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(54) Title: FUSION PROTEINS OF GDF15 AND USE THEREOF

(57) Abstract: Provided herein are fusion polypeptides that comprise GDF15 and single domain antibody binding to serum albumin, and the polypeptide complexes thereof, pharmaceutical compositions that comprise the fusion polypeptides and use of the fusion polypeptides to treat diseases.

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FUSION PROTEINS OF GDF15 AND USE THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to fusion proteins, pharmaceutical compositions thereof, and methods of using such to prevent and/or treat diseases.

BACKGROUND

[0002] Growth differentiation factor 15 (GDF15), also named as Macrophage inhibitory cytokine-1 or MIC-1, is a member of the transforming growth factor beta superfamily. The function of GDF15 is not fully understood. Existing studies show that GDF15 may have a role in regulating inflammatory pathways, participating in regulating apoptosis, cell repair and cell growth, which are biological processes observed in cardiovascular and neoplastic disorders. Other studies show that GDF15 possesses therapeutic utility in metabolic disorders such as obesity.

[0003] Metabolic disorders are commonly associated with insulin resistance, visceral adiposity, atherogenic dyslipidemia, etc., which pose major and escalating public health and clinical challenge worldwide. However, existing treatment for metabolic diseases faces problems such as short half-life and/or low efficacy.

[0004] Therefore, there is a need for an improved therapeutic solution for treating metabolic diseases.

SUMMARY OF THE INVENTION

[0005] Provided herein are fusion proteins of GDF15, and pharmaceutical compositions and methods of use for treating/preventing metabolic disorders.

[0006] In one aspect, the present disclosure provides a fusion polypeptide comprising:

- a) a first polypeptide fragment comprising a serum albumin binding single domain antibody; and
 - b) a second polypeptide fragment comprising a GFRAL receptor agonist;
- wherein the first polypeptide fragment and the second polypeptide fragment are linked to one another directly or via a linker.

- [0007]** In some embodiments, the single domain antibody comprises a VHH domain.
- [0008]** In some embodiments, the VHH domain is humanized.
- [0009]** In some embodiments, the VHH domain comprises a complementarity determining region 1 (CDR1), CDR2 and CDR3, wherein the CDR1 comprises the sequence of SEQ ID NO: 1 or a variant thereof having up to 3, 2, or 1 amino acid mutation, a CDR2 comprising the sequence of SEQ ID NO: 2 or a variant thereof having up to 3, 2, or 1 amino acid mutation, and/or a CDR3 comprising the sequence of SEQ ID NO: 3 or a variant thereof having up to 3, 2, or 1 amino acid mutation, wherein the VHH domain retains the binding specificity to serum albumin, optionally to human serum albumin.
- [0010]** In some embodiments, the VHH domain comprises a complementarity determining region 1 (CDR1) comprising the sequence of SEQ ID NO: 1, a CDR2 comprising the sequence of SEQ ID NO: 2, and a CDR3 comprising the sequence of SEQ ID NO: 3.
- [0011]** In some embodiments, the VHH domain comprises the sequence of SEQ ID NO: 4, or a variant thereof having at least 70% (e.g. at least 75%, 80%, 85%, 90%, 95%, 99% identity to SEQ ID NO: 4, wherein the variant retains the binding specificity and/or affinity to serum albumin.
- [0012]** In some embodiments, the variant of SEQ ID NO: 4 has up to 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid mutation relative to SEQ ID NO: 4.
- [0013]** In some embodiments, the serum albumin binding single domain antibody further comprises an N-terminal extension attached to the VHH domain.
- [0014]** In some embodiments, the N-terminal extension comprises amino acid residues of SG, AG, S or A.
- [0015]** In some embodiments, the serum albumin binding single domain antibody comprises a sequence selected from SEQ ID NOs: 4-8.
- [0016]** In some embodiments, the GFRAL receptor agonist comprises GDF15.
- [0017]** In some embodiments, the GDF15 comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 9 while retaining substantial biological activity of SEQ ID NO: 9.

[0018] In some embodiments, the GDF15 comprises no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 mutations relative to SEQ ID NO: 9 while retaining substantial biological activity of SEQ ID NO: 9.

[0019] In some embodiments, the GDF15 comprises one or more mutations at a position selected from the group consisting of: A1, R2, N3, H6, P11, H18, T19, V20, R21, A30, M43, A47, R53, A54, M57, H66, R67, L68, A75, A81, P85, M86, Q90, T92, and L105, or any combination thereof, relative to SEQ ID NO: 9.

[0020] In some embodiments, the one or more mutations in GDF15 comprises:

- 1) mutation of N3 selected from the group consisting of: N3Q, N3S, N3E, N3A, N3T, N3P, N3G, N3V, N3H, N3Y and deletion of N3; and/or
- 2) substitution of M57 selected from the group consisting of: M57A, M57E and M57L; and/or
- 3) substitution of M86L or M86A.

[0021] In some embodiments, the one or more mutations in GDF15 are selected from the group consisting of: R2S, R2A, R2E, N3S, N3E, N3A, N3T, N3P, N3G, N3V, N3H, N3Y, N3Q, H6D, P11E, H18E, H18Q, T19S, V20L, R21E, A30E, M43L, M43E, A47E, R53E, A54E, M57A, M57E, M57L, H66E, R67E, L68E, A75E, A81E, P85E, M86F, M86A, M86L, Q90E, T92E, L105E, deletion of N3, and deletion of N-terminal 1-3 residues, or any combination thereof, relative to SEQ ID NO: 9.

[0022] In some embodiments, the GDF15 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-12 and 14.

[0023] In some embodiments, the fusion polypeptide comprises from N terminus to C terminus, the serum albumin binding single domain antibody, the linker and the GDF15.

[0024] In some embodiments, the linker comprises a polypeptide linker.

[0025] In some embodiments, the polypeptide linker has a length of at least 4 amino acid residues (e.g. at least 4, 12, 24, 40, 50, 60, 70, 80, 90, 100, 110, or 120 amino acid residues).

[0026] In some embodiments, the polypeptide linker has a length of 4-80 or 20-80 or 30-80 or 40-80 amino acid residues.

[0027] In some embodiments, the polypeptide linker has a length of about 4, 8, 20, 40, 50, 60, 70 or 80 amino acid residues.

[0028] In some embodiments, the polypeptide linker comprises at least one acidic amino acid residue.

[0029] In some embodiments, the at least one acidic amino acid residue comprises glutamic acid (E).

[0030] In some embodiments, the polypeptide linker comprises one or more repeats of a repeating sequence.

[0031] In some embodiments, the repeating sequence consists of no more than 4, 5 or 6 types of amino acid residues selected from the group consisting of: G, Q, A, E, P, T and S.

[0032] In some embodiments, the repeating sequence comprises Q.

[0033] In some embodiments, the repeating sequence consists of G, Q, A, E, and P.

[0034] In some embodiments, the repeating sequence consists of a sequence selected from the group consisting of: SEQ ID NO: 15 (GQEPGAQP), SEQ ID NO: 16 (GAQPGAQP), SEQ ID NO: 17 (GQEP), SEQ ID NO: 18 (GAQP), SEQ ID NO: 40 (SEPATSGSETPGTSESATPESGPGTSTEPSEG), SEQ ID NO: 19 (GAQPGQEPGAQP), SEQ ID NO: 20 (GAQPGQEP), SEQ ID NO: 21 (GEQP), SEQ ID NO: 22 (GPQE), SEQ ID NO: 23 (GPEQ), SEQ ID NO: 24 (GSEP), SEQ ID NO: 25 (GESP), SEQ ID NO: 26 (GPSE), SEQ ID NO: 27 (GPES), SEQ ID NO: 28 (GQAP), SEQ ID NO: 29 (GPAQ), SEQ ID NO: 30 (GPQA), SEQ ID NO: 31 (GSQP), SEQ ID NO: 32 (GASP), SEQ ID NO: 33 (GPAS), SEQ ID NO: 34 (GPSA), SEQ ID NO: 35 (GGGS), SEQ ID NO: 36 (GSGS), SEQ ID NO: 37 (GGGS), SEQ ID NO: 38 (GQEPPGQAP), SEQ ID NO: 39: (GQAPGQEP), SEQ ID NO: 41 (SEPATS), SEQ ID NO: 42 (GSETPG), SEQ ID NO: 43 (TSESAT), SEQ ID NO: 44 (PESGPG), SEQ ID NO: 45 (TSTEPS) and GS.

[0035] In some embodiments, the polypeptide linker comprises (Repeat1)_r(Repeat2)_s(Repeat3)_x(Repeat4)_y, wherein Repeat1, Repeat2, Repeat3 and Repeat4 are linked via a peptide bond or via one or more amino acid residues, Repeat1, Repeat2, Repeat3 and Repeat4 independently, comprise or consist of a sequence selected from the group consisting of: SEQ ID NO: 15 (GQEPGAQP), SEQ ID NO: 16

(GAQPGAQP), SEQ ID NO: 17(GQEP), SEQ ID NO: 18 (GAQP), SEQ ID NO: 40 (SEPATSGSETPGTSESATPESGPGTSTEPSEG), SEQ ID NO: 19 (GAQPGQEPGAQP), SEQ ID NO: 219 (GAQPGQEP), SEQ ID NO: 21 (GEQP), SEQ ID NO: 22 (GPQE), SEQ ID NO: 23 (GPEQ), SEQ ID NO: 24 (GSEP), SEQ ID NO: 25 (GESP), SEQ ID NO: 26 (GPSE), SEQ ID NO: 27 (GPES), SEQ ID NO: 28 (GQAP), SEQ ID NO: 29 (GPAQ), SEQ ID NO: 30 (GPQA), SEQ ID NO: 31 (GSQP), SEQ ID NO: 32 (GASP), SEQ ID NO: 33 (GPAS), SEQ ID NO: 34 (GPSA), SEQ ID NO: 35 (GGGS), SEQ ID NO: 36 (GSGS), SEQ ID NO: 37 (GGGS), SEQ ID NO: 38 (GQEPGQAP), SEQ ID NO: 39 (GQAPGQEP), SEQ ID NO: 41 (SEPATS), SEQ ID NO: 42 (GSETPG), SEQ ID NO: 43 (TSESAT), SEQ ID NO: 44 (PESGPG), SEQ ID NO: 45 (TSTEPS) and GS, and r, s, x and y are independently an integer selected from 0 to 30, provided that r, s, x and y are not 0 at the same time.

[0036] In some embodiments, x and y are 0, r, and s are independently an integer selected from 1 to 30, and Repeat1 and Repeat2 are a combination selected from the group consisting of:

- a) Repeat1 comprises or consists of a sequence of SEQ ID NO: 15, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18;
- b) Repeat1 comprises or consists of a sequence of SEQ ID NO: 17, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18;
- c) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 17;
- d) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15;
- e) Repeat1 comprises or consists of a sequence of SEQ ID NO: 19, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18;
- f) Repeat1 comprises or consists of a sequence of SEQ ID NO: 40, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18;
- g) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 38 and
- h) Repeat1 comprises or consists of a sequence of SEQ ID NO: 40, Repeat2 comprises or consists of a sequence of SEQ ID NO: 17.

[0037] In some embodiments, r, x and y are 0, s is an integer selected from 1 to 30, and Repeat1 comprises or consists of a sequence of SEQ ID NO: 17.

