GGGS (SEQ ID NO: 1182)—Fc (SEQ ID NO: 1119)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)

[0665] PD-L2/CD155 Stack 2 (SEQ ID NO: 1122): PD-L2 (SEQ ID NO: 880)—2×GGGS (SEQ ID NO: 1182)—Fc (SEQ ID NO: 1119)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)

[0666] PD-L2/CD155 Stack 3 (SEQ ID NO: 1123): CD155 variant (SEQ ID NO: 1271)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)—2×GGGS (SEQ ID NO: 1182)—Fc (SEQ ID NO: 1119)

[0667] PD-L2/CD155 Stack 4 (SEQ ID NO: 1124): PD-L2 (SEQ ID NO: 880)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—2×GGGS (SEQ ID NO: 1182)—Fc (SEQ ID NO: 1119)

[0668] PD-L2/CD155 Stack 5 (SEQ ID NO: 1125): Fe (SEQ ID NO: 1119)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—3×GGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)

[0669] PD-L2/CD155 Stack 6 (SEQ ID NO: 1126): Fe (SEQ ID NO: 1119)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)—3×GGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)

[0670] Heterodimeric stacks were generated in two ways. The first way was by co-expression of the variant PD-L2 IgV and/or variant CD155 IgV fused to (1) a first "knob" Fc subunit (set forth in SEQ ID NO:1117 containing the mutations S354C and T366W by EU numbering, corresponding to S139C and T151W with reference to wild-type human IgG1 Fc set forth in SEQ ID NO:56); and (2) a second "hole" Fc subunit (set forth in SEQ ID NO:1118 containing the mutations Y349C, T366S, L368A and Y407V by EU numbering, corresponding to Y134C, T151S, L153A and Y192V with reference to wild-type human IgG1 Fc set forth in SEQ ID NO:56) for expression of a heterodimeric molecule by "knobs-into-hole" engineering. In addition, both the knob and hole Fe also contained mutations L19A, L20E, G22A to reduce effector function and contained replacement of the cysteine residue to a serine residue at position 5 (C5S), each compared to the wild-type or unmodified Fc set forth in SEQ ID NO: 56 (corresponding to C220S, L234A, L235E and G237A by EU numbering, respectively). In a second way, PDL2 and/or CD155 were fused to both knob and hole Fc's to generate stacks where each chain contains Fc with fused IgV domain(s). For constructs in which the Fc sequence was the N-terminal portion of the sequence, a stuffer sequence HMSSVSAQ (SEQ ID NO:1120) was added immediately preceding the Fc sequence.

[0671] The heterodimeric variant IgV-stacked-Fc fusion molecules containing various configurations of variant IgV domains from PD-L2 (SEQ ID NO: 880) and CD155 (SEQ ID NO:1271) were expressed and purified substantially as described in Example 5. For each stack, the encoding nucleic acid molecule of the knob and hole were designed to produce heterodimeric stacks in various configurations with sequences in the order shown:

[0672] PD-L2/CD155 Stack 7 containing (1) knob Fc fusion (SEQ ID NO:1127): CD155 variant (SEQ ID NO: 1271)—2×GGGS (SEQ ID NO: 1182)—knob Fc (SEQ ID NO: 1117)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880) and (2) hole Fc (SEQ ID NO: 1118 plus N-terminal HMSSVSAQ set forth in SEQ ID NO:1120)

[0673] PD-L2/CD155 Stack 8 containing (1) knob Fc fusion (SEQ ID NO:1128): PD-L2 (SEQ ID NO: 880)—2×GGGS (SEQ ID NO: 1182)—knob Fc (SEQ ID NO: 1117)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271) and (2) hole Fc (SEQ ID NO:1118 plus N-terminal HMSSVSAQ set forth in SEQ ID NO:1120)

[0674] PD-L2/CD155 Stack 9 containing (1) knob Fc fusion (SEQ ID NO:1129): CD155 variant (SEQ ID NO: 1271)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—2×GGGS (SEQ ID NO: 1182)—knob Fc (SEQ ID NO: 1117)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880); and (2) hole Fc (SEQ ID NO:1118 plus N-terminal HMSSVSAQ set forth in SEQ ID NO:1120)

[0675] PD-L2/CD155 Stack 10 containing (1) knob Fc fusion (SEQ ID NO:1130): PD-L2 (SEQ ID NO: 880)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO:880)—2×GGGS (SEQ ID NO: 1182)—knob Fc (SEQ ID NO: 1117)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271); and (2) hole Fc (SEQ ID NO: 1118 plus N-terminal HMSSVSAQ set forth in SEQ ID NO:1120)

[0676] PD-L2/CD155 Stack 11 containing (1) knob fc fusion (SEQ ID NO: 1131): CD155 variant (SEQ ID NO: 1271)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—2×GGGS (SEQ ID NO: 1182)—knob Fc (SEQ ID NO: 1117); and (2) hole Fc fusion (SEQ ID NO:1132):PD-L2 (SEQ ID NO: 880)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)—2×GGGS (SEQ ID NO: 1182)—hole Fc (SEQ ID NO: 1118)

[0677] PD-L2/CD155 Stack 12 containing (1) knob fc fusion (SEQ ID NO: 1133): knob Fc (SEQ ID NO: 1117 plus N-terminal HMSSVSAQ set forth in SEQ ID NO:1120)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—3×GGGGS (SEQ ID NO: 1181) CD155 variant (SEQ ID NO: 1271); and (2) hole Fc (SEQ ID NO:1134): hole Fc (SEQ ID NO: 1118 plus N-terminal HMSSVSAQ set forth in SEQ ID NO: 1120)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)

[0678] PD-L2/CD155 Stack 13 containing (1) knob Fc fusion (SEQ ID NO:1129): CD155 variant (SEQ ID NO: 1271)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—2×GGGS (SEQ ID NO: 1182)—knob Fc (SEQ ID NO: 1117)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880); and (2) hole Fc (SEQ ID NO:1134): hole Fc (SEQ ID NO: 1118 plus N-terminal HMSSVSAQ set forth in SEQ ID NO:1120)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)

[0679] PD-L2/CD155 Stack 14 containing (1) knob Fc fusion (SEQ ID NO:1129): CD155 variant (SEQ ID NO: 1271)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—2×GGGS (SEQ ID NO:

1182)—knob Fc (SEQ ID NO: 1117)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880); and (2) hole Fc fusion (SEQ ID NO:1132): PD-L2 (SEQ ID NO: 880)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)—2×GGGS (SEQ ID NO: 1182)—hole Fc (SEQ ID NO: 1118)

[0680] PD-L2/CD155 Stack 15 containing (1) knob Fc fusion (SEQ ID NO:1130): PD-L2 (SEQ ID NO: 880)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO:880)—2×GGGS (SEQ ID NO: 1182)—knob Fc (SEQ ID NO: 1117)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271); and (2) hole Fc fusion (SEQ ID NO:1132): PD-L2 (SEQ ID NO: 880)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)

[0681] 2×GGGS (SEQ ID NO: 1182)—hole Fe (SEQ ID NO: 1118)

[0682] PD-L2/CD155 Stack 16 containing (1) knob Fc fusion (SEQ ID NO:1130): PD-L2 (SEQ ID NO: 880)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO:880)—2×GGGS (SEQ ID NO: 1182)—knob Fe (SEQ ID NO: 1117)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271); and (2) hole Fe (SEQ ID NO: 1134): hole Fe (SEQ ID NO: 1118 plus N-terminal HMSSVSAQ set forth in SEQ ID NO:1120)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)—3×GGGGS (SEQ ID NO: 1181)—PD-L2 (SEQ ID NO: 880)

Example 12

Assessment of Binding to Cell-Expressed Counter Structures and Bioactivity of Affinity-Matured IgSF Domain-Containing Stack Molecules

[0683] This Example describes Fc-fusion binding studies to show specificity and affinity of exemplary PD-L2/CD155 stack immunomodulatory proteins generated in Example 11 for cognate binding partners. The exemplary PD-L2/CD155 stack immunomodulatory proteins generated in Example 11 also were assessed for Fc-fusion variant protein bioactivity characterization in a human primary T cell in vitro assay.

A. Binding to Cell-Expressed Counter Structure

[0684] Binding studies were carried out using Jurkat/IL-2 reporter cells (purchased from Promega Corp. USA) that were transduced to stably express human PD-1 (Jurkat/PD-1 cells), human TIGIT (Jurkat/TIGIT cells) or both PD-1 and TIGIT (Jurkat/PD-1/TIGIT cells). For staining by flow cytometry, 100,000 Jurkat/PD-1, Jurkat/TIGIT, Jurkat/PD-1/TIGIT cells or negative control (Jurkat only) were plated in 96-well round-bottom plates. Cells were spun down and resuspended in staining buffer (PBS (phosphate buffered saline), 1% BSA (bovine serum albumin), and 0.1% sodium azide) for 20 minutes to block non-specific binding. Afterwards, cells were centrifuged again and resuspended in 50 μL staining buffer containing 100 nM to 46 pM of each candidate Fc fusion protein. Primary staining was performed on ice for 90 minutes, before washing cells twice in 200 μL staining buffer. PE-conjugated anti-human Fe (Jackson ImmunoResearch, USA) was diluted 1:150 in 50 μL staining buffer and added to cells and incubated another 30 minutes on ice. Secondary antibody was washed out twice, cells were fixed in 400 formaldehyde/PBS, and samples were analyzed on Intellicyt flow cytometer (Intellicyt Corp., USA).