[0038] In some embodiments, y is 0, r, s and x are independently an integer selected from 1 to 30, and Repeat1, Repeat2, and Repeat 3 are a combination selected from the group consisting of:

- (a) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 19, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
- (b) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 17, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
- (c) Repeat1 comprises or consists of a sequence of SEQ ID NO: 40, Repeat2 comprises or consists of a sequence of SEQ ID NO: 17, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
- (d) Repeat1 comprises or consists of a sequence of SEQ ID NO: 40, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 17;
- (e) Repeat1 comprises or consists of a sequence of SEQ ID NO: 15, Repeat2 comprises or consists of a sequence of SEQ ID NO: 28, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
- (f) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 38, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 15;
- (g) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
- (h) Repeat1 comprises or consists of a sequence of SEQ ID NO: 17, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 17; and
- (i) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 38.

[0039] In some embodiments, r, s, x and y are independently an integer selected from 1 to 30, and Repeat1, Repeat2, Repeat 3 and Repeat 4 are a combination selected from the group consisting of:

- (a) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 20, Repeat3 comprises or consists of a sequence of SEQ ID NO: 17, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 18;
- (b) Repeat1 comprises or consists of a sequence of SEQ ID NO: 15, Repeat2 comprises or consists of a sequence of SEQ ID NO: 38, Repeat3 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 18;
- (c) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 38, Repeat3 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 18;
- (d) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 28, Repeat3 comprises or consists of a sequence of SEQ ID NO: 18, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 15; and
- (e) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15, Repeat3 comprises or consists of a sequence of SEQ ID NO: 39, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 38.

[0040] In some embodiments, the polypeptide linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 17, 46-49 and 61.

[0041] In some embodiments, the fusion polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 50-60 and 13.

[0042] In another aspect, the present disclosure provides a polypeptide complex comprises a dimer of the fusion polypeptide provided herein.

[0043] In some embodiments, the polypeptide complex is a homo-dimer.

[0044] In some embodiments, the dimer is associated with a disulfide bond.

[0045] In some embodiments, the disulfide bond is formed between the second polypeptide fragments comprising the GDF15.

[0046] In another aspect, the present disclosure provides a polynucleotide encoding the fusion polypeptide provided herein, or the polypeptide complex provided herein.

[0047] In another aspect, the present disclosure provides a vector comprising the polynucleotide provided herein.

[0048] In another aspect, the present disclosure provides a host cell comprising the vector provided herein.

[0049] In some embodiments, the host cell is a prokaryotic cell or eukaryotic cell.

[0050] In another aspect, the present disclosure provides a method of producing the polypeptide complex provided herein, comprising culturing the host cell provided herein under a condition that allows expression of the polynucleotide provided herein.

[0051] In some embodiments, the host cell is prokaryotic cell or a eukaryotic cell.

[0052] In some embodiments, the polypeptide complex is expressed as inclusion bodies.

[0053] In some embodiments, the method further comprises renaturing the polypeptide complex from the inclusion bodies.

[0054] In another aspect, the present disclosure provides a pharmaceutical composition comprising the fusion polypeptide provided herein, or the polypeptide complex provided herein and a pharmaceutically acceptable carrier.

[0055] In another aspect, the present disclosure provides a method of preventing or treating a metabolic disorder in a subject in need thereof, comprising administering a therapeutically effective amount of the fusion polypeptide provided herein, or the polypeptide complex provided herein.

[0056] In some embodiments, the metabolic disorder is diabetes, obesity, non-alcoholic steatohepatitis (NASH), cardiovascular like dyslipidemia, arteriosclerosis, alcoholic steatohepatitis (ASH), diabetic nephropathy, gestational diabetes, metabolic syndrome such as metabolic syndrome X, nonalcoholic fatty liver disease (NAFLD), end-

stage liver disease, hepatic steatosis (fatty liver), liver cirrhosis, or primary biliary cirrhosis (PBC).

[0057] Throughout the present disclosure, the articles “a,” “an,” and “the” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “a fusion polypeptide” means one fusion polypeptide or more than one fusion polypeptides.

[0058] In all occurrences in this application where there are a series of recited numerical values, it is to be understood that any of the recited numerical values may be the upper limit or lower limit of a numerical range. It is to be further understood that the invention encompasses all such numerical ranges, i.e., a range having a combination of an upper numerical limit and a lower numerical limit, wherein the numerical value for each of the upper limit and the lower limit can be any numerical value recited herein. Ranges provided herein are understood to include all values within the range. For example, 1-10 is understood to include all of the values 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, and fractional values as appropriate. Similarly, ranges delimited by “at least” are understood to include the lower value provided and all higher numbers.

[0059] As used herein, “about” is understood to include within three standard deviations of the mean or within standard ranges of tolerance in the specific art. In certain embodiments, about is understood a variation of no more than 0.5.

[0060] The articles “a” and “an” are used herein to refer to one or more than one (i.e. to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[0061] The term “including” is used herein to mean, and is used interchangeably with, the phrase “including but not limited to”. Similarly, “such as” is used herein to mean, and is used interchangeably, with the phrase “such as but not limited to”.

[0062] The term “or” is used inclusively herein to mean, and is used interchangeably with, the term “and/or,” unless context clearly indicates otherwise.

BRIEF DESCRIPTION OF THE FIGURES

[0063] **Figures 1a to 1d** show in vivo activities of the tested fusion proteins in C57BL/6 mice. **Figure 1a** shows food intake upon the treatment of Fusion protein 1 or 2 from Day 1 to Day 11, with semaglutide as a control. **Figure 1b** shows body weight loss

upon the treatment of Fusion protein 1 or 2 from Day 1 to Day 11, with semaglutide as a control. **Figure 1c** shows food intake upon the treatment of Fusion protein 5 or 12 from Day 1 to Day 11. **Figure 1d** shows body weight loss upon the treatment of Fusion protein 5 or 12 from Day 1 to Day 11.

[0064] **Figure 2** shows pharmacokinetic measurement of the tested fusion proteins in C57BL/6 mice.

[0065] **Figures 3a and 3b** show in vivo efficacy of the tested fusion proteins in DIO mice. **Figure 3a** shows food intake upon the treatment of Fusion protein 5 or 12 from Day 1 to Day 12. **Figure 3b** shows body weight loss upon the treatment of Fusion protein 5 or 12 from Day 1 to Day 12.

[0066] **Figures 4a to 4d** show the sequences disclosed in the present disclosure.

FIGURE DETAILED DESCRIPTION

[0067] The following description of the invention is merely intended to illustrate various embodiments of the invention. As such, the specific modifications discussed are not to be construed as limitations on the scope of the invention. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is understood that such equivalent embodiments are to be included herein. All references cited herein, including publications, patents and patent applications are incorporated herein by reference in their entirety.

[0068] Definitions

[0069] The term “amino acid” as used herein refers to an organic compound containing amine (-NH₂) and carboxyl (-COOH) functional groups, along with a side chain specific to each amino acid. The names of amino acids are also represented as standard single letter or three-letter codes in the present disclosure.

[0070] “Percent (%) sequence identity” is defined as the percentage of amino acid (or nucleic acid) residues in a candidate sequence that are identical to the amino acid (or nucleic acid) residues in a reference sequence, after aligning the sequences and, if necessary, introducing gaps, to achieve the maximum number of identical amino acids (or nucleic acids). In other words, percent (%) sequence identity of an amino acid sequence

(or nucleic acid sequence) can be calculated by dividing the number of amino acid residues (or bases) that are identical relative to the reference sequence to which it is being compared by the total number of the amino acid residues (or bases) in the candidate sequence or in the reference sequence, whichever is shorter. Conservative substitution of the amino acid residues is not considered as identical residues. Alignment for purposes of determining percent amino acid (or nucleic acid) sequence identity can be achieved, for example, using publicly available tools such as BLASTN, BLASTp (available on the website of U.S. National Center for Biotechnology Information (NCBI), see also, Altschul S.F. et al, *J. Mol. Biol.*, 215:403–410 (1990); Stephen F. et al, *Nucleic Acids Res.*, 25:3389–3402 (1997)), ClustalW2 (available on the website of European Bioinformatics Institute, see also, Higgins D.G. et al, *Methods in Enzymology*, 266:383-402 (1996); Larkin M.A. et al, *Bioinformatics (Oxford, England)*, 23(21): 2947-8 (2007)), and ALIGN or Megalign (DNASTAR) software. Those skilled in the art may use the default parameters provided by the tool, or may customize the parameters as appropriate for the alignment, such as for example, by selecting a suitable algorithm.

[0071] A “conservative substitution” with reference to amino acid sequence refers to replacing an amino acid residue with a different amino acid residue having a side chain with similar physiochemical properties. For example, conservative substitutions can be made among amino acid residues with hydrophobic side chains (e.g. Met, Ala, Val, Leu, and Ile), among residues with neutral hydrophilic side chains (e.g. Cys, Ser, Thr, Asn and Gln), among residues with acidic side chains (e.g. Asp, Glu), among amino acids with basic side chains (e.g. His, Lys, and Arg), or among residues with aromatic side chains (e.g. Trp, Tyr, and Phe). As known in the art, conservative substitution usually does not cause significant change in the protein conformational structure, and therefore could retain the biological activity of a protein.

[0072] The term “functional form” as used herein, refers to different forms (such as variants, fragments, fusions, derivatives and mimetics) of the parent molecule, which, despite of having difference in amino acid sequences or in chemical structures, still retains substantial biological activity of the parent molecule. The expression “retain substantial biological activity”, as used herein, means exhibiting at least part of (for example, no less than about 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%) or all of the biological activity of the parent molecule. A functional form of a parent polypeptide may include both naturally-occurring variant forms and non-naturally occurring forms such as

those obtained by recombinant methods or chemical synthesis. The functional forms may contain non-natural amino acid residues.

[0073] The term "variant" as used herein refers to a polypeptide having at least 70% sequence identity to the parent polypeptide. A variant may differ from the parent peptide by one or more amino acid residues. For example, a variant may have substitutions, additions, deletions, insertions, or truncations of one more amino acid residue of the parent polypeptide.

[0074] The term "fragment" as used herein refers to partial sequence of the parent polypeptide of any length. A fragment can still retain at least partial function of the parent polypeptide.

[0075] The term "derivative" as used herein refers to a chemically modified polypeptide or fusion polypeptide, in which one or more well-defined number of substituent groups have been covalently attached to one or more specific amino acid residues of the polypeptide or fusion polypeptide. Exemplary chemical modification can be, e.g. alkylation, acylation, esterification, amidation, phosphorylation, glycosylation, labeling, methylation of one or more amino acids, or conjugation with one or more moieties.

[0076] The term "mimetics" as used herein refers to molecular structures that serve as substitutes for amino acids, peptides, polypeptides, or fusion polypeptide. For example, amino acid mimetics, as used herein, can be synthetic structures (either known or yet unknown), which may or may not be an amino acid, but retain the functional features of the parent amino acids while the structure of the amino acid mimetic is different from the structure of the parent amino acid. Examples include a methacryloyl or acryloyl derivative of an amide, β -, γ -, δ -imino acids (such as piperidine-4-carboxylic acid) and the like.