[0685] Mean Fluorescence Intensity (MFI) was calculated with FlowJo Version 10 software (FlowJo LLC, USA). Table 17 sets forth binding activity as measured by the Mean Fluorescence Intensity (MFI) value for binding of 6.25 nM of each stack Fc-fusion molecule to Jurkat/PD-1, Jurkat/TIGIT, and Jurkat/PD-1/TIGIT cells. As shown in Table 17, several stack proteins bind both PD-i and TIGIT with high affinity.

TABLE 17

Binding of PD-L2/CD155 Stacks to Cell-Expressed Counter Structure

			Binding to Jurkat Transfectants MFI at 6.25 nM		
Category	Description	SEQ ID NO	PD1	TIGIT	TIGIT + PD1
Homodimers	(CD155 IgV)-(G4S)2-Fc-(G4S)3-(PD-L2 IgV)	1121	242	19948	5336
	(PD-L2 IgV)-(G4S)2-Fc-(G4S)3-(CD155 IgV)	1122	16344	9095	16458
	(CD155 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-Fc	1123	91	22342	8135
	(PD-L2 IgV)-(G4S)3-(CD155 IgV)-(G4S)2-Fc	1124	9218	14516	19256
	Fc-(G4S)3-(CD155 IgV)-(G4S)3-(PD-L2 IgV)	1125	108	5486	2905
	Fc-(G4S)3-(PD-L2 IgV)-(G4S)3-(CD155 IgV)	1126	66	9974	5202
Heterodimers	(CD155 IgV)-(G4S)2-knob Fc-(G4S)3-(PD-L2 IgV) and hole Fc	1127 + 1118	107	2544	1512
	(PD-L2 IgV)-(G4S)2-knob Fc-(G4S)3-(CD155 IgV) and hole Fc	1128 + 1118	1658	360	6762
	(CD155 IgV)-(G4S)3-(CD155 IgV)-(G4S)2-knob Fc-(G4S)3-(PD-L2 IgV)-(G4S)3-(PD-L2 IgV) and hole Fc	1129 + 1118	289	8677	4371

TABLE 17-continued

Binding of PD-L2/CD155 Stacks to Cell-Expressed Counter Structure

Category	Description	SEQ ID NO	Binding to Jurkat Transfectants MFI at 6.25 nM		
			PD1	TIGIT	TIGIT + PD1
	(PD-L2 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-knob Fc-(G4S)3-(CD155 IgV)-(G4S)3-(CD155 IgV) and hole Fc	1130 + 1118	1594	2554	5509
	(CD155 IgV)-(G4S)3-(CD155 IgV)-(G4S)2-knob Fc and (PD-L2 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-hole Fc	1131 + 1132	1758	9642	9343
	(CD155 IgV)-(G4S)3-(CD155 IgV)-(G4S)2-knob Fc-(G4S)3-(PD-L2 IgV)-(G4S)3-(PD-L2 IgV) and (PD-L2 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-hole Fc	1129 + 1132	4821	7596	8081
	(CD155 IgV)-(G4S)3-(CD155 IgV)-(G4S)2-knob Fc-(G4S)3-(PD-L2 IgV)-(G4S)3-(PD-L2 IgV) and hole Fc-(G4S)3-(PD-L2 IgV)-(G4S)3-(PD-L2 IgV)	1129 + 1134	515	8299	4228
	(PD-L2 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-knob Fc-(G4S)3-(CD155 IgV)-(G4S)3-(CD155 IgV) and (PD-L2 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-hole Fc	1130 + 1132	10970	3339	9014
	(PD-L2 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-knob Fc-(G4S)3-(CD155 IgV)-(G4S)3-(CD155 IgV) and hole Fc-(G4S)3-(PD-L2 IgV)-(G4S)3-(PD-L2 IgV)	1130 + 1134	3785	1475	5989
Controls	PDL2-IgV	880	22448	73	20280
	CD155-IgV	1271	133	22342	4893
	Fc Control (homodimer)	1119	44	86	66
	Fc Control (heterodimer)	1117 + 1118	42	48	48
	Wild Type CD155 full ECD Fc	20 (ECD)	64	4547	249
	Wild Type PD-L2 full ECD Fc	4 (ECD)	392	46	314
	Irrelevant Ig Control	hIgG	41	48	41

B. Assessment of Bioactivity of Affinity-Matured IgSF Domain-Containing Molecules Using Mixed Lymphocyte Reaction (MLR)

[0686] Soluble PD-L2/CD155 stack protein bioactivity was tested in a human Mixed Lymphocyte Reaction (MVLR). Human primary dendritic cells (DC) were generated by culturing monocytes isolated from PBMC (BenTech Bio, USA) in vitro for 7 days with 50 ng/mL rIL-4 (R&D Systems, USA) and 80 ng/mL rGM-CSF (R&D Systems, USA) in Ex-Vivo 15 media (Lonza, Switzerland). To induce DC maturation, lipopolysaccharide (LPS) (InvivoGen Corp., USA) was added to the DC cultures on day 6 and cells were incubated for an additional 24 hours. Approximately 10,000 matured DC and 100,000 purified allogeneic CD3+ T cells (BenTech Bio, USA) were co-cultured with several concentrations of PD-L2/CD155 stack or control proteins in 96 well round-bottom plates in 200 μL final volume of Ex-Vivo 15 media. Irrelevant human IgG and homodimeric and heterodimeric empty Fc proteins were used as negative controls. As positive controls, PD-L2-Fc (full PD-L2 extracellular domain), wildtype CD155-Fc (full CD155 extracellular domain) were assessed. Variant PD-L2 IgV-Fc fusion proteins were tested at 20 nM. On day 5, IFN-gamma secretion in culture supernatants was analyzed using the Human IFN-gamma Duoset ELISA kit (R&D Systems, USA). Optical density was measured on a BioTek Cytation Multimode Microplate Reader (BioTek Corp., USA) and quantitated against titrated rIFN-gamma standard included in the IFN-gamma Duo-set kit (R&D Systems, USA).

[0687] Results for the bioactivity studies for exemplary tested PD-L2/CD155 stack proteins are summarized in Table 18, which sets forth the calculated levels of IFN-gamma in culture supernatants (pg/ml). The sequence identifier (SEQ ID NO) for each stack proteins is set forth in column 3. As shown in Table 18, culture supernatants incubated in the presence of exemplary PD-L2/CD155 stack proteins exhibited altered levels of IFNg production in the MLR assay.

TABLE 18

Bioactivity Data of PD-L2/CD155 Stacks

Category	Description	SEQ ID NO	Mixed Lymphocyte Reaction: IFNg at 96 hours IFNg [pg/mL]	Mixed Lymphocyte Reaction: IFNg at 96 hours Fold Increase compared to IgG Control
Homodimers	(CD155 IgV)-(G4S)2-Fc-(G4S)3-(PD-L2 IgV)	1121	3097.0	1.3
	(PD-L2 IgV)-(G4S)2-Fc-(G4S)3-(CD155 IgV)	1122	3700.3	1.6
	(CD155 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-Fc	1123	3061.6	1.3
	(PD-L2 IgV)-(G4S)3-(CD155 IgV)-(G4S)2-Fc	1124	2270.0	1.0
	Fc-(G4S)3-(CD155 IgV)-(G4S)3-(PD-L2 IgV)	1125	2003.5	0.9
	Fc-(G4S)3-(PD-L2 IgV)-(G4S)3-(CD155 IgV)	1126	2951.0	1.3
Heterodimers	(CD155 IgV)-(G4S)2-knob Fc-(G4S)3-(PD-L2 IgV) and hole Fc	1127 + 1118	2040.4	0.9
	(PD-L2 IgV)-(G4S)2-knob Fc-(G4S)3-(CD155 IgV) and hole Fc	1128 + 1118	3768.6	1.6
	(CD155 IgV)-(G4S)3-(CD155 IgV)-(G4S)2-knob Fc-(G4S)3-(PD-L2 IgV)-(G4S)3-(PD-L2 IgV) and hole Fc	1129 + 1118	3549.7	1.5
	(PD-L2 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-knob Fc-(G4S)3-(CD155 IgV)-(G4S)3-(CD155 IgV) and hole Fc	1130 + 1118	2568.6	1.1
	(CD155 IgV)-(G4S)3-(CD155 IgV)-(G4S)2-knob Fc and (PD-L2 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-hole Fc	1131 + 1132	2572.1	1.1
	(CD155 IgV)-(G4S)3-(CD155 IgV)-(G4S)2-knob Fc-(G4S)3-(PD-L2 IgV)-(G4S)3-(PD-L2 IgV) and (PD-L2 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-hole Fc	1129 + 1132	3216.7	1.4
	(CD155 IgV)-(G4S)3-(CD155 IgV)-(G4S)2-knob Fc-(G4S)3-(PD-L2 IgV)-(G4S)3-(PD-L2 IgV) and hole Fc-(G4S)3-(PD-L2 IgV)-(G4S)3-(PD-L2 IgV)	1129 + 1134	2673.4	1.2
	(PD-L2 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-knob Fc-(G4S)3-(CD155 IgV)-(G4S)3-(CD155 IgV) and (PD-L2 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-hole Fc	1130 + 1132	2361.6	1.0
	(PD-L2 IgV)-(G4S)3-(PD-L2 IgV)-(G4S)2-knob Fc-(G4S)3-(CD155 IgV)-(G4S)3-(CD155 IgV) and hole Fc-(G4S)3-(PD-L2 IgV)-(G4S)3-(PD-L2 IgV)	1130 + 1134	3311.8	1.4
Controls	PDL2-IgV	880	2367.2	1.0
	CD155-IgV	1271	2590.7	1.1
	Fc Control (homodimer)	1119	2617.9	1.1
	Fc Control (heterodimer)	1117 + 1118	2861.5	1.2
	Wild Type CD155 full ECD Fc	20 (ECD)	2481.0	1.1
	Wild Type PD-L2 full ECD Fc	4 (ECD)	3298.5	1.4
	Irrelevant Ig Control	hIgG	2297.6	1.0

Example 13

Generation of Multi-Domain Stacked Molecules Containing Different Affinity-Modified Domains

[0688] This Example describes immunomodulatory proteins that were generated as multi-domain stack constructs containing at least two different affinity modified IgV domains from identified variant PD-L1 polypeptides, identified variant CD112 polypeptides and identified variant CD155 polypeptides described above. Specifically, the exemplary variant PD-L1 IgV D43G/N45D/L56Q/V58A/G101G-ins (G101GG) (SEQ ID NO: 659), CD112 IgV molecule S118F (SEQ ID NO: 1374) and/or the exemplary variant CD155 IgV molecule P18S/S65W/S67A/L104Q/G111R (SEQ ID NO: 1271) were linked together and fused to an Fc in various configurations.

[0689] Homodimeric stacks were generated in various configurations as summarized in FIGS. **5**A and **5**B and as follows. In the generated homodimeric stack constructs, the variant CD155 IgV variant, CD112 IgV and/or variant PD-L1 IgV were variously linked to the N- or C-terminus of a human IgG1 Fc region via a 2×GGGS (SEQ ID NO:1182) or 3×GGGGS (SEQ ID NO: 1181) peptide linker. In this study, the exemplary IgG1 Fc region is set forth in SEQ ID NO:1119 and contained the mutations L234A, L235E, G237A, E356D and M358L by EU numbering (corresponding to L19A, L20E, G22A, E141D and M143L with reference to wild-type human IgG1 Fc set forth in SEQ ID NO:56). Further, the Fc region contained replacement of the cysteine residues to a serine residue at position 5 (C5S) compared to the wild-type or unmodified Fc set forth in SEQ ID NO: 56 (corresponding to C220S by EU numbering). In some examples, the Fc was further modified to remove the C-terminal lysine at position 232 of the wild-type or unmodified Fc set forth in SEQ ID NO: 56 (corresponding to K447del by EU numbering). The exemplary IgG1 Fc region comprising a lysine deletion is set forth in SEQ ID NO: 1253. Other Fc regions also are suitable for generation of stack molecules. Exemplary generated stacks are set forth below.

[0690] Expression constructs encoding Fc fusion proteins of interest were transiently expressed in Expi293 HEK293 cells from Invitrogen using the manufacturer's commercial Expifectamine reagents and media. Supernatants were harvested and protein was captured and eluted from a Protein A column using an AKTA protein purification system. The eluted material was then separated by an additional preparative SEC step to generate monomeric, highly purified material. The purified proteins were formulated in 15 mM acetate, 200 mM NaCl, 9% sucrose, pH 5.0 (ASUS). The protein was vialed in a sterile biosafety cabinet and frozen at −80 C. A vial was thawed and assessed by analytical SEC to demonstrate the material was stable and predominantly monomeric after thaw.

[0691] For each stack, the encoding nucleic acid molecule was designed to produce homodimeric stacks in various configurations with sequences in the order shown:

A. Stack Constructs Containing PD-L1 and CD155

[0692] PD-L1/CD155 Stack 1 (SEQ ID NO: 1254): CD155 variant (SEQ ID NO: 1271)—2×GGGS (SEQ ID NO: 1182)—Fc (SEQ ID NO: 1119)—3×GGGGS (SEQ ID NO: 1181)—PD-L1 (SEQ ID NO: 659)

[0693] PD-L1/CD155 Stack 2 (SEQ ID NO: 1255): PD-L1 (SEQ ID NO: 659)—2×GGGS (SEQ ID NO: 1182)—Fc (SEQ ID NO: 1119)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)

[0694] PD-L1/CD155 Stack 3 (SEQ ID NO: 1256): CD155 variant (SEQ ID NO: 1271)—3×GGGGS (SEQ ID NO: 1181)—PD-L1 (SEQ ID NO: 659)—2×GGGS (SEQ ID NO: 1182)—Fc (SEQ ID NO: 1119)

[0695] PD-L1/CD155 Stack 4 (SEQ ID NO: 1257): PD-L1 (SEQ ID NO: 659)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—2×GGGS (SEQ ID NO: 1182)—Fc (SEQ ID NO: 1119)

[0696] PD-L1/CD155 Stack 5 (SEQ ID NO: 1258): N-terminal HMSSVSAQ set forth in SEQ ID NO:1120—Fc (SEQ ID NO: 1119)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—3×GGGS (SEQ ID NO: 1181)—PD-L1 (SEQ ID NO: 659)

[0697] PD-L1/CD155 Stack 6 (SEQ ID NO: 1259): N-terminal HMSSVSAQ set forth in SEQ ID NO:1120—Fc (SEQ ID NO: 1119)—3×GGGGS (SEQ ID NO: 1181)—PD-L1 (SEQ ID NO: 659)—3×GGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)

B. Stack Constructs Containing CD112 and CD155

[0698] CD112/CD155 Stack 1 (SEQ ID NO: 1260): CD155 variant (SEQ ID NO: 1271)—2×GGGS (SEQ ID NO: 1182)—Fc with lysine removed (SEQ ID NO: 1253)—3×GGGGS (SEQ ID NO: 1181)—CD112 (SEQ ID NO: 1374)

[0699] CD112/CD155 Stack 2 (SEQ ID NO: 1261): CD112 (SEQ ID NO: 1374)—2×GGGS (SEQ ID NO: 1182)—Fc with lysine removed (SEQ ID NO: 1253)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)

[0700] CD112/CD155 Stack 3 (SEQ ID NO: 1262): CD155 variant (SEQ ID NO: 1271)—3×GGGGS (SEQ ID NO: 1181)—CD112 (SEQ ID NO: 1374)—2×GGGS (SEQ ID NO: 1182)—Fe (SEQ ID NO: 1119)

[0701] CD112/CD155 Stack 4 (SEQ ID NO: 1263): CD112 (SEQ ID NO: 1374)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—2×GGGS (SEQ ID NO:1182)—Fc (SEQ ID NO:1119)

[0702] CD112/CD155 Stack 5 (SEQ ID NO: 1264): N-terminal HMSSVSAQ set forth in SEQ ID NO:1120—Fc with lysine removed (SEQ ID NO: 1253)—3×GGGGS (SEQ ID NO: 1181)—CD112 (SEQ ID NO: 1374)—3×GGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)

[0703] CD112/CD155 Stack 6 (SEQ ID NO: 1265): N-terminal HMSSVSAQ set forth in SEQ ID NO: 1120—Fc with lysine removed (SEQ ID NO: 1253)—3×GGGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—3×GGGGS (SEQ ID NO: 1181)

[0704] CD112 (SEQ ID NO: 1374)

C. Stack Constructs Containing PD-L1, CD112 and CD155

[0705] PD-L1/CD112/CD155 Stack 1 (SEQ ID NO: 1266): PD-L1 (SEQ ID NO: 659)—3×GGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—2×GGGS (SEQ ID NO: 1182)—Fc with lysine removed (SEQ ID NO: 1253)—3×GGGGS (SEQ ID NO: 1181)—CD112 (SEQ ID NO: 1374)

[0706] PD-L1/CD112/CD155 Stack 2 (SEQ ID NO: 1267): PD-L1 (SEQ ID NO: 659)—3×GGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—3×GGGS (SEQ ID NO: 1181)—CD112 (SEQ ID NO: 1374)—2×GGGS (SEQ ID NO: 1182)—Fe (SEQ ID NO: 1119)