[0077] "Treating" or "treatment" of a condition as used herein includes preventing or alleviating a condition, slowing the onset or rate of development of a condition, reducing the risk of developing a condition, preventing or delaying the development of symptoms associated with a condition, reducing or ending symptoms associated with a condition, generating a complete or partial regression of a condition, curing a condition, or some combination thereof.

[0078] The term "vector" as used herein refers to a vehicle into which a polynucleotide encoding a protein may be operably inserted so as to bring about the

expression of that protein. A vector may be used to transform, transduce, or transfect a host cell so as to bring about expression of the genetic element it carries within the host cell. Examples of vectors include plasmids, phagemids, cosmids, artificial chromosomes such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or P1-derived artificial chromosome (PAC), bacteriophages such as lambda phage or M13 phage, and animal viruses. Categories of animal viruses used as vectors include retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (*e.g.*, herpes simplex virus), poxvirus, baculovirus, papillomavirus, and papovavirus (*e.g.*, SV40). A vector may contain a variety of elements for controlling expression, including promoter sequences, transcription initiation sequences, enhancer sequences, selectable elements, and reporter genes. In addition, the vector may contain an origin of replication. A vector may also include materials to aid in its entry into the cell, including but not limited to a viral particle, a liposome, or a protein coating. A vector can be an expression vector or a cloning vector. The present disclosure provides vectors (*e.g.*, expression vectors) containing the nucleic acid sequence provided herein encoding the fusion polypeptide, at least one promoter (*e.g.*, SV40, CMV, EF-1 α) operably linked to the nucleic acid sequence, and at least one selection marker. Examples of vectors include, but are not limited to, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (*e.g.*, herpes simplex virus), poxvirus, baculovirus, papillomavirus, papovavirus (*e.g.*, SV40), lambda phage, and M13 phage, plasmid pcDNA3.3, pMD18-T, pOptivec, pCMV, pEGFP, pIRES, pQD-Hyg-GSeu, pALTER, pBAD, pcDNA, pCal, pL, pET, pGEMEX, pGEX, pCI, pEGFT, pSV2, pFUSE, pVITRO, pVIVO, pMAL, pMONO, pSELECT, pUNO, pDUO, Psg5L, pBABE, pWPXL, pBI, p15TV-L, pPro18, pTD, pRS10, pLexA, pACT2.2, pCMV-SCRIPT.RTM., pCDM8, pCDNA1.1/amp, pcDNA3.1, pRc/RSV, PCR 2.1, pEF-1, pFB, pSG5, pXT1, pCDEF3, pSVSPORT, pEF-Bos etc.

[0079] The phrase “host cell” as used herein refers to a cell into which an exogenous polynucleotide and/or a vector has been introduced.

[0080] The term “pharmaceutically acceptable” indicates that the designated carrier, vehicle, diluent, excipient(s), and/or salt is generally chemically and/or physically compatible with the other ingredients comprising the formulation, and physiologically compatible with the recipient thereof.

[0081] The term “subject” or “individual” or “animal” or “patient” as used herein refers to human or non-human animal, including a mammal or a primate, in need of

diagnosis, prognosis, amelioration, prevention and/or treatment of a disease or disorder. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, swine, cows, bears, and so on.

[0082] Fusion polypeptides

[0083] Provided herein are fusion proteins or fusion polypeptides comprising a serum albumin binding single domain antibody and a glial cell line-derived neurotrophic factor (GDNF) receptor alpha like (GFRAL receptor) agonist.

[0084] The term "fusion" or "fused" when used with respect to amino acid sequences (e.g. peptide, polypeptide or protein) refers to combination of two or more amino acid sequences, for example by chemical bonding or recombinant means, into a single amino acid sequence which does not exist naturally. A fusion amino acid sequence may be produced by genetic recombination of two encoding polynucleotide sequences, and can be expressed by a method of introducing a construct containing the recombinant polynucleotides into a host cell.

[0085] The term "protein" and "polypeptide" are used interchangeably herein and refer a polymer of amino acid residues linked by covalent bonds such as peptide bonds. A protein or polypeptide as provided herein can comprise naturally occurring or non-natural amino acid residues, or both. Polypeptides and proteins provided herein can comprise any suitable length of amino acid residues, for example, from at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or more amino acid residues in length.

[0086] The term "naturally occurring" amino acid residue, as used herein, refers to an amino acid residue found in native proteins or peptides, all in their D and L stereoisomers if their structure allows such stereoisomeric forms. Examples of naturally occurring amino acid residues include, but not limited to, 20 standard amino acids, including, glycine (Gly or G), alanine (Ala or A), valine (Val or V), leucine (Leu or L), isoleucine (Ile or I), serine (Ser or S), cysteine (Cys or C), threonine (Thr or T), methionine (Met or M), proline (Pro or P), phenylalanine (Phe or F), tyrosine (Tyr or Y), tryptophan (Trp or W), histidine (His or H), lysine (Lys or K), arginine (Arg or R), aspartate (Asp or D), glutamate (Glu or E), asparagine (Asn or N), and glutamine (Gln or Q), and their natural analogs, such as canavanine, pyrrolysine (PYL), selenocysteine, pyrroline-carboxy-lysine

(PCL), Sarcosine, beta-Alanine, phosphoserine, γ -carboxyglutamate, and ornithine. Examples of naturally occurring amino acid residues in their D stereoisomer include, for example, D-aspartate, D-Serine, D-Cysteine, D-Alanine, D-glutamate and so on.

[0087] An "amino acid analog" is a compound that has the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs can have modified R groups {e.g., norleucine) or modified peptide backbones, but will retain the same basic chemical structure as a naturally occurring amino acid.

[0088] A "non-natural" amino acid residue, as used herein, refers to any amino acid residues that are not found in nature, including without limitation, a modified amino acid residue, and/or an amino acid mimetic, which is not one of the known naturally occurring amino acids, yet functions in a manner similar to the naturally occurring amino acids. Modified amino acid or a mimetic can be generated by addition of a chemical entity such as a carbohydrate group, a phosphate group, a farnesyl group, an isofarnesyl group, a fatty acid group, a linker for conjugation, functionalization, or other modification, etc. A non-natural amino acid can also refer to an amino acid manufactured by chemical synthesis. Exemplary non-natural amino acids include, but not limited to, 2-Aminoisobutyric acid (Aib), imidazole-4-acetate (IA), imidazolepropionic acid (IPA), α -aminobutyric acid (Abu), tert-butylglycine (Tle), β -alanine, 3-aminomethyl benzoic acid, anthranilic acid, des-amino-histidine (abbreviated DesaminoHis, alternative name imidazopropionic acid, abbreviated Impr), the beta analogues of amino acids such as β -alanine, 2-amino-histidine, β -hydroxy-histidine, homohistidine, N α -acetyl-histidine, α -fluoro-methyl-histidine, α -methyl-histidine, α,α -dimethyl-glutamic acid, m-CF₃-phenylalanine, α,β -diaminopropionic acid (abbreviated Dap), 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine, (1-aminocyclopropyl)carboxylic acid, (1-aminocyclobutyl)-carboxylic acid, (1-aminocyclopentyl)carboxylic acid, (1-aminocyclohexyl)carboxylic acid, (1-aminocycloheptyl)carboxylic acid, and (1-aminocyclooctyl)carboxylic acid.

[0089] Introduction of non-natural amino acids into a fusion polypeptide, polypeptide fragment, and/or polypeptide complex may be realized by the technology described in Wang et al., Science 292:498-500, 2001 ; Deiters et al., J Am Chem Soc 125:1 1782-1 1783, 2003; Wang and Schultz, Science 301 :964- 967, 2003; Zhang et al.,

Science 303:371-373, 2004 or in US Patent No. 7,083,970. Briefly, some of these expression systems involve site-directed mutagenesis to introduce a stop codon, such as an amber (UAG), ochre (UAA), and opal (UGA) codons) into the open reading frame encoding a fusion polypeptide of the present disclosure. Other codons, such as a four-base codon (e.g. AGGA, AGGU, CGGU, CGCU, CGAU, CCCU, CUCU, CUAU, and GGGU), a five- base codon, a six-base codon, etc. can also be introduced into the expression systems for non-natural amino acids. Such expression vectors are then introduced into a host that can utilize a tRNA specific for the introduced stop codon or other codons and carried with the non-natural amino acid of choice.

[0090] In certain embodiments, the fusion polypeptide is a single chain polypeptide. A single chain polypeptide can still have intrachain bonds within the molecule but does not include complexes formed by one or more interchain bonds.

[0091] In one aspect, the present disclosure provides a fusion polypeptide comprising: a) a first polypeptide fragment comprising a serum albumin binding single domain antibody; and b) a second polypeptide fragment comprising a glial cell line-derived neurotrophic factor (GDNF) receptor alpha like (GFRAL receptor) agonist; wherein the first polypeptide fragment and the second polypeptide fragment are linked to one another directly or via a linker.

[0092] The GFRAL receptor agonist in the fusion polypeptide functions as a therapeutic moiety and the serum albumin binding single domain antibody functions as a fusion partner to increase the half-life of the GFRAL receptor agonist. In some embodiments, the fusion polypeptide has a half-life that is at least 1.5 times (e.g., at least 2 times, at least 5 times, at least 10 times, at least 20 times, at least 30 times, at least 40 times, at least 50 times, at least 60 times, at least 70 times, at least 80 times, at least 90 times, at least 100 times, at least 150 times or at least 200 times) greater than the half-life of the corresponding therapeutic moiety per se in a given animal (e.g. a mouse, a monkey, a human, among others). In some embodiments, the fusion polypeptide has a half-life that is increased with more than 1 hour (e.g., more than 2 hours, more than 6 hours, more than 12 hours, more than 18 hours, more than 24 hours, more than one day, more than two days, more than 3 days, more than 4 days, more than 5 days, more than 6 days, more than 1 weeks, more than 2 weeks, more than three weeks, more than one month, more than two months ,more than three months or more than 6 months) compared to the half-life of the corresponding therapeutic moiety per se in a given animal (e.g. a mouse, a

monkey, a human, among others). In some embodiments, the fusion protein has a half-life that is more than 5 hours (e.g., more than 6 hours, more than 12 hours, more than 18 hours, of about one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, one month, two months, three months or 6 months) in a given animal (e.g. a mouse, a monkey, a human, among others). In some embodiments, the fusion protein has a half-life that permits administration of the fusion protein at a frequency of no more than once per day, once every two days, once every three days, once per week, once every two weeks, once every three weeks, once every month, once every two months, once every three months, once every four months, or once six months in a given animal (e.g. a mouse, a monkey, a human, among others), yet still capable of proving therapeutic benefit.

[0093] The term “half-life” refers to the time taken for the serum concentration of a polypeptide (e.g., a fusion polypeptide) to be reduced by 50%. Methods for pharmacokinetic analysis and determination of half-life are familiar to those skilled in the art. Details may be found in Kenneth, A et al: Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and in Peters et al, Pharmacokinetic analysis: A Practical Approach (1996). Reference is also made to “Pharmacokinetics”, M Gibaldi & D Perron, published by Marcel Dekker, 2nd revised edition (1982).

[0094] Without being bound to any theory, the serum albumin binding single domain antibody may also provide for improved tissue targeting, tissue distribution, tissue penetration, diffusion within the tissue, and enhanced efficacy of the fusion polypeptide as compared with the therapeutic moiety per se.