[0707] PD-L1/CD112/CD155 Stack 3 (SEQ ID NO: 1268): PD-L1 (SEQ ID NO: 659)—3×GGGS (SEQ ID NO: 1181)—CD112 (SEQ ID NO: 1374)—3×GGGS (SEQ ID NO: 1181)—CD155 variant (SEQ ID NO: 1271)—2×GGGS (SEQ ID NO: 1182)—Fe (SEQ ID NO: 1119)

Example 14

Assessment of Binding and Bioactivity of PD-L1/CD155 Stacked Affinity-Matured IgSF Domain-Containing Molecules

[0708] This Example describes binding studies to assess specificity and affinity of exemplary PD-L1/CD155 variant IgV stack immunomodulatory proteins (PD-L1/CD155 stacked IgV-Fc), generated in Example 13, for binding to cognate binding partners. In addition, a Jurkat/IL2/PD1/TIGIT reporter assay was used to assess PD-1 and TIGIT blocking activity of PD-L1/CD155 stacked IgV-Fc molecules. As a control, binding and blocking activity also was assessed of the non-stack variant PD-L1 IgV-Fc or CD155 IgV-Fc fusion molecules containing the same variant PD-L1 IgV (SEQ ID NO:659) or variant CD155 IgV (SEQ ID NO:1271), respectively, used in the stacks. Wild-type CD155-ECD-Fc and wild-type PD-L1-ECD-Fc containing the wildtype CD155 ECD (SEQ ID NO:20) or the wildtype PD-L1 ECD (SEQ ID NO:3), respectively, also were assessed.

A. Binding to Cell-Expressed Counter Structure

[0709] Binding studies were carried out using Jurkat/IL-2 reporter cells (purchased from Promega Corp. USA) that were transduced to stably express human PD-1 (Jurkat/PD-1 cells), human TIGIT (Jurkat/TIGIT cells) or both PD-1 and TIGIT (Jurkat/PD-1/TIGIT cells). For staining by flow cytometry, 100,000 Jurkat/PD-1, Jurkat/TIGIT, Jurkat/PD-I/TIGIT cells or negative control (Jurkat only) were plated in 96-well round-bottom plates. Cells were spun down and resuspended in staining buffer (PBS (phosphate buffered saline), 1% BSA (bovine serum albumin), and 0.1% sodium azide) for 20 minutes to block non-specific binding. Afterwards, cells were centrifuged again and resuspended in 50 μL staining buffer containing 100 nM to 6 pM of each candidate Fc fusion protein, either variant PD-L1 IgV-Fc or CD155 IgV-Fc fusion molecules or PD-L1/CD155 stacked IgV-Fc fusion molecules described above. Primary staining was performed on ice for 90 minutes, before washing cells twice in 200 μL staining buffer. PE-conjugated anti-human Fc (Jackson ImmunoResearch, USA) was diluted 1:150 in 50 μL staining buffer and added to cells and incubated another 30 minutes on ice. Secondary antibody was washed out twice, cells were fixed in 4% formaldehyde/PBS, and samples were analyzed on Intellicyt flow cytometer (Intellicyt Corp., USA).

[0710] Mean Fluorescence Intensity (MFI) was calculated with FlowJo Version 10 software (FlowJo LLC, USA). Table 19 sets forth the binding activity as measured by the Mean Fluorescence Intensity (MFI) value for binding of 20 nM of each stack Fc-fusion molecule, non-stack variant PD-L1 IgV-Fc or CD155 IgV-Fc controls or wild-type ECD controls, to Jurkat/PD-1, Jurkat/TIGIT, and Jurkat/PD-1/TIGIT cells. As shown in Table 19, several stack proteins bound both PD-1 and TIGIT with high affinity.

TABLE 19

Binding of Stacks to Cell-Expressed Counter Structure

		Flow Binding to Jurkat Cells Stably Expressing:		
SEQ ID NO	Description	PD-1	TIGIT	PD-1 + TIGIT
1254	(CD155 IgV) (G4S)2 Fc (G4S)3 (PD-L1 IgV)	61805	80658	35128
1255	(PD-L1 IgV) (G4S)2 Fc(G4S)3 (CD155 IgV)	69813	36485	52538
1256	(CD155 IgV) (G4S)3 (PD-L1 IgV) (G4S)2 Fc	47261	81840	32188
1257	(PD-L1 IgV) (G4S)3 (CD155 IgV) (G4S)2 Fc	77959	60515	51615
659	non-stack variant PD-L1 IgV-Fc control	111746	630	41390
1271	non-stack variant CD155 IgV-Fc control	460	79152	7910
20 (ECD)	CD155-ECD-Fc	511	28790	1196
3 (ECD)	PD-L1-ECD-Fc (R&D Systems)	35005	557	10358
1119	Fc Control (homodimer)	437	483	478
		MFI at 20 nM	MFI at 20 nM	MFI at 20 nM

B. Assessment of Bioactivity of Affinity-Matured IgSF Domain-Containing Molecules

[0711] Jurkat effector cells expressing an IL-2-luciferase reporter and cell-surface PD-I and TIGIT were suspended at 2×10^6 cells/mL in Jurkat Assay buffer (RPMI1640+5% FBS) and anti-CD28 was added to a final concentration of 3 μg/mL. Jurkat cells were then plated at 50 μL/well for a total of 100,000 cells per well.

[0712] To each well, 25 μL of PD-L1/CD155 stacked IgV-Fc test protein was added to the Jurkat cells. As a control, non-stack variant PD-L1 IgV-Fc or CD155 IgV-Fc fusion molecules, alone or in combination, also were assessed for comparison. Anti-TIGIT antibody (clone MBSA43), anti-PD-1 antibody (nivolumab) or an empty Fc molecule were also used as controls. All proteins were added at five concentrations: 400 nM, 100 nM, 25 nM, 6.25 nM, and 1.56 nM. The Jurkat cells with test or control proteins were incubated for 15 minutes at room temperature. CHO-derived artificial antigen presenting cells (aAPC) displaying transduced cell surface anti-CD3 single chain Fv (OKT3), PD-L1 and CD155 were brought to 0.8×10^6 cells/mL and 25 μL of cells was added to each well bringing the final volume of each well to 100 μL. Each well had a final ratio of 5:1 Jurkat:CHO cells and a test protein concentration of 100, 25, 6.25, 1.56 or 0.47 nM and an anti-CD28 concentration of 1.5 μg/mL. Jurkat cells and CHO cells were incubated for 5

hours at 37 degrees Celsius in a humidified 5% CO_2 incubation chamber. Plates are then removed from the incubator and acclimated to room temperature for 15 minutes. 100 μL of a cell lysis and luciferase substrate solution (BioGlo luciferase reagent, Promega) was added to each well and the plates were incubated on an orbital shaker for 10 minutes. Luminescence was measured with a 1 second per well integration time using a BioTek Cytation luminometer.

[0713] An average relative luminescence value was determined for each test sample and a fold increase (or decrease) in IL-2 reporter signal was calculated for each stack molecule compared to non-stack variant PD-L1 IgV-Fc and variant CD155 IgV-Fc proteins. Because the assay is a measure of blockade of inhibitory signals, an increase in luminescent signal compared to control indicates the presence of blocking activity.

[0714] As shown in Table 20, the luciferase activity of the Jurkat effector cells co-cultured with anti-CD3/PD-L1/CD155 aAPC and 100 nM PD-L1/CD155 stack Fc molecules was altered (increased) for each molecule tested compared to control. The differences in luminescence signals demonstrate the differences in binding of the PD-L1/CD155 stack-Fc molecules to PD-1 and TIGIT and the resulting co-blockade of inhibitory activity. In the Table, Column 1 sets forth the SEQ ID NO identifier for each PD-L1/CD155 stack-Fc variant tested.

TABLE 20

Jurkat/IL2/PD1/TIGIT + CHO/OKT3/PD-L1/CD155 Reporter Assay Results

SEQ ID NO	Description	RLU	Fold Increase Compared to non-stack variant PD-L1 IgV-Fc control	Fold Increase Compared to non-stack variant CD155 IgV-Fc control	Fold Increase Compared to variant PD-L1 IgV-Fc and variant CD155 IgV-Fc
1254	(CD155 IgV) (G4S)2 Fc (G4S)3 (PD-L1 IgV)	573	0.6	1.7	0.8
1255	(PD-L1 IgV) (G4S)2 Fc(G4S)3 (CD155 IgV)	962	1.0	2.8	1.4
1256	(CD155 IgV) (G4S)3 (PD-L1 IgV) (G4S)2 Fc	434	0.5	1.3	0.6
1257	(PD-L1 IgV) (G4S)3 (CD155 IgV) (G4S)2 Fc	1923	2.0	5.6	2.7
659	non-stack variant PD-L1 IgV-Fc control	958	1.0	2.8	1.3
1271	non-stack variant CD155 IgV-Fc control	345	0.4	1.0	0.5
—	Anti-TIGIT antibody (clone MBSA43), anti-PD-1 antibody (nivolumab)	2192	2.3	6.4	3.1
659 + 1271	PD-L1 IgV + CD155 IgV	710	0.7	2.1	1.0
1119	Fc Control (homodimer)	235	0.2	0.7	0.3

Example 15

Assessment of Binding and Bioactivity of CD112/CD155 Stacked Affinity-Matured IgSF Domain-Containing Molecules

[0715] This Example describes binding studies to assess specificity and affinity of CD112/CD155 variant stack immunomodulatory proteins (CD112/CD155 stacked IgV-Fc), generated in Example 13, for binding to cognate binding partners. In addition, a Jurkat/IL2/CD112R/TIGIT reporter assay was used to assess CD112R and TIGIT blocking activity of CD112/CD155 stacked IgV-Fc molecules. As a comparison, binding and blocking activity also was assessed of the non-stack variant CD112 IgV-Fc or CD155 IgV-Fc fusion molecules containing the same variant CD112 IgV (SEQ ID NO:1374) or variant CD155 IgV (SEQ ID NO:1271), respectively, used in the stacks. Wild-type CD155-ECD-Fc and wild-type CD112-ECD-Fc containing the wildtype CD155 ECD (SEQ ID NO:20) or the wildtype CD112 ECD (SEQ ID NO:21), respectively, also were assessed.

A. Binding to Cell-Expressed Counter Structure

[0716] Binding studies were carried out using Jurkat/IL-2 reporter cells which endogenously express CD112R (purchased from Promega Corp. USA) that were transduced to stably express human TIGIT (Jurkat/TIGIT cells). For staining by flow cytometry, 100,000 Jurkat parental (CD112R), or Jurkat/TIGIT cells were plated in 96-well round-bottom plates. Cells were spun down and resuspended in staining buffer (PBS (phosphate buffered saline), 1% BSA (bovine serum albumin), and 0.1% sodium azide) for 20 minutes to block non-specific binding. Afterwards, cells were centrifuged again and resuspended in 50 μL staining buffer containing 100 nM to 6 pM of each candidate Fc fusion protein. Primary staining was performed on ice for 90 minutes, before washing cells twice in 200 μL staining buffer. PE-conjugated anti-human Fc (Jackson ImmunoResearch, USA) was diluted 1:150 in 50 μL staining buffer and added to cells and incubated another 30 minutes on ice. Secondary antibody was washed out twice, cells were fixed in 4% formaldehyde/PBS, and samples were analyzed on an LSRII flow cytometer (Becton Dickinson Corp., USA).

[0717] Binding values, expressed as Mean Fluorescence Intensity (MFI), were determined for a 33.3 nM concentration of each stack Fc fusion protein and non-stack variant CD112 IgV-Fc or CD155 IgV-Fc fusion molecules. Data was analyzed using FlowJo Version 10 software (FlowJo LLC, USA). Results for the binding studies for exemplary tested CD112/CD155 stack Fc fusion molecules (tested at 33.3 nM) are shown in Table 21. As shown in Table 21, several stack proteins bound TIGIT and/or CD112R with high affinity

TABLE 21

Binding of Stacks to Cell-Expressed Counter Structure

Category	Description	SEQ ID NO	Binding to Jurkat Transfectants Expressing: TIGIT	CD112R
Stacks	(CD155 IgV) (G4S)2 Fc1.1 (G4S)3 (CD112 IgV)	1260	6430	570
	(CD112 IgV) (G4S)2 Fc1.1 (G4S)3 (CD155 IgV)	1261	2003	677
	(CD155 IgV) (G4S)3 (CD112 IgV) (G4S)2 Fc1.1	1262	5958	448
	(CD112 IgV) (G4S)3 (CD155 IgV) (G4S)2 Fc1.1	1263	1734	475
	Fc1.1 (G4S)3 (CD112 IgV) (G4S)3 (CD155 IgV)	1264	765	458
Controls	non-stack variant CD155 IgV-Fc control	1271	6294	55
	non-stack variant CD112 IgV-Fc control	1374	358	516
	Fc Control	1119	23.3	27
			MFI at 33.3 nM	

B. Assessment of Bioactivity of Affinity-Matured IgSF Domain-Containing Molecules

[0718] Jurkat effector cells expressing an IL-2-luciferase reporter and cell-surface CD112R and TIGIT were suspended at 2×10^6 cells/mL in Jurkat Assay buffer (RPMI1640+5% FBS). Jurkat cells were then plated at 50 μL/well for a total of 100,000 cells per well.

[0719] To each well, 25 μL of CD112/CD155 stacked IgV-Fc test protein was added to the Jurkat cells. As a control, non-stack variant CD112 IgV-Fc or CD155 IgV-Fc fusion molecules, alone or in combination, also were assessed for comparison. An empty Fc molecule was used as a negative control. All proteins were added at three concentrations: 400 nM, 100 nM, and 25 nM. The Jurkat cells with test or control proteins were incubated for 15 minutes at room temperature. K562-derived artificial antigen presenting cells (aAPC) displaying endogenous CD155 and CD112 and transduced cell surface anti-CD3 single chain Fv (OKT3) and CD80 were brought to 0.8×10^6 cells/mL and 25 μL of cells was added to each well bringing the final volume of each well to 100 μL. Each well had a final ratio of 5:1 Jurkat:K562 and a test protein concentration of 100, 25, or 6.25 nM. Jurkat cells and K562 cells were incubated for 5 hours at 37 degrees Celsius in a humidified 5% CO_2 incubation chamber. Plates are then removed from the incubator and acclimated to room temperature for 15 minutes. 100 μL of a cell lysis and luciferase substrate solution (BioGlo luciferase reagent, Promega) was added to each well and the plates were incubated on an orbital shaker for 10 minutes. Luminescence was measured with a 1 second per well integration time using a BioTek Cytation luminometer.

[0720] An average relative luminescence value was determined for each test sample and a fold increase (or decrease) in IL-2 reporter signal was calculated for each stack molecule compared to non-stack variant CD112 IgV-Fc and variant CD155 IgV-Fc proteins. Because the assay is a measure of blockade of inhibitory signals, an increase in luminescent signal compared to control indicates the presence of blocking activity.

[0721] As shown in Table 22, the luciferase activity of the Jurkat effector cells co-cultured with anti-CD3/CD112/CD155 aAPC and CD112/CD155 stack Fc molecules was altered (increased) for each molecule tested compared to control. The differences in luminescence signals demonstrate the differences in binding of the CD112/CD155 stack-Fc molecules to CD112R and TIGIT and the resulting co-blockade of inhibitory activity. In the Table, Column 2 sets forth the SEQ ID NO identifier for each CD112/CD155 stack-Fc variant tested.

TABLE 22

Jurkat/IL2/TIGIT + K562/OKT3/CD80 Reporter Assay Results

Description	SEQ ID NO	RLU	Fold Increase Compared to Fc Control	Fold Increase Compared to CD112IgV-Fc	Fold Increase Compared to CD155 IgV-Fc	Fold Increase Compared to CD112IgV-Fc + CD155 IgV-Fc
(CD155 IgV) (G4S)2 Fc (G4S)3 (CD112 IgV)	1260	6013	2.12	1.32	1.50	1.27
(CD112 IgV) (G4S)2 Fc (G4S)3 (CD155 IgV)	1261	3777	1.33	0.83	0.94	0.80
(CD155 IgV) (G4S)3 (CD112 IgV) (G4S)2 Fc	1262	3863	1.36	0.85	0.96	0.82
(CD112 IgV) (G4S)3 (CD155 IgV) (G4S)2 Fc	1263	3525	1.25	0.78	0.88	0.75
Fc (G4S)3 (CD112 IgV) (G4S)3 (CD155 IgV)	1264	3457	1.22	0.76	0.86	0.73

TABLE 22-continued

Jurkat/IL2/TIGIT + K562/OKT3/CD80 Reporter Assay Results

Description	SEQ ID NO	RLU	Fold Increase Compared to Fc Control	Fold Increase Compared to CD112IgV-Fc	Fold Increase Compared to CD155 IgV-Fc	Fold Increase Compared to CD112IgV-Fc + CD155 IgV-Fc
non-stack variant CD112 IgV-Fc control	1374	4542	1.60	1.00	1.13	0.96
non-stack variant CD155 IgV-Fc control	1271	4004	1.41	0.88	1.00	0.85
CD112IgV-Fc + CD155 IgV-Fc	1271 + 1374	4720	1.67	1.04	1.18	1.00
Fc Control	1119	2831	1.00	0.62	0.71	0.60

Example 16

Assessment of Binding and Bioactivity of PD-L1/CD112/CD155 Stacked Affinity-Matured IgSF Domain-Containing Molecules

[0722] This Example describes binding studies to assess specificity and affinity of PD-L1/CD112/CD155 stack immunomodulatory proteins (PD-L1/CD112/CD155 stacked IgV-Fc), generated in Example 13, for binding to cognate binding partners. In addition, a Jurkat/IL2/PD1/CD112R/TIGIT reporter assay was used to assess PD-1, CD112R, and TIGIT blocking activity of PD-L1/CD112/CD155 stacked IgV-Fc molecules. As a comparison, binding and blocking activity also was assessed of the non-stack variant PD-L1 IgV-Fc, CD112 IgV-Fc or CD155 IgV-Fc fusion molecules containing the same variant PD-L1 (SEQ ID NO:659), CD112 IgV (SEQ ID NO:1374) or variant CD155 IgV (SEQ ID NO:1271), respectively, used in the stacks.