[0095] Serum albumin binding single domain antibody

[0096] The term “serum albumin” refers to an albumin (a type of globular protein) found in vertebrate blood. Serum albumin is produced by the liver, occurs dissolved in blood plasma and is the most abundant blood protein in mammals. Serum albumin typically has a half-life of around three weeks, which is mainly regulated by neonatal Fc receptor (FcRn). FcRn protects serum albumin from intracellular degradation by binding it with high affinity and diverting it from a lysosomal pathway, returning it to the extracellular compartment. In some embodiments, the serum albumin is selected from human serum albumin (HSA), cynomolgus monkeys serum albumin and mouse serum albumin. In some embodiments, the serum albumin provided herein is HSA.

[0097] The term “antibody” as used herein includes any immunoglobulin that binds to a specific antigen. conventional antibody (e.g., antibodies from human or mice) comprises two heavy (H) chains and two light (L) chains. The heavy chains are classified as α , δ , ϵ , γ , and μ , each heavy chain consists of a variable domain (VH domain) and a first, second, third, and optionally fourth constant region (CH1, CH2, CH3, CH4 respectively); The light chains are classified as λ or κ , while each light chain consists of a variable domain (VL domain) and a constant domain. The antibody has a “Y” shape, with the stem of the Y consisting of the second and third constant domain of two heavy chains bound together via disulfide bonding. Each arm of the Y includes the variable domain and first constant domain of a single heavy chain bound to the variable and constant domains of a single light chain. The variable domains of the light and heavy chains are responsible for antigen binding. The variable domains in both chains generally contain three hypervariable regions called the complementarity determining regions (CDRs, namely CDR1, CDR2, and CDR3 of light chain or heavy chain). CDR boundaries for the antibodies and antigen-binding fragments disclosed herein may be defined or identified by the conventions of Kabat, IMGT, Chothia, or Al-Lazikani (Al-Lazikani, B., Chothia, C., Lesk, A. M., *J. Mol. Biol.*, 273(4), 927 (1997); Chothia, C. *et al.*, *J Mol Biol.* Dec 5;186(3):651-63 (1985); Chothia, C. and Lesk, A.M., *J. Mol. Biol.*, 196,901 (1987); Chothia, C. *et al.*, *Nature.* Dec 21-28;342(6252):877-83 (1989); Kabat E.A. *et al.*, Sequences of Proteins of immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991); Marie-Paule Lefranc *et al.*, *Developmental and Comparative Immunology*, 27: 55-77 (2003); Marie-Paule Lefranc *et al.*, *Immunome Research*, 1(3), (2005); Marie-Paule Lefranc, *Molecular Biology of B cells* (second edition), chapter 26, 481-514, (2015)). The three CDRs are interposed between flanking stretches known as framework regions (FRs, namely FR1, FR2, FR3 and FR4 of light chain or heavy chain), which are more highly conserved than the CDRs and form a scaffold to support the highly variable loops. The constant domains of the heavy and light chains are not involved in antigen-binding, but exhibit various effector functions. Conventional antibodies are assigned to classes based on the amino acid sequences of the constant domains of their heavy chains.

[0098] The term “single domain antibody” refers to an antibody fragment containing a single variable domain of a heavy chain or a single variable domain of a light

chain. A single domain antibody contains 3 complementarity determining regions (CDRs) and is capable of binding to a specific antigen (e.g., serum albumin).

[0099] The single variable domain can be derived from the variable domain of a camelid antibody (VHH domain), or the variable domain of cartilaginous fish antibody (VNAR domain). Both camelid antibodies and cartilaginous fish antibodies naturally lack light chains and consist of a pair of heavy chains. Alternatively, the single variable domain can be derived from the variable domain of a conventional antibody (e.g., from humans or mice) heavy chain (VH domain) or the variable domain of a common antibody light chain (VL domain). It is contemplated that a single domain antibody is fairly small in size, for example, has a molecular weight of no more than 25 kD, no more than 20 kD, no more than 15 kD, or no more than 10 kD.

[00100] The term “single domain antibody” is used herein in its broadest sense and is not limited to a specific biological source or to a specific method of preparation. For example, a single domain antibody can be obtained, for example (1) by isolating the VHH domain or VNAR domain of a naturally occurring heavy chain antibody; (2) by expression of a nucleotide sequence encoding a naturally occurring VHH domain or VNAR domain; (3) by “humanization” (as described below) of a naturally occurring VHH domain or VNAR domain or by expression of a nucleic acid encoding a such humanized VHH domain or VNAR domain; (4) by “camelization” of a naturally occurring VH domain from any animal species, in particular a species of mammal, such as from a human being, or by expression of a nucleic acid encoding such a camelized VH domain; 5) using synthetic or semi-synthetic techniques for preparing proteins, polypeptides or other amino acid sequences; and/or (6) by any combination of the foregoing. Suitable methods and techniques for performing the foregoing will be clear to the skilled person.

[00101] In some embodiments, the single domain antibody described herein comprises a VHH domain derived from antibodies raised in Camelidae species, for example in camel, dromedary, alpaca and guanaco. A single domain antibody comprising a VHH domain are highly soluble and highly stable to heat, pH, proteases and other denaturing agents or conditions.

[00102] In some embodiments, the first polypeptide fragment comprises one or more single domain antibody that is capable of specifically binding to serum albumin.

[00103] The term “binding specificity”, “specific binding” or “specifically binds” in reference to the interaction of a binding molecule, e.g., an antibody, and its binding partner, e.g., an antigen, means that the interaction is dependent upon the presence of a particular structure, e.g., an antigenic determinant or epitope, on the binding partner. In other words, the antibody preferentially binds or recognizes the binding partner even when the binding partner is present in a mixture of other molecules or organisms. Antibodies or fragments thereof that immunospecifically bind to an antigen may be cross-reactive with related antigens, carrying the same epitope. The “binding specificity” is generally measured against nonspecific background binding. Typically, an antibody is considered specific when it binds to the target antigen at least 10 times above background binding.

[00104] The serum binding single domain antibody contemplated herein can bind to or otherwise associate with serum albumin in such a way that the binding of said serum albumin molecule to FcRn is not (significantly) reduced or inhibited (i.e. compared to the binding of said serum albumin molecule to FcRn when the single domain antibody is not bound thereto). In this aspect of the invention, by “not significantly reduced or inhibited” is meant that the binding affinity for serum albumin to FcRn (as measured using a suitable assay, such as SPR) is not reduced by more than 50%, preferably not reduced by more than 30%, even more preferably not reduced by more than 10%, such as not reduced by more than 5%, or essentially not reduced at all. In this aspect “not significantly reduced or inhibited” may also mean that the half-life of the serum albumin molecule is not significantly reduced (e.g., is not reduced by more than 50%, preferably not reduced by more than 30%, even more preferably not reduced by more than 10%, such as not reduced by more than 5%, or essentially not reduced at all, as measured using a suitable technique known per se). In some embodiments, the single domain antibody are capable of binding to amino acid residues on serum albumin that are not involved in binding of serum albumin to FcRn.

[00105] In some embodiments, the single domain antibody described herein binds to serum albumin selected from HSA, cynomolgus monkeys serum albumin and mouse serum albumin. In some embodiments, the binding affinity towards mouse serum albumin is about weaker than that towards human or cynomolgus serum albumin. In some embodiments, the single domain antibody specifically binds to HSA.

[00106] In some embodiments, the single domain antibody described herein binds to serum albumin with sufficient binding affinity. The term “affinity” as used herein

refers to the strength of non-covalent interaction between an immunoglobulin molecule (i.e. antibody) or fragment thereof and an antigen. Affinity can be expressed numerically using “Kd” values. In general, a lower Kd value corresponds to a stronger binding. Kd may be determined by using any conventional method known in the art, including but are not limited to radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), surface plasmon resonance (SPR) method, microscale thermophoresis method, HPLC-MS method and flow cytometry (such as FACS) method. In some embodiment, the antibody disclosed here has a K_d value of $\leq 10^{-6}$ M (e.g. $\leq 5 \times 10^{-7}$ M, $\leq 2 \times 10^{-7}$ M, $\leq 10^{-7}$ M, $\leq 5 \times 10^{-8}$ M, $\leq 2 \times 10^{-8}$ M, $\leq 10^{-8}$ M, $\leq 5 \times 10^{-9}$ M, $\leq 4 \times 10^{-9}$ M, $\leq 3 \times 10^{-9}$ M, $\leq 2 \times 10^{-9}$ M, or $\leq 10^{-9}$ M) with the specific antigen.

[00107] In certain embodiments, the single domain antibody provided herein are capable of binding to HSA at a K_d value of 10^{-5} M to 1×10^{-12} M or less, of 10^{-7} M to 1×10^{-12} M or less, or of 10^{-8} M to 1×10^{-12} M or less. In some embodiments, the K_d is no more than 1×10^{-7} M (e.g. no more than 5×10^{-7} M, no more than 2×10^{-7} M, no more than 10^{-7} M, no more than 5×10^{-8} M, no more than 2×10^{-8} M, no more than 10^{-8} M, no more than 5×10^{-9} M, no more than 4×10^{-9} M, no more than 3×10^{-9} M, no more than 2×10^{-9} M, or no more than 10^{-9} M).

[00108] In some embodiments, the single domain antibody provided herein is a humanized antibody. The term “humanized” as used herein means that the single domain antibody comprises CDRs derived from a non-human animal, and FR regions derived from human. A humanized antibody polypeptide is desirable in its reduced immunogenicity in human. A humanized antibody polypeptide is chimeric in its variable regions, as non-human CDR sequences are grafted to human or substantially human FR sequences. Humanization of an antibody polypeptide can be essentially performed by substituting the non-human (such as camelid) CDR genes for the corresponding human CDR genes in a human immunoglobulin gene (see, for example, Jones et al. (1986) Nature 321:522-525; Riechmann et al. (1988) Nature 332:323-327; Verhoeyen et al. (1988) Science 239:1534-1536).

[00109] Various single domain antibodies that are capable of specifically binding to HSA with a high affinity are known in the art, such as fully human domain antibodies isolated using phase display, VHH antibodies developed from camelidae family, and VNAR antibodies developed from cartilaginous fish, see Zorzi, A et al, Med Chem

Commun, 2019,10, 1068. Exemplary HSA-binding single domain antibodies are disclosed in US8188223B2, US9067991B2, US9321832B2, PCT application WO2008028977A2, WO2008043822A2, WO2020099871A1, G. Winter, et al, Annu. Rev. Immunol., 1994, 12, 433–455., L. J. Holt, et al., Protein Eng., Des. Sel., 2008, 21(5), 283–288, A. Walker, et al., Protein Eng., Des. Sel., 2010, 23(4),271–278, L. J. Goodall, et al.,PLoS One, 2015, 10(9), e0137065, R. L. O'Connor-Semmes, et al., Clin. Pharmacol. Ther., 2014, 96(6), 704–712, C. Read, et al., Basic Clin. Pharmacol.Toxicol., 2019, 1–8, R. Adams, et al., mAbs, 2016, 8(7),1336–1346, E. Dave, et al., mAbs, 2016, 8(7), 1319–1335, S. Steeland, et al., Drug Discovery Today, 2016, 21(7), 1076–1113, K. Coppieters, et al., Arthritis Rheum., 2006, 54(6), 1856–1866, M. Van Roy, , et al., Arthritis Res. Ther., 2015, 17, 135, C. McMahon, et al., Nat. Struct. Mol. Biol., 2018, 25(3), 289–296, M. R. Muller, et al., mAbs, 2012, 4(6), 673–685, all of which are contemplated within the scope of the disclosure and incorporated by reference.