A. Binding to Cell-Expressed Counter Structure

[0723] Binding studies were carried out using Jurkat/IL-2 reporter cells which endogenously express CD112R (purchased from Promega Corp. USA) that were transduced to stably express human PD-1 (Jurkat/PD-1 cells), human TIGIT (Jurkat/TIGIT cells) or both PD-1 and TIGIT (Jurkat/PD-1/TIGIT cells). For staining by flow cytometry, 100,000 Jurkat parental (CD112R), Jurkat/PD-1, Jurkat/TIGIT, Jurkat/PD-I/TIGIT cells were plated in 96-well round-bottom plates. Cells were spun down and resuspended in staining buffer (PBS (phosphate buffered saline), 1% BSA (bovine serum albumin), and 0.1% sodium azide) for 20 minutes to block non-specific binding. Afterwards, cells were centrifuged again and resuspended in 50 μL staining buffer containing 100 nM to 6 pM of each candidate Fc fusion protein. Primary staining was performed on ice for 90 minutes, before washing cells twice in 200 μL staining buffer. PE-conjugated anti-human Fc (Jackson ImmunoResearch, USA) was diluted 1:150 in 50 μL staining buffer and added to cells and incubated another 30 minutes on ice. Secondary antibody was washed out twice, cells were fixed in 4% formaldehyde/PBS, and samples were analyzed on an LSRII flow cytometer (Becton Dickinson Corp., USA).

[0724] Binding values, expressed as Mean Fluorescence Intensity (MFI), were determined for a 33.3 nM concentration of each stack Fc fusion protein and non-stack variant PD-L1 IgV-Fc, CD112-IgV-Fc and CD155-IgV-Fc proteins. Data was analyzed using FlowJo Version 10 software (FlowJo LLC, USA). Results for the binding studies for exemplary tested PD-L1/CD112/CD155 stack Fc fusion molecules (tested at 33.3 nM) are shown in Table 23. As shown, several stack proteins bound PD-1, TIGIT and/or CD112R with high affinity.

TABLE 23

Binding of Stacks to Cell-Expressed Counter Structure

			Binding to Jurkat Transfectants			
Category	Description	SEQ ID NO	TIGIT	CD112R	PD1	TIGIT/CD112R/PD1
Stacks	(PD-L1 IgV) (G4S)3 (CD155 IgV) (G4S)3 (CD112 IgV) (G4S)2 Fc	1267	2457	969	16989	19041
	(PD-L1 IgV) (G4S)3 (CD112 IgV) (G4S)3 (CD155 IgV) (G4S)2 Fc	1268	1504	289	21968	18727
Controls	non-stack variant PD-L1 IgV-Fc control	659	101	100	20713	18468
	non-stack variant CD155 IgV-Fc control	1271	6294	55	31	961
	non-stack variant CD112 IgV-Fc control	1374	358	516	459	477

TABLE 23-continued

Binding of Stacks to Cell-Expressed Counter Structure						
			Binding to Jurkat Transfectants			
Category	Description	SEQ ID NO	TIGIT	CD112R	PD1	TIGIT/ CD112R/ PD1
	Fc Control	1119	23.3	27 MFI at 33.3 nM	22	35

B. Assessment of Bioactivity of Affinity-Matured IgSF Domain-Containing Molecules

[0725] Jurkat effector cells expressing an IL-2-luciferase reporter and cell-surface PD-1, CD112R, and TIGIT were suspended at 2×10^6 cells/mL in Jurkat Assay buffer (RPMI1640+5% FBS) and anti-CD28 was added to a final concentration of 3 μg/mL. Jurkat cells were then plated at 50 μL/well for a total of 100,000 cells per well.

[0726] To each well, 25 μL of PD-L1/CD112/CD155 stacked IgV-Fc test protein was added to the Jurkat cells. As a control, non-stack variant PD-L1 IgV-Fc, CD112 IgV-Fc or CD155 IgV-Fc fusion molecules, alone or in combination, also were assessed for comparison. Anti-TIGIT antibody (clone MBSA43), anti-PD-1 antibody (nivolumab) or an empty Fc molecule are used as controls. All proteins were added at five concentrations: 400 nM, 100 nM, 25 nM, 6.25 nM, 1.56 nM, or 0.49 nM. The Jurkat cells with test or control proteins were incubated for 15 minutes at room temperature. CHO-derived artificial antigen presenting cells (aAPC) displaying transduced cell surface anti-CD3 single chain Fv (OKT3), PD-L1 and CD112 were brought to 0.8×10^6 cells/mL and 25 μL of cells was added to each well bringing the final volume of each well to 100 μL. Each well had a final ratio of 5:1 Jurkat:CHO cells and a test protein concentration of 100, 25, 6.25, 1.56, 0.47 or 0.12 nM and an anti-CD28 concentration of 1.5 μg/mL. Jurkat cells and CHO cells were incubated for 5 hours at 37 degrees Celsius in a humidified 5% CO_2 incubation chamber. Plates are then removed from the incubator and acclimated to room temperature for 15 minutes. 100 μL of a cell lysis and luciferase substrate solution (BioGlo luciferase reagent, Promega) was added to each well and the plates were incubated on an orbital shaker for 10 minutes. Luminescence was measured with a 1 second per well integration time using a BioTek Cytation luminometer.

[0727] An average relative luminescence value (RLU) was determined for each test sample and a fold increase (or decrease) in IL-2 reporter signal was calculated for each stack molecule compared to non-stack variant PD-L1 IgV-Fc, variant CD112 IgV-Fc and variant CD155 IgV-Fc proteins. Because the assay is a measure of blockade of inhibitory signals, an increase in luminescent signal compared to control indicates the presence of blocking activity.

[0728] As shown in Table 24, the luciferase activity of the Jurkat effector cells co-cultured with anti-CD3/PD-L1/CD112 aAPC and the PD-L1/CD112/CD155 stack Fc molecules was altered (increased) for each molecule tested. The differences in luminescence signals demonstrate the differences in binding of the PD-L1/CD112/CD155 stack-Fc molecules to PD-1, CD112R and TIGIT and the resulting co-blockade of inhibitory activity. In the Table, Column 1 sets forth the SEQ ID NO identifier for each PD-L1/CD112/CD155 stack-Fc variant tested.

TABLE 24

Jurkat/IL2/TIGIT/PD1 Reporter Assay						
Description	SEQ ID NO	RLU	Fold Increase compared to Fc Control	Fold Increase compared to PD-L1 IgV-Fc	Fold Increase compared to CD155 IgV-Fc	Fold Increase compared to CD112-IgV -Fc
(PD-L1 IgV) (G4S)3 (CD155 IgV) (G4S)3 (CD112 IgV) (G4S)2 Fc	1267	896	1.44	1.12	1.04	1.40
(PD-L1 IgV) (G4S)3 (CD112 IgV) (G4S)3 (CD155 IgV) (G4S)2 Fc	1268	961	1.54	1.20	1.12	1.50
non-stack variant PD-L1 IgV-Fc control	659	800	1.28	1.00	0.93	1.25
non-stack variant CD155 IgV-Fc control	1271	859	1.38	1.07	1.00	1.34
non-stack variant CD112 IgV-Fc control	1374	640	1.03	0.80	0.75	1.00
Fc Control	1119	624	1.00	0.78	0.73	0.98

[0729] The present invention is not intended to be limited in scope to the particular disclosed embodiments, which are provided, for example, to illustrate various aspects of the invention. Various modifications to the compositions and methods described will become apparent from the description and teachings herein. Such variations may be practiced without departing from the true scope and spirit of the disclosure and are intended to fall within the scope of the present disclosure.

SEQUENCE LISTING

The patent application contains a lengthy sequence listing. A copy of the sequence listing is available in electronic form from the USPTO web site (https://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20230381229A1). An electronic copy of the sequence listing will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

1. A variant CD155 polypeptide, comprising an IgV domain or a specific binding fragment thereof, an IgC domain or a specific binding fragment thereof, or both, wherein the variant CD155 polypeptide comprises one or more amino acid modifications at one or more positions in an unmodified CD155 or a specific binding fragment thereof corresponding to position(s) selected from 7, 8, 9, 10, 11, 12, 13, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 67, 68, 69, 70, 72, 73, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 87, 88, 89, 90, 91, 92, 94, 95, 96, 97, 98, 99, 100, 102, 104, 106, 107, 108, 110, 111, 112, 113, 114, 115, or 116 with reference to positions set forth in SEQ ID NO:47.