[00110] In some embodiments, the single domain antibody comprises a VHH domain. In some embodiments, the VHH domain is humanized.

[00111] In some embodiments, the VHH domain comprises a complementarity determining region 1 (CDR1), CDR2 and CDR3, wherein the CDR1 comprises the sequence of **SEQ ID NO: 1 (SFGMS)** or a variant thereof having up to 3, 2, or 1 amino acid mutation, a CDR2 comprising the sequence of **SEQ ID NO: 2 (SISGSGSDTLYADSVKG)** or a variant thereof having up to 3, 2, or 1 amino acid mutation, and/or a CDR3 comprising the sequence of **SEQ ID NO: 3 (GGSLSR)** or a variant thereof having up to 3, 2, or 1 amino acid mutation, wherein the VHH domain retains the binding specificity to serum albumin, optionally to human serum albumin.

[00112] In some embodiments, the VHH domain comprises a complementarity determining region 1 (CDR1) comprising the sequence of **SEQ ID NO: 1**, a CDR2 comprising the sequence of **SEQ ID NO: 2**, and a CDR3 comprising the sequence of **SEQ ID NO: 3**.

[00113] In some embodiments, the VHH domain comprises a complementarity determining region 1 (CDR1) consisting of the sequence of **SEQ ID NO: 1**, a CDR2 consisting of the sequence of **SEQ ID NO: 2**, and a CDR3 consisting of the sequence of **SEQ ID NO: 3**.

[00114] In some embodiments, the VHH domain comprises the sequence of **SEQ ID NO: 4**, or a variant thereof having at least 70% (e.g. at least 75%, 80%, 85%, 90%, 95%, 99% identity to **SEQ ID NO: 4**, wherein the variant retains the binding specificity and/or affinity to serum albumin.

[00115] In some embodiments, the variant of **SEQ ID NO: 4** has up to 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid mutation relative to **SEQ ID NO: 4**.

[00116] In certain embodiments, the serum albumin binding single domain antibody further comprises an N-terminal extension attached to the VHH domain. In certain embodiments, the N-terminal extension comprises amino acid residues of SG, AG, S or A. In certain embodiments, the N-terminal extension comprises a tag, optionally a cleavable tag. Without wishing to be bound by any theory, it is believed that certain N-terminal extension can be useful for expression and post-translational processing.

[00117] In certain embodiments, the serum albumin binding single domain antibody does not comprise an N-terminal extension attached to the VHH domain. For example, the VHH domain can be attached to a cleavable tag which, after cleavage, is no longer present in the final product.

[00118] In certain embodiments, the serum albumin binding single domain antibody comprises a sequence selected from **SEQ ID NO: 4-8**.

[00119] In some embodiments, the first polypeptide fragment comprising one or more serum albumin binding single domain antibody.

[00120] One of ordinary skill in the art will appreciate that various amino acid substitutions, e.g., conservative amino acid substitutions, may be made in the sequence of any of the polypeptide fragment described herein, without necessarily decreasing its activity. Examples of amino acid substitutions include substituting an L-amino acid for its corresponding D-amino acid, substituting cysteine for homocysteine or other non-natural amino acids having a thiol- containing side chain, substituting a lysine for homolysine, diaminobutyric acid, diaminopropionic acid, ornithine or other non-national amino acids having an amino containing side chain, or substituting an alanine for norvaline or the like.

[00121] **GDF15**

[00122] The term "GDNF receptor alpha like (GFRAL) receptor" or "GFRAL receptor" or "glial cell line-derived neurotrophic factor receptor alpha like (GFRAL)

receptor" refers to a receptor for GDF15 to recognize and bind so as to initiate a signal transduction as described in WO2017/121865, the disclosure of which is incorporated herein to its entirety.

[00123] The term "GDNF receptor alpha like (GFRAL) receptor agonist" or "GFRAL receptor agonist" as used herein refers to a molecule which, by itself or by its dimer or multimer, is capable of binding to and activating the GFRAL receptor. A GFRAL receptor agonist may elicit a magnitude of GFRAL receptor response that is similar to or partial of a natural ligand.

[00124] A GFRAL receptor agonist can include both a natural ligand of the receptor and an artificially designed or modified molecules that exhibits agonist activity comparable to or no less than 30%, 40% or 50% of that of the natural ligand. In certain embodiments, the GFRAL receptor agonist include, without limitation, GDF15 (including its monomer and dimer), and those disclosed in WO 2017/109706, WO 2013/148117, WO 2014/120619, WO 2012/138919, WO 2013/1 13008, WO 2015/017710, the disclosure of which are incorporated herein to their entirety.

[00125] In certain embodiments, the GFRAL receptor agonist comprises GDF15.

[00126] The term "Growth Differentiation Factor 15" or "GDF15" as used herein is intended to broadly encompass the mature domain of the native GDF15 monomer peptide, its homodimer, and all its functional forms such as its functional variants, fragments, fusions, derivatives and mimetics. The full length native GDF15 peptide consists of 308 amino acid residues (NCI Ref. Seq. NP_004855.2), with amino acids 1-29 being a signal peptide, amino acids 30-196 being a pro-domain, and amino acids 197-308 being a mature domain of 112 amino acids. The GDF15 provided in the present disclosure can be the 112 amino acid mature domain of the full length native GDF15 monomer, dimer, or functional variants, fragments, fusions, derivatives or mimetics thereof.

[00127] The mature GDF15 peptide contains nine cysteine residues that form four intrachain disulfide bonds and one interchain disulfide bond that gives rise to a covalently linked homodimer. In other words, biologically active GDF15 is a homodimer of the mature peptide covalently linked by one interchain disulfide bond. The term "GDF15" as used herein also includes monomer, multimer, more particularly dimer of the protein.

[00128] A functional form of the mature domain of the native GDF15 polypeptide is capable of activating the GFRAL receptor at a level comparable to, or no

less than about 20% (or no less than 30%, 40%, 50%, 60%, 70%, 80%, 90%) of, that of the mature domain of the native GDF15 polypeptide. Activation of the GFRAL receptor can lead to biological activities such as, for example, the ability to lower blood glucose, insulin, triglyceride, or cholesterol levels; the ability to reduce body weight; or the ability to improve glucose tolerance, lipid tolerance, or insulin sensitivity; the ability to lower urine glucose and protein excretion. Many functional forms of the mature domain of the native GDF15 polypeptide are known in the art, for example, without limitation, those disclosed in WO 2015/197446, WO2017/121865, WO2017/109706, WO2013/148117, WO2014/120619(definition to the diseases), WO2012/138919, WO2013/113008, WO2015/017710, disclosure of which are incorporated herein by their entirety.

[00129] In certain embodiments, the GDF15 provided herein comprises an amino acid sequence having at least 90% sequence identity to **SEQ ID NO: 9** while retaining substantial biological activity of **SEQ ID NO: 9**.

[00130] In certain embodiments, the GDF15 comprises no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 substitutions relative to **SEQ ID NO: 9** while retaining substantial biological activity of **SEQ ID NO: 9**. In certain embodiments, the GDF15 comprises at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 substitutions relative to **SEQ ID NO: 9** while retaining substantial biological activity of **SEQ ID NO: 9**.

[00131] In certain embodiments, the GDF15 comprises one or more mutations at a position selected from the group consisting of: A1, R2, N3, H6, P11, H18, T19, V20, R21, A30, M43, A47, R53, A54, M57, H66, R67, L68, A75, A81, P85, M86, Q90, T92, and L105, or any combination thereof. For example, it is believed that substitution at N3 is useful to prevent deamidation and substitution at M57 is useful to reduce oxidation. In certain embodiments, the one or more additional substitutions in GDF15 is at a position of N3 and/or M57. In certain embodiments, the one or more mutations comprise a conservative substitution. As used herein, the numbering of the residues in GDF15 is referred to with reference to the 112 amino acid monomer sequence as set forth in **SEQ ID NO: 9**, where residue 1 is Alanine (A1) and residue 112 is Isoleucine (I112).

[00132] In certain embodiments, the GDF15 comprises a mutation of N3 which is selected from the group consisting of: N3Q, N3S, N3E, N3A, N3T, N3P, N3G, N3V, N3H, N3Y, and deletion of N3. In certain embodiments, the GDF15 comprises a

substitution of M57 which is M57A, M57E or M57L. In certain embodiments, the GDF15 comprises a substitution of R2 which is R2S, R2A, or R2E.

[00133] In certain embodiments, the one or more mutations in GDF15 are selected from the group consisting of: R2S, R2A, R2E, N3S, N3E, N3A, N3T, N3P, N3G, N3V, N3H, N3Y, N3Q, H6D, P11E, H18E, H18Q, T19S, V20L, R21E, A30E, M43L, M43E, A47E, R53E, A54E, M57A, M57E, M57L, H66E, R67E, L68E, A75E, A81E, P85E, M86F, M86A, M86L, Q90E, T92E, L105E, deletion of N3, deletion of N-terminal 1-3 residues (i.e. A1R2N3), or any combination thereof. More suitable mutations that can be made to GDF15 without significantly reducing its biological activity are described in WO2012138919, WO2013148117, WO2014120619, WO2016018931, WO2017202936, and WO2018215525, disclosure of which is incorporated herein by their entirety.

[00134] In certain embodiments, the second polypeptide fragment provided herein comprises GDF15 comprising or consisting of an amino acid sequence selected from the group consisting of **SEQ ID NO: 9-12 and 14**.

[00135] **Linker**

[00136] In certain embodiments, the fusion polypeptide comprises, from N terminus to C terminus, a serum albumin binding single domain antibody operably linked to GDF15, directly or via a linker.

[00137] The term "linker" as used herein can be any suitable bifunctional moiety capable of reacting with at least two entities to be linked, thereby bonding the entities to form one molecule or maintaining association of the entities in sufficiently close proximity. The linker can be integrated in the resulting linked molecule or structure, with or without its reacted functional groups. In certain embodiments, the linker separates a first polypeptide fragment from a second polypeptide fragment, without substantial interference to the respective biological activities of either fragment. Suitable linkers can be, for example without limitation, polypeptide linkers and non-peptide linkers, such as bifunctional chemical moieties, or polymers such as PEG.

[00138] A direct linkage can be, for example, a covalent bond such as a peptide bond and a linker can be, for example, a polypeptide linker. In certain embodiments, the fusion polypeptide comprises from N terminus to C terminus, a serum albumin binding single domain antibody, a polypeptide linker and the GDF15.

[00139] In certain embodiments, the linker comprises a polypeptide linker. The polypeptide linker can be made up of amino acid residues linked together by peptide bonds. The polypeptide linker can further comprise one or more non-natural amino acids.

[00140] In certain embodiments, the polypeptide linker comprises at least 1, 4, 5, 10, 12, 20, 24, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150 or 172 amino acid residues, or more. In certain embodiments, the polypeptide linker has a length of from 1 to 200 (1-200), 1-180, 1-170, 1-160, 1-150, 1-140, 1-130, 1-120, 1-110, 1-100, 1-90, 1-80, 1-70, 1-60, 1-50, 1-40, 4-80, 20-80, 30-80, 40-200, 40-180, 40-170, 40-160, 40-150, 40-140, 40-130, 40-120, 40-110, 40-100, 40-90, or 40-80 amino acid residues. Without wishing to be bound by any theory, it is believed that a suitable length of the polypeptide linker can further improve the biological activity or stability or pharmacokinetic parameters of the linked polypeptide molecule.

[00141] In certain embodiments, the polypeptide linker has a length of 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 50, 52, 56, 60, 64, 68, 70, 72, 76 or 80 amino acid residues.

[00142] Any suitable polypeptide linkers can be used. For example, the polypeptide linker may comprise or consist of amino acid residues selected from the amino acids glycine (G), glutamine (Q), alanine (A), glutamic acid (E), proline (P), Threonine (T), serine (S), methionine (M), or asparagine (N). In some embodiments, the polypeptide linker can be made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. In some embodiments, linkers are polyglycines, polyalanines, combinations of glycine and alanine (such as poly(Gly-Ala)), or combinations of glycine and serine (such as poly(Gly-Ser)).

[00143] In certain embodiments, the polypeptide linker comprises at least one acidic amino acid residue. Acidic amino acid residue refers to an amino acid residue having an acidic side chain that contains a carboxylic acid group with a pKa between 3.5 and 4.5. Exemplary acidic amino acid residues include, but not limited to, aspartic acid and glutamic acid. In certain embodiments, the at least one acidic amino acid residue comprises aspartic acid or glutamic acid.

[00144] In certain embodiments, the polypeptide linker comprises or consists of one or more repeats of a repeating sequence. In certain embodiments, the polypeptide linker comprises or consists of 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, or 30 repeats of a repeating sequence, or within any numerical range defined by any two numbers listed above.

[00145] In certain embodiments, the repeating sequence comprises or consists of no more than 4, 5 or 6 types of amino acid residues selected from the group consisting of: G, Q, A, E, P, T and S. In certain embodiments, the repeating sequence comprises or consists of no more than 4, 5 or 6 types of amino acid residues selected from the group consisting of: G, Q, A, E, P, and S. In certain embodiments, the repeating sequence comprises Q. In certain embodiments, the repeating sequence consists of Q and no more than 3, 4 or 5 types of amino acid residues selected from the group consisting of: G, A, E, P, and S.

[00146] In certain embodiments, the repeating sequence consists of G, Q, A, E, and P.

[00147] In certain embodiments, the repeating sequence comprises or consists of a sequence selected from the group consisting of: **SEQ ID NOs: 15-45** and GS.

[00148] In certain embodiments, the polypeptide linker comprises or consists of more than one repeating sequence. For example, the polypeptide linker comprises or consists of 2, 3, or 4 different repeating sequences. In certain embodiments, the polypeptide linker comprises or consists of sequential or tandem repeats of the different repeating sequences.

[00149] In certain embodiments, the polypeptide linker comprises or consists of (Repeat1)_r(Repeat2)_s(Repeat3)_x(Repeat4)_y, wherein:

Repeat1, Repeat2, Repeat3 and Repeat4 are linked via a peptide bond or via one or more amino acid residues;

Repeat1, Repeat2, Repeat3 and Repeat4 independently, comprise or consist of a sequence selected from the group consisting of: **SEQ ID NOs: 15-45** and GS, and

r, s, x and y are independently an integer selected from 0 to 30 (e.g. from 0-29, 0-28, 0-27, 0-26, 0-25, 0-24, 0-23, 0-22, 0-21, 0-20, 0-19, 0-18, 0-17, 0-16, 0-15, 0-14, 0-13, 0-12, 0-11, 0-10, 0-9, 0-8, 0-7, 0-6, 0-5, 0-4, 0-3, 0-2, or 0-1), provided that r, s, x and y are not 0 at the same time.

[00150] In certain embodiments, x and y are 0, r, and s are independently an integer selected from 1 to 30 (e.g. from 1-29, 1-28, 1-27, 1-26, 1-25, 1-24, 1-23, 1-22, 1-21,

1-20, 1-19, 1-18, 1-17, 1-16, 1-15, 1-14, 1-13, 1-12, 1-11, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, or 1-2), and Repeat1 and Repeat2 are a combination selected from the group consisting of:

- (a) Repeat1 comprises or consists of a sequence of SEQ ID NO: 15, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18;
- (b) Repeat1 comprises or consists of a sequence of SEQ ID NO: 17, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18;
- (c) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 17;
- (d) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15;
- (e) Repeat1 comprises or consists of a sequence of SEQ ID NO: 19, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18;
- (f) Repeat1 comprises or consists of a sequence of SEQ ID NO: 40, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18;
- (g) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 38 and
- (h) Repeat1 comprises or consists of a sequence of SEQ ID NO: 40, Repeat2 comprises or consists of a sequence of SEQ ID NO: 17.

[00151] In certain embodiments, r, x and y are 0, s is an integer selected from 1 to 30 (e.g. from 1-29, 1-28, 1-27, 1-26, 1-25, 1-24, 1-23, 1-22, 1-21, 1-20, 1-19, 1-18, 1-17, 1-16, 1-15, 1-14, 1-13, 1-12, 1-11, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, or 1-2), and Repeat1 comprises or consists of a sequence of **SEQ ID NO: 7**.

[00152] In certain embodiments, y is 0, r, s and x are independently an integer selected from 1 to 30 (e.g. from 1-29, 1-28, 1-27, 1-26, 1-25, 1-24, 1-23, 1-22, 1-21, 1-20, 1-19, 1-18, 1-17, 1-16, 1-15, 1-14, 1-13, 1-12, 1-11, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, or 1-2), and Repeat1, Repeat2 and Repeat3 are a combination selected from the group consisting of:

- (j) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 19, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;

- (k) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 17, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
- (l) Repeat1 comprises or consists of a sequence of SEQ ID NO: 40, Repeat2 comprises or consists of a sequence of SEQ ID NO: 17, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
- (m) Repeat1 comprises or consists of a sequence of SEQ ID NO: 40, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 17;
- (n) Repeat1 comprises or consists of a sequence of SEQ ID NO: 15, Repeat2 comprises or consists of a sequence of SEQ ID NO: 28, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
- (o) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 38, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 15;
- (p) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
- (q) Repeat1 comprises or consists of a sequence of SEQ ID NO: 17, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 17; and
- (r) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 38.

[00153] In certain embodiments, r, s, x and y are independently an integer selected from 1 to 30 (e.g. from 1-29, 1-28, 1-27, 1-26, 1-25, 1-24, 1-23, 1-22, 1-21, 1-20, 1-19, 1-18, 1-17, 1-16, 1-15, 1-14, 1-13, 1-12, 1-11, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, or 1-2), and Repeat1, Repeat2, Repeat 3 and Repeat 4 are a combination selected from the group consisting of:

- (a) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 20, Repeat3 comprises or consists of a sequence of SEQ ID NO: 17, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 18;

(b) Repeat1 comprises or consists of a sequence of SEQ ID NO: 15, Repeat2 comprises or consists of a sequence of SEQ ID NO: 38, Repeat3 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 18;

(c) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 38, Repeat3 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 18;

(d) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 28, Repeat3 comprises or consists of a sequence of SEQ ID NO: 18, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 15; and

(e) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15, Repeat3 comprises or consists of a sequence of SEQ ID NO: 39, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 38.

[00154] In certain embodiments, the polypeptide linker comprises an amino acid sequence selected from the group consisting of **SEQ ID NOs: 17 and 46-49**. In certain embodiments, the polypeptide linker comprises an amino acid sequence selected from the group consisting of **SEQ ID NOs: 48-49**.

[00155] In certain embodiments, the fusion polypeptide provided herein comprise an amino acid sequence selected from the group consisting of: **SEQ ID NOs: 50-59 and 13**.

[00156] Table 1 below shows the SEQ ID NOs of the exemplary fusion polypeptide sequences, and the SEQ ID NOs for the serum albumin binding single domain antibody, linker and GDF15 sequences contained in the fusion polypeptides provided herein. Table 1 below also shows the mutations in the serum albumin binding single domain antibody, and in the GDF15, as well as the repeating sequences and number of repeats in the linker sequences.

Table 1. Exemplary fusion polypeptide sequences

Molecule	SEQ ID NO	Mutations in single domain	Linker	Mutations in GDF15 [#]
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		antibody**		
Fusion Protein 1	50	-1A (SEQ ID NO: 5)	(GAQP)3(GQEP)7 (SEQ ID NO: 49)	N3Q, M57L (SEQ ID NO:10)
Fusion Protein 2	51	-1A (SEQ ID NO: 5)	(GAQP)8(GQEPGAQP)6 (SEQ ID NO: 48)	N3Q, M57L (SEQ ID NO:10)
Fusion Protein 3	52	-1S (SEQ ID NO: 6)	(GAQP)3(GQEP)7 (SEQ ID NO: 49)	N3Q, M57L (SEQ ID NO:10)
Fusion Protein 4	53	-2A, -1G (SEQ ID NO: 7)	(GAQP)3(GQEP)7 (SEQ ID NO: 49)	N3Q, M57L (SEQ ID NO:10)
Fusion Protein 5	54	-2S, -1G (SEQ ID NO: 8)	(GAQP)3(GQEP)7 (SEQ ID NO: 49)	N3Q, M57L (SEQ ID NO:10)
Fusion Protein 6	55	-2S, -1G (SEQ ID NO: 8)	(GAQP)8(GQEPGAQP)6 (SEQ ID NO: 48)	N3Q, M57L (SEQ ID NO:10)
Fusion Protein 7	56	-2S, -1G (SEQ ID NO: 8)	(GQEP)5 (SEQ ID NO: 47)	N3Q, M57L (SEQ ID NO:10)
Fusion Protein 8	57	-2S, -1G (SEQ ID NO: 8)	(GQEP)2 (SEQ ID NO: 46)	N3Q, M57L (SEQ ID NO:10)
Fusion Protein 9	58	-2S, -1G (SEQ ID NO: 8)	(GQEP) (SEQ ID NO: 17)	N3Q, M57L (SEQ ID NO:10)
Fusion Protein 10	59	Native (SEQ ID NO: 4)	(GAQP)3(GQEP)7 (SEQ ID NO: 49)	N3Q, M57L (SEQ ID NO:10)
Fusion Protein 11	13	Native (SEQ ID NO: 4)	(GAQP)8(GQEPGAQP)6 (SEQ ID NO: 48)	N3Q, M57L (SEQ ID NO:10)
Fusion Protein 12	60	-2S, -1G (SEQ ID NO: 8)	(GAQP)2(GQEP)7(GAQP) (SEQ ID NO: 61)	N3Q, M57L (SEQ ID NO:10)

** : Mutations in single domain antibody means mutations relative to **SEQ ID NO: 4**, wherein the first residue is E1, and the last residue is S116; -1 position refers to the first residue extended from the N-terminus of **SEQ ID NO: 4**, and -n position refers to the nth residue extended from the N-terminus of **SEQ ID NO: 4**.

: Mutations in GDF15 means mutations relative to **SEQ ID NO: 9**, wherein the first residue is A1, and the last residue is I112;

[00157] Polypeptide complex

[00158] In another aspect, the present disclosure provides a polypeptide complex comprising a dimer of the fusion polypeptide provided herein.

[00159] The term “polypeptide complex(es)” as used herein, means a complex comprises two or more fusion polypeptides, polypeptides or polypeptide fragments held together by covalent interactions (e.g. disulfide bond between cysteines) or non-covalent interactions. A polypeptide complex can be generated naturally or synthetically. The two or more fusion polypeptides, polypeptides or polypeptide fragments in the polypeptide complex may be the same or different. In certain embodiments, the polypeptide complex is a homo-dimer.

[00160] In certain embodiments, the dimer is associated with a disulfide bond.

In certain embodiments, the disulfide bond is formed between the second polypeptide fragments comprising the GDF15.

[00161] Method of preparation

[00162] The present disclosure provides isolated nucleic acids or polynucleotides that encode the fusion polypeptide provided herein or the polypeptide complex provided herein.

[00163] The term “nucleic acid” or “polynucleotide” as used herein refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses polynucleotides containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular polynucleotide sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. 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 (see Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

[00164] The nucleic acids or polynucleotides encoding the fusion polypeptide provided herein or the polypeptide complex provided herein can be constructed using recombinant techniques. To this end, DNA encoding the serum albumin binding single domain antibody (such as VHH domain specific for HSA) and DNA encoding the GFARL agonist (such as GFD 15) fusion polypeptide or the polypeptide complex can be obtained and operably linked to allow transcription and expression in a host cell to produce the fusion polypeptide. If needed, polynucleotide sequences encoding for one or more linkers are also operably linked to allow expression of the desired product.

[00165] The encoding polynucleotide sequences can be further operably linked to one or more regulatory sequences, optionally in an expression vector, such that the expression or production of the fusion polypeptides or polypeptide complexes is feasible and under proper control.

[00166] The encoding polynucleotide sequence(s) can be inserted into a vector for further cloning (amplification of the DNA) or for expression, using recombinant techniques known in the art. Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter (e.g. prokaryotic promoters such as T7, T7lac, Sp6, araBAD, trp, lac, tac, pLm, A3, lac, lpp, npr, pac, syn, trc and T3, or eukaryotic promoters such as SV40, CMV, and EF-1 α), and a transcription termination sequence.

[00167] **Vectors and host cells**

[00168] In another aspect, the present disclosure provides a vector comprising the polynucleotide of provided herein.

[00169] Vectors comprising the polynucleotide sequence(s) provided herein can be introduced to a host cell for cloning or gene expression. The phrase "host cell" as used herein refers to a cell into which an exogenous polynucleotide and/or a vector has been introduced. In other embodiments, the vectors are extra-chromosomal. The host cells can be isolated if desired. In certain embodiments, the host cell is a prokaryotic cell or eukaryotic cell.

[00170] Suitable host cells for cloning or expressing the DNA in the vectors herein are mainly prokaryotes. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae

such as Escherichia, e.g., E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, e.g., Salmonella typhimurium, Serratia, e.g., Serratia marcescans, and Shigella, as well as Bacilli such as B. subtilis and B. licheniformis, Pseudomonas such as P. aeruginosa, and Streptomyces. In some embodiments, host cells are eukaryotes, such as yeast and mammalian cells (e.g., immortalized mammalian cells).

[00171] A vector comprising the polynucleotide sequence(s) provided herein can be introduced into a host cell using any suitable method known to a skilled person in the art, e.g., transformation, transfection or transduction. In one example, the polynucleotide sequence encoding the serum albumin binding single domain antibody/GDF15 fusion polypeptide can be subcloned into an expression vector, which is expressed as inclusion bodies in the host cells. The vector can be a viral vector, and any suitable viral vector can be used in this capacity.

[00172] In another aspect, the present disclosure provides a host cell comprising the vector provided herein. The host cell is prokaryotic cell or a eukaryotic cell. Host cells transformed with the above-described expression or cloning vectors can be cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the cloning vectors.

[00173] In another aspect, the present disclosure provides a method of producing the polypeptide complex provided herein, comprising culturing the host cell provided herein under a condition that allows expression of the polynucleotide provided herein.

[00174] For production of the fusion polypeptide or polypeptide complex provided herein, the host cells transformed with the expression vector may be cultured in a variety of media. Commercially available bacteria growth media such as Terrific Broth, LB Broth, LB Agar, M9 minimal media, MagiaMedia Medium, and ImMedia Medium (ThermoFisher) are suitable for culturing the bacterial host cells. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium (MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM), Sigma) are suitable for culturing the eukaryotic host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine),

antibiotics (such as GENTAMYCINTM drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[00175] In one aspect, the present disclosure provides a method of expressing the fusion polypeptide and/or polypeptide complex provided herein, comprising culturing the host cell provided herein under the condition at which the fusion polypeptide and/or the polypeptide complex is expressed.

[00176] In certain embodiments, the polypeptide complex is expressed as inclusion bodies. In certain embodiments, the method further comprises renaturing the polypeptide complex from the inclusion bodies.

[00177] When using recombinant techniques, the fusion polypeptide and the polypeptide complex provided herein can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the product is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Carter et al., *Bio/Technology* 10:163-167 (1992) describe a procedure for isolating proteins which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonyl fluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the product is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[00178] In certain embodiments, the method further comprises isolating the fusion polypeptide and/or polypeptide complex.

[00179] The fusion polypeptide and the polypeptide complex provided herein prepared from the cells can be purified using, for example, hydroxylapatite

chromatography, gel electrophoresis, dialysis, DEAE-cellulose ion exchange chromatography, ammonium sulfate precipitation, salting out, and affinity chromatography.

[00180] Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the protein to be recovered.

[00181] **Pharmaceutical composition**

[00182] In another aspect, the present disclosure also provides a pharmaceutical composition comprising the fusion polypeptide, or polypeptide complex provided herein and a pharmaceutically acceptable carrier.

[00183] The term “pharmaceutically acceptable” indicates that the designated carrier, vehicle, diluent, excipient(s), and/or salt is generally chemically and/or physically compatible with the other ingredients comprising the formulation, and physiologically compatible with the recipient thereof.

[00184] A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is bioactivity acceptable and nontoxic to a subject. Pharmaceutical acceptable carriers for use in the pharmaceutical compositions disclosed herein may include, for example, pharmaceutically acceptable liquid, gel, or solid carriers, aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, anesthetics, suspending/dispersing agents, sequestering or chelating agents, diluents, adjuvants, excipients, or non-toxic auxiliary substances, other components known in the art, or various combinations thereof.

[00185] Suitable components may include, for example, antioxidants, fillers, binders, disintegrants, buffers, preservatives, lubricants, flavorings, thickeners, coloring agents, emulsifiers or stabilizers such as sugars and cyclodextrins. Suitable antioxidants may include, for example, methionine, ascorbic acid, EDTA, sodium thiosulfate, platinum, catalase, citric acid, cysteine, thioglycerol, thioglycolic acid, thiosorbitol, butylated hydroxyanisole, butylated hydroxytoluene, and/or propyl gallate. As disclosed herein, inclusion of one or more antioxidants such as methionine in a pharmaceutical composition

provided herein decreases oxidation of the polypeptide complex or the bispecific polypeptide complex. This reduction in oxidation prevents or reduces loss of binding affinity, thereby improving protein stability and maximizing shelf-life. Therefore, in certain embodiments, compositions are provided that comprise the fusion polypeptide, the polypeptide complex or the conjugate disclosed herein and one or more antioxidants such as methionine.

[00186] To further illustrate, pharmaceutical acceptable carriers may include, for example, aqueous vehicles such as sodium chloride injection, Ringer's injection, isotonic dextrose injection, sterile water injection, or dextrose and lactated Ringer's injection, nonaqueous vehicles such as fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil, or peanut oil, antimicrobial agents at bacteriostatic or fungistatic concentrations, isotonic agents such as sodium chloride or dextrose, buffers such as phosphate or citrate buffers, antioxidants such as sodium bisulfate, local anesthetics such as procaine hydrochloride, suspending and dispersing agents such as sodium carboxymethylcellulose, hydroxypropyl methylcellulose, or polyvinylpyrrolidone, emulsifying agents such as Polysorbate 80 (TWEEN-80), sequestering or chelating agents such as EDTA (ethylenediaminetetraacetic acid) or EGTA (ethylene glycol tetraacetic acid), ethyl alcohol, polyethylene glycol, propylene glycol, sodium hydroxide, hydrochloric acid, citric acid, or lactic acid. Antimicrobial agents utilized as carriers may be added to pharmaceutical compositions in multiple-dose containers that include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Suitable excipients may include, for example, water, saline, dextrose, glycerol, or ethanol. Suitable non-toxic auxiliary substances may include, for example, wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, or agents such as sodium acetate, sorbitan monolaurate, triethanolamine oleate, or cyclodextrin.

[00187] The pharmaceutical compositions can be a liquid solution, suspension, emulsion, pill, capsule, tablet, sustained release formulation, or powder. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrrollidone, sodium saccharine, cellulose, magnesium carbonate, etc.

[00188] In embodiments, the pharmaceutical compositions are formulated into an injectable composition. The injectable pharmaceutical compositions may be prepared

in any conventional form, such as for example liquid solution, suspension, emulsion, or solid forms suitable for generating liquid solution, suspension, or emulsion. Preparations for injection may include sterile and/or non-pyretic solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use, and sterile and/or non-pyretic emulsions. The solutions may be either aqueous or nonaqueous.

[00189] In certain embodiments, unit-dose parenteral preparations are packaged in an ampoule, a vial or a syringe with a needle. All preparations for parenteral administration should be sterile and not pyretic, as is known and practiced in the art.

[00190] In certain embodiments, a sterile, lyophilized powder is prepared by dissolving the fusion polypeptide, the polypeptide complex or the conjugate as disclosed herein in a suitable solvent. The solvent may contain an excipient which improves the stability or other pharmacological components of the powder or reconstituted solution, prepared from the powder. Excipients that may be used include, but are not limited to, water, dextrose, sorbital, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent. The solvent may contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those of skill in the art at, in one embodiment, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides a desirable formulation. In one embodiment, the resulting solution will be apportioned into vials for lyophilization. Each vial can contain a single dosage or multiple dosages of the fusion polypeptide, the polypeptide complex, or the conjugate provided herein or composition thereof. Overfilling vials with a small amount above that needed for a dose or set of doses (e.g., about 10%) is acceptable so as to facilitate accurate sample withdrawal and accurate dosing. The lyophilized powder can be stored under appropriate conditions, such as at about 4 °C to room temperature.

[00191] Reconstitution of a lyophilized powder with water for injection provides a formulation for use in parenteral administration. In one embodiment, for reconstitution the sterile and/or non-pyretic water or other liquid suitable carrier is added to lyophilized powder. The precise amount depends upon the selected therapy being given, and can be empirically determined.

[00192] Administration of the pharmaceutical composition as described herein may be via any route known to be effective by the physician of ordinary skill. One example is peripheral parenteral administration by a sterile syringe or some other mechanical device such as an infusion pump. In certain embodiments, peripheral parenteral route is intravenous, intramuscular, subcutaneous, or intraperitoneal routes of administration.

[00193] In certain embodiments, the fusion polypeptide, the polypeptide complex, or the conjugate described herein is formulated in a form suitable for non-parenteral routes administration, such as oral, rectal, nasal, or lower respiratory routes administration.

[00194] In certain embodiments, the fusion polypeptide or the polypeptide complex described herein is formulated in a solid formulation such as lyophilization or spray drying, which is then reconstituted in a suitable diluent solution prior to administration. Standard pharmaceutical formulation techniques, such as those described in Remington: The Science and Practice of Pharmacy (D.B. Troy, Editor, 21st Edition, Lippincott, Williams & Wilkins, 2006), may be employed. Alternatively, the fusion polypeptide, the polypeptide complex, or the conjugate described herein can be formulated for administration through the lingual, sublingual, buccal, in the mouth, oral, in the stomach and intestine, nasal, pulmonary, for example, through the bronchioles and alveoli or a combination thereof, epidermal, dermal, transdermal, vaginal, rectal, ocular, for examples through the conjunctiva, uretal, transdermal, or pulmonary route. As a still further option, the fusion polypeptide, the polypeptide complex, or the conjugate described herein can be formulated for administration through transdermal administration, for example, by needle-free injection or from a patch, optionally an iontophoretic patch, or transmucosal, for example, buccal, administration.

[00195] **Method of treatment**

[00196] In another aspect, the present disclosure provides a method of preventing or treating a metabolic disorder in a subject in need thereof, comprising administering a therapeutically effective amount of the fusion polypeptide provided herein, or the polypeptide complex provided herein.

[00197] Therapeutic methods are also provided, comprising: administering a therapeutically effective amount of the fusion polypeptide, or the polypeptide complex provided herein to a subject in need thereof, thereby treating or preventing a condition or a

disorder. In certain embodiments, the subject has been identified as having a disorder or condition likely to respond to the fusion polypeptide, or the polypeptide complex provided herein.

[00198] In certain embodiments, the metabolic disorder is diabetes, obesity, non-alcoholic steatohepatitis (NASH), cardiovascular like dyslipidemia, arteriosclerosis, alcoholic steatohepatitis (ASH), diabetic nephropathy, gestational diabetes, metabolic syndrome such as metabolic syndrome X, nonalcoholic fatty liver disease (NAFLD), end-stage liver disease, hepatic steatosis (fatty liver), liver cirrhosis, or primary biliary cirrhosis (PBC).

[00199] For example, a metabolic condition or disorder that can be treated or ameliorated using the fusion polypeptide, or the polypeptide complex provided herein, includes a condition where a human subject has a fasting blood glucose level of 125 mg/dL or greater, for example 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200 or greater than 200 mg/dL. Blood glucose levels can be determined in the fed or fasted state, or at random. The metabolic condition or disorder can also comprise a condition in which a subject is at increased risk of developing a metabolic condition. For a human subject, such conditions include a fasting blood glucose level of 100 mg/dL. Conditions that can be treated using a pharmaceutical composition comprising a GDF15 mutant polypeptide can also be found in the American Diabetes Association Standards of Medical Care in Diabetes Care-2011, American Diabetes Association, Diabetes Care Vol. 34, No. Supplement 1, S1-S61, 2010, incorporated herein by reference.

[00200] The therapeutically effective amount of the fusion polypeptide and the polypeptide complex provided herein will depend on various factors known in the art, such as for example body weight, age, past medical history, present medications, state of health of the subject and potential for cross-reaction, allergies, sensitivities and adverse side-effects, as well as the administration route and extent of disease development. Dosages may be proportionally reduced or increased by one of ordinary skill in the art (e.g., physician or veterinarian) as indicated by these and other circumstances or requirements. The therapeutically effective amount can be an amount of the fusion polypeptide, the polypeptide complex and the conjugate provided herein, that elicits a biological or medicinal response in a tissue system, animal, or human being sought by a researcher, medical doctor, or other clinician, which includes alleviation or amelioration of the symptoms of the disease or disorder being treated, i.e., an amount that supports an

observable level of one or more desired biological or medicinal response, for example lowering blood glucose, insulin, triglyceride, or cholesterol levels; reducing body weight; or improving glucose tolerance, energy expenditure, or insulin sensitivity.

[00201] In certain embodiments, the fusion polypeptide, or the polypeptide complex o provided herein may be administered at a therapeutically effective dosage of about 0.01 mg/kg to about 100 mg/kg (e.g., about 0.01 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 2 mg/kg, about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 55 mg/kg, about 60 mg/kg, about 65 mg/kg, about 70 mg/kg, about 75 mg/kg, about 80 mg/kg, about 85 mg/kg, about 90 mg/kg, about 95 mg/kg, or about 100 mg/kg). In certain of these embodiments, the fusion polypeptide, or the polypeptide complex provided herein is administered at a dosage of about 50 mg/kg or less, and in certain of these embodiments the dosage is 10 mg/kg or less, 5 mg/kg or less, 1 mg/kg or less, 0.5 mg/kg or less, or 0.1 mg/kg or less. In certain embodiments, the administration dosage may change over the course of treatment. For example, in certain embodiments the initial administration dosage may be higher than subsequent administration dosages. In certain embodiments, the administration dosage may vary over the course of treatment depending on the reaction of the subject.

[00202] Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single dose may be administered, or several divided doses may be administered over time.

[00203] The fusion polypeptide, or the polypeptide complex provided herein may be administered by any route known in the art, such as for example parenteral (e.g., subcutaneous, intraperitoneal, intravenous, including intravenous infusion, intramuscular, or intradermal injection) or non-parenteral (e.g., oral, intranasal, intraocular, sublingual, rectal, or topical) routes.

[00204] The fusion polypeptide, or the polypeptide complex may be administered alone or in combination with one or more additional therapeutic means or agents.

[00205] In certain embodiments, when used for treating a metabolic disease, the fusion polypeptide, or the polypeptide complex provided herein may be administered in combination with any other therapeutic agent for use in the treatment of a metabolic

disease or any medical disorder that related. "Administered in combination" as used herein includes administration simultaneously as part of the same pharmaceutical composition, simultaneously as separate compositions, or at different timings as separate compositions. A composition administered prior to or after another agent is considered to be administered "in combination" with that agent as the phrase is used herein, even if the composition and the second agent are administered via different routes. Where possible, additional therapeutic agents administered in combination with the fusion polypeptide, the polypeptide complex or the conjugate provided herein are administered according to the schedule listed in the product information sheet of the additional therapeutic agent, or according to the Physicians' Desk Reference (Physicians' Desk Reference, 70th Ed (2016)) or protocols well known in the art. A non-limiting list of examples of compounds that can be administered in combination with a pharmaceutical composition comprising include rosiglitazone, pioglitazone, repaglinide, nateglinide, metformin, exenatide, stagliptin, pramlintide, glipizide, glimepirideacarbose, and miglitol.

[00206] Kit

[00207] Also provided are kits for practicing the disclosed methods. Such kits can comprise a pharmaceutical composition such as those described herein, including nucleic acids encoding the fusion polypeptides, or the polypeptide complex provided herein, vectors and cells comprising such nucleic acids, and pharmaceutical compositions comprising such nucleic acid-containing compounds, which can be provided in a sterile container. Optionally, instructions on how to employ the provided pharmaceutical composition in the treatment of a metabolic disorder can also be included or be made available to a patient or a medical service provider.

[00208] In one aspect, a kit comprises (a) a pharmaceutical composition comprising a therapeutically effective amount of a fusion polypeptide, or a polypeptide complex; and (b) one or more containers for the pharmaceutical composition. Such a kit can also comprise instructions for the use thereof; the instructions can be tailored to the precise metabolic disorder being treated. The instructions can describe the use and nature of the materials provided in the kit. In certain embodiments, kits include instructions for a patient to carry out administration to treat a metabolic disorder, such as elevated glucose levels, elevated insulin levels, diabetes, obesity, non-alcoholic steatohepatitis (NASH), cardiovascular like dyslipidemia, arteriosclerosis, alcoholic steatohepatitis (ASH), diabetic nephropathy, metabolic syndrome such as metabolic syndrome X, nonalcoholic

fatty liver disease (NAFLD), end-stage liver disease, hepatic steatosis (fatty liver), liver cirrhosis, or primary biliary cirrhosis (PBC).

[00209] Instructions can be printed on a substrate, such as paper or plastic, etc, and can be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (e.g., associated with the packaging), etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. CD-ROM, diskette, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, such as over the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. Often it will be desirable that some or all components of a kit are packaged in suitable packaging to maintain sterility. The components of a kit can be packaged in a kit containment element to make a single, easily handled unit, where the kit containment element, e.g., box or analogous structure, may or may not be an airtight container, e.g., to further preserve the sterility of some or all of the components of the kit.

[00210] Example 1: Recombinant expression of fusion proteins

[00211] The GDF15 fusion polypeptides listed in Table 1 were produced from bacterial E. coli expression system as homodimers, using BL21(DE3) derivative strain. The DNA coding for the GDF15 fusion precursors was codon optimized for E. coli expression, de novo synthesized and subcloned into PET derivative expression vectors (Novagen). Amino acid substitutions were accomplished by modification of the corresponding genetic codes. Overexpression of GDF15 fusion protein complexes as inclusion bodies was induced with 0.5 mM isopropyl b-d-thiogalactoside (IPTG) when the cell density reached an OD600 of 2.0 in Terrific Broth (TB) medium. The cells were harvested after protein induction at 37 °C for 20-22 hours. The quality of inclusion bodies was analyzed with microscopic imaging.

[00212] Example 2: Refolding and purification

[00213] Cells were harvested as described in Example 1 and lysed in 20mM Tris pH8.0, 0.15M NaCl buffer by cell disruptor (900 bar, for twice). The insoluble fractions, containing the fusion proteins (i.e. homodimers), were collected and washed in the same buffer twice by centrifugation (8,000 X g, for 30min). The 8M urea with 10mM DTT buffer

was then used to solubilize the inclusion bodies. After 1 hours, the solutions were diluted 20-fold with refolding buffer. The refolding samples were stirred at the room temperature for 12 hours. After removing tag by protease, the proteins were loaded on to anion exchange chromatography using Source 30Q resin (GE healthcare) running with gradient elution with 20mM Tris 9.0 and 0.5 M NaCl. In some instances, further polishing was performed by hydrophobic interaction chromatography. The samples in each step were characterized by LC/MS to confirm the correct molecular weight.

[00214] Example 3: *In vitro* activities of the fusion proteins.

[00215] Method:

[00216] The *in vitro* GDF15 activities of the fusion proteins (i.e. homodimers) were assessed using a cell line overexpressing both human GFRAL, c-Ret receptor and SRE luciferase reporter. The fusion proteins were tested with 3-fold serial dilutions with 5nM as top concentration. To assess the impact of albumin binding, the fusion proteins were also tested in the presence of 1% HSA. After 3-5 hours treatment, luciferase activities were measured by SteadyLite plus kit (Perkin Elmer, 6066751).

[00217] The activity of each protein was represented by EC₅₀, derived from non-linear regression analysis. Data for a few exemplary protein conjugates were summarized in below **Table 2**, with native GDF15 as reference.

[00218] Table 2: *In vitro* activities of the fusion proteins.

		HSA free	1% HSA