2-4. (canceled)

5. The variant CD155 polypeptide of claim **1**, wherein the unmodified CD155 comprises (i) the sequence of amino acids set forth in SEQ ID NO:47, (ii) a sequence of amino acids that has at least 95% sequence identity to SEQ ID NO:47; or (iii) a portion of (i) or (ii) comprising an IgV domain or IgC domain or specific binding fragment thereof or both.

6. (canceled)

7. The variant CD155 polypeptide of claim **1**, wherein the variant CD155 comprises up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acid modifications compared to the unmodified CD155 polypeptide.

8. The variant CD155 of claim **1**, wherein the variant CD155 comprises a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:47 or a specific binding fragment thereof.

9-11. (canceled)

12. The variant CD155 polypeptide of claim **1**, wherein the one or more amino acid modifications are selected from G7E, D8G, V9A, V9D, V9I, V9L, V10F, V10G, V10I, V11A, V11E, V11M, Q12H, Q12K, Q12L, A13E, A13R, T15I, T15S, Q16H, P18C, P18F, P18H, P18L, P18S, P18T, P18Y, G19D, F20I, F20S, F20Y, L21S, L21M, G22S, D23A, D23G, D23N, D23Y, S24A, S24P, V25A, V25E, T26M, C29R, Y30C, Y30F, Y30H, Q32L, Q32R, V33M, P34S, N35D, N35F, N35S, M36I, M36R, M36T, E37G, E37P, E37S, E37V, V38A, V38G, T39A, T39S, H40Q, H40R, H40T, V41A, V41M, S42A, S42C, S42G, S42L, S42N, S42P, S42Q, S42T, S42V, S42W, L44P, L44V, T45A, T45G, T45I, T45S, T45Q, T45V, W46C, W46R, A47E, A47G, A47V, R48Q, H49L, H49Q, H49R, G50S, E51G, E51K, E51V, S52A, S52E, S52G, S52K, S52L, S52M, S52P, S52Q, S52R, S52T, S52W, G53R, S54C, S54G, S54H, S54N, S54R, M55I, M55L, M55V, A56V, V57A, V57L, V57T, F58L, F58Y, H59E, H59N, N59R, Q60H, Q60K, Q60P, Q60R, T61A, T61G, T61K, T61M, T61R, T61S, Q62F, Q62H, Q62K, Q62L, Q62M, Q62R, Q62Y, P64S, S65A, S65C, S65G, S65D, S65T, S65Y, S65H, S65N, S65T, S65W, S67A, S67E, S67G, S67H, S67L, S67T, S67V, S67W, E68D, E68G, S69L, S69P, K70E, K70R, K70Q, L72Q, E73D, E73G, E73R, V75A, V75L, A76E, A76G, A76T, A77T, A77V, R78G, R78K, R78S, L79P, L79Q, L79V, G80D, G80S, A81E, A81P, A81T, A81V, E82D, E82G, L83P, L83Q, R84W, N85D, N85Y, N87T, L88P, R89K, M90I, M90L, M90V, F91S, F91T, F91P, G92A, G92E, G92W, R94H, V95A, E96D, D97G, E98D, E98S, G99D, G99Y, N100Y, T102S, L104E, L104M, L104N, L104P, L104Q, L104T, L104Y, V106A, V1061, V106L, T107A, T107L, T107M, T107S, T107V, F108H, F108L, F108Y, Q110R, G111D, G111R, S112I, S112N, S112V, R113G, R113W, S114N, S114T, V115A, V115M, D116G, or D116N, or a conservative amino acid substitution thereof.

13. The variant CD155 polypeptide of claim **1**, wherein the one or more amino acid modifications are selected from P18S/P64S/F91S, P18S/F91S/L104P, P18L/L79V/F91S, P18S/F91S, P18T/F91S, P18T/S42P/F91S, G7E/P18T/Y30C/F91S, P18T/F91S/G111D, P18S/F91P, P18T/F91S/F108L, P18S/F91S, P18T/T45A/F91S, P18T/F91S/R94H, P18S/Y30C/F91S, A81V/L83P, A13E/P18S/A56V/F91S, P18T/F91S/V115A, P18T/Q60K, S52M, T45Q/S52L/L104E/G111R, S42G, Q62F, S52Q, S42A/L104Q/G111R, S42A/S52Q/L104Q/G111R, S52W/L104E, S42C, S52W, S52M/L104Q, S42L/S52L/Q62F/L104Q, S42W, S42Q, S52L, S52R, L104E, G111R, S52E, Q62Y, T45Q/S52M/L104E, S42N/L104Q/G111R, S52M/V57L, S42N/S52Q/Q62F, S42A/S52L/L104E/G111R, S42W/S52Q/V57L/Q62Y, L104Q, S42L/S52Q/L104E, S42C/S52L, S42W/S52R/Q62Y/L104Q, T45Q/S52R/L104E, S52R/Q62F/L104Q/G111R, T45Q/S52L/V57L/L104E, S52M/Q62Y, Q62F/L104E/G111R, T45Q/S52Q, S52L/L104E, S42V/S52E, T45Q/S52R/G111R, S42G/S52Q/L104E/G111R, S42N/S52E/V57L/L104E, S42C/S52M/Q62F, S42L, S42A, S42G/S52L/Q62F/L104Q, S42N, P18T/S65A/S67V/F91S, P18F/T39A/T45Q/T61R/S65N/S67L/E73G/R78G, P18T/T45Q/T61R/S65N/S67L, P18F/S65A/S67V/F91S, P18F/

T45Q/T61R/S65N/S67L/F91S/L104P, P18S/L79P/L104M, P18S/L104M, L79P/L104M, P18T/T45Q/L79P, P18T/T45Q/T61R/S65H/S67H, P18T/A81E, P18S/D23Y/E37P/S52G/Q62M/G80S/A81P/G99Y/S 112N, A13R/D23Y/E37P/S42P/Q62Y/A81E, A13R/D23Y/E37P/G99Y/S112N, A13R/D23Y/E37P/Q62M/A77V/G80S/A81P/G99Y, P18L/E37S/Q62M/G80S/A81P/G99Y/S112N, P18S/L104T, P18S/Q62H/L79Q/F91S, T45Q/S52K/Q62F/L104Q/G111R, T45Q/S52Q/Q62Y/L104Q/G111R, T45Q/S52Q/Q62Y/L104E/G111R, V57A/T61M/S65W/S67A/E96D/L104T, P18L/V57T/T61S/S65Y/S67A/L104T, P18T/T45Q, P18L/V57A/T61M/S65W/S67A/L104T, T61M/S65W/S67A/L104T, P18S/V41A/S42G/T45G/L104N, P18H/S42G/T45I/S52T/G53R/S54H/V57L/H59E/T61S/S65D/E68G/L104N, P18S/S42G/T45V/F58L/S67W/L104N, P18S/T45I/L104N, P18S/S42G/T45G/L104N/V106A, P18H/H40R/S42G/T45I/S52T/G53R/S54H/V57L/H59E/T61S/S65D/E68G/L104Y/V106L/F108H, E37V/S42G/T45G/L104N, P18S/T45Q/L79P/L104T, P18L/Q62R, A13R/D23Y/E37P/S42L/S52G/Q62Y/A81E, P18L/H49R/L104T/D116N, A13R/D23Y/E37P/Q62M/G80S/A81P/L104T, S65T/L104T, A13R/D23Y/E37P/S52G/V57A/Q62M/K70E/L104T, P18L/A47V/Q62Y/E73D/L104T, H40T/V41M/A47V/S52Q/Q62L/S65T/E73R/D97G/E98S/L104T/D116N, P18L/S42P/T45Q/T61G/S65H/S67E/L104T/D116N, P18S/H40T/V41M/A47V/S52Q/Q62L/S65T/E73R/L104M/V106A, H40T/V41M/A47V/S52Q/Q62L/S65T/E68G/E73R/D97G/E98S/L104T, T45Q/S52E/L104E, T45Q/S52E/Q62F/L104E, P18F/T26M/L44V/Q62K/L79P/F91S/L104M/G111D, P18S/T45S/T61K/S65W/S67A/F91S/G111R, P18S/L79P/L104M/T107M, P18S/S65W/S67A/M90V/V95A/L104Q/G111R, P18S/A47G/L79P/F91S/L104M/T107A/R113W, P18T/D23G/S24A/N35D/H49L/L79P/F91S/L104M/G111R, V9L/P18S/Q60R/V75L/L79P/R89K/F91S/L104E/G111R, P18S/H49R/E73D/L79P/N85D/F91S/V95A/L104M/G111R, V11A/P18S/L79P/F91S/L104M/G111R, V11A/P18S/S54R/Q60P/Q62K/L79P/N85D/F91S/T107M, P18T/S52P/S65A/S67V/L79P/F91S/L104M/G111R, P18T/M36T/L79P/F91S/G111R, D8G/P18S/M36I/V38A/H49Q/A76E/F91S/L104M/T107A/R113W, P18S/S52P/S65A/S67V/L79P/F91S/L104M/T107S/R113W, T15I/P18T/L79P/F91S/L104M/G111R, P18F/T26M/L44V/Q62K/L79P/E82D/F91S/L104M/G111D, P18T/E37G/G53R/Q62K/L79P/F91S/E98D/L104M/T107M, P18L/K70E/L79P/F91S/V95A/G111R, V9I/Q12K/P18F/S65A/S67V/L79P/L104T/G111R/S112I, P18F/S65A/S67V/F91S/L104M/G111R, V9I/V10I/P18S/F20S/T45A/L79P/F91S/L104M/F108Y/G111R/S112V, V9L/P18L/L79P/M90I/F91S/T102S/L104M/G111R, P18C/T26M/L44V/M55I/Q62K/L79P/F91S/L104M/T107M, V9I/P18T/D23G/L79P/F91S/G111R, P18F/L79P/M90L/F91S/V95A/L104M/G111R, P18T/M36T/S65A/S67E/L79Q/A81T/F91S/G111R, V9L/P18T/Q62R/L79P/F91S/L104M/G111R, P18S/S65W/S67A/L104Q/G111R, P18T/G19D/M36T/S54N/L79P/L83Q/F91S/T107M/F108Y, V9L/P18L/M55V/S69L/L79P/A81E/F91S/T107M, P18F/H40Q/T61K/Q62K/L79P/F91S/L104M/T107V, P18S/Q32R/Q62K/R78G/L79P/F91S/T107A/R113W, Q12H/P18T/L21S/G22S/V57A/Q62R/L79P/F91S/T107M, V9I/P18S/S24P/H49Q/F58Y/Q60R/Q62K/L79P/F91S/T107M, P18T/W46C/H49R/S65A/S67V/A76T/L79P/S87T/L104M, P18S/S42T/E51G/L79P/F91S/G92W/T107M, V10F/T15S/P18L/R48Q/L79P/F91S/T107M/V115M, P18S/L211M/Y30F/N35D/R84W/F91S/T107M/D116G, P18F/E51V/S54G/Q60R/L79Q/E82G/S87T/M901/F91S/G92R/T107M, Q16H/P18F/F91S/T107M, P18T/D23G/Q60R/S67L/L79P/F91S/T107M/V115A, D8G/V9I/V11A/P18T/T26M/S52P/L79P/F91S/G92A/T107L/V115A, V9I/P18F/A47E/G50S/E68G/L79P/F91S/T107M, P18S/M55I/Q62K/S69P/L79P/F91S/T107M, P18T/T39S/S52P/S54R/L79P/F91S/T107M, P18S/D23N/L79P/F91S/T107M/S114N, P18S/P34S/E51V/L79P/F91S/G111R, P18S/H59N/V75A/L79P/A81T/F91S/L104M/T107M, P18S/W46R/E68D/L79P/F91S/T107M/R113G, V9L/P18F/T45A/S65A/S67V/R78K/L79V/F91S/T107M/S114T, P18T/M55L/T61R/L79P/F91S/V106I/T107M, T15I/P18S/V33M/N35F/T39S/M55L/R78S/L79P/F91S/T107M, P18S/Q62K/K70E/L79P/F91S/G92E/R113W, P18F/F20I/T26M/A47V/E51K/L79P/F91S, P18T/D23A/Q60H/L79P/M90V/F91S/T107M, P18S/D23G/C29R/N35D/E37G/M55I/Q62K/S65A/S67G/R78G/L79P/F91S/L104M/T107M/Q110R, A13E/P18S/M36R/Q62K/S67T/L79P/N85D/F91S/T107M, V9I/P18T/H49R/L79P/N85D/F91S/L104T/T107M, V9A/P18F/T61S/Q62L/L79P/F91S/G111R, D8E/P18T/T61A/L79P/F91S/T107M, P18S/V41A/H49R/S54C/L79S/N85Y/L88P/F91S/L104M/T107M, V11E/P18H/F20Y/V25E/N35S/H49R/L79P/F91S/T107M/G111R, V11A/P18F/D23A/L79P/G80D/V95A/T107M, P18S/K70R/L79P/F91S/G111R, V9L/V11M/P18S/N35S/S54G/Q62K/L79P/L104M/T107M/V115M, V9L/P18Y/V25A/V38G/M55V/A77T/L79P/M90I/F91S/L104M, V10G/P18T/L72Q/L79P/F91S/T107M, P18S/H59R/A76G/R78S/L79P, V9A/P18S/M36T/S65G/L79P/F91S/L104T/G111R/S112I, P18T/S52A/V57A/Q60R/Q62K/S65C/L79P/F91T/N100Y/T107M, V11A/P18F/N35D/A47E/Q62K/L79P/F91S/G99D/T107M/S114N, V11A/P18T/N35S/L79P/S87T/F91S, V9D/V11M/Q12L/P18S/E37V/M55I/Q60R/K70Q/L79P/F91S/L104M/T107M, or T15S/P18S/Y30H/Q32L/Q62R/L79P/F91S/T107M.

14. (canceled)

15. The variant CD155 polypeptide of claim **1**, comprising the sequence of amino acids set forth in any of SEQ ID NOS: 59-80, 178-274, 1230-1252, 1269, and 1610-1655 or a specific binding fragment thereof, or a sequence of amino acids that exhibits at least 95% sequence identity to any of SEQ ID NOS: 59-80, 178-274, 1230-1252, 1269, and 1610-1655 or a specific binding fragment thereof and that contains the one or more of the amino acid modifications thereof; or

the sequence of amino acids set forth in any of SEQ ID NOS: 81-102, 156-177, 275-468, 1184-1229, 1270-1271, 1656-1747 or a specific binding fragment thereof, or a sequence of amino acids that exhibits at least 95% sequence identity to any of SEQ ID NOS: 81-102, 156-177, 275-468, 1184-1229, 1270-1271, 1656-1747 or a specific binding fragment thereof and that contains the one or more of the amino acid modifications thereof.

16-19. (canceled)

20. The variant CD155 polypeptide of claim **1**, wherein the variant CD155 polypeptide specifically binds to the ectodomain of one or more of TIGIT, CD226 or CD96 with increased affinity compared to the binding of the unmodified CD155 to the same ecotodomain of TIGIT, CD226 or CD96.

21-35. (canceled)

36. The variant CD155 polypeptide of claim **1**, wherein the variant CD155 polypeptide is linked to a multimerization domain.

37. The variant CD155 polypeptide of claim **36**, wherein the multimerization domain is an Fc domain or a variant Fc domain with reduced effector function.

38-42. (canceled)

43. The variant CD155 polypeptide of claim **1** wherein the variant CD155 polypeptide is a transmembrane immunomodulatory protein further comprising a transmembrane domain.

44-49. (canceled)

50. An immunomodulatory protein, comprising the variant CD155 of claim **1** linked to a second polypeptide comprising an immunoglobulin superfamily (IgSF) domain.

51-82. (canceled)

83. A conjugate, comprising a variant CD155 polypeptide of claim **1** linked to a targeting moiety that specifically binds to a molecule on the surface of a cell.

84-90. (canceled)

91. A nucleic acid molecule, encoding the variant CD155 polypeptide of claim **1**.

92-99. (canceled)

100. A method of producing a variant CD155 polypeptide or an immunomodulatory protein, comprising introducing the nucleic acid molecule of claim **91** or a vector comprising the nucleic acid molecule of claim **91** into a host cell under conditions to express the protein in the cell.

101. (canceled)

102. A method of engineering a cell expressing a variant CD155 polypeptide, comprising introducing a nucleic acid molecule encoding the variant CD155 polypeptide of claim **1** into a host cell under conditions in which the polypeptide is expressed in the cell.

103. An engineered cell, comprising the variant CD155 polypeptide of claim **1** or a nucleic acid molecule encoding a sequence of amino acids comprising the variant CD155 polypeptide of claim **1**.

104-115. (canceled)

116. The engineered cell of claim **103**, wherein the cell further comprises a chimeric antigen receptor (CAR) or an engineered T-cell receptor (TCR).

117. An infectious agent, comprising a variant CD155 polypeptide of claim **1** or a nucleic acid molecule encoding a sequence of amino acids comprising the variant CD155 polypeptide of claim **1**.

118-128. (canceled)

129. A pharmaceutical composition, comprising the variant CD155 polypeptide of claim **1**; and immunomodulatory protein or conjugate comprising the variant CD155 polypeptide of claim **1**; or an engineered cell or infectious agent comprising the variant CD155 polypeptide of claim **1** or a nucleic acid molecule encoding the variant CD155 polypeptide of claim **1**.

130-135. (canceled)

136. A method of modulating an immune response in a subject, comprising administering the pharmaceutical composition of claim **129** to the subject.

137. A method of modulating an immune response in a subject, comprising administering the engineered cell of claim **103**.

138-159. (canceled)

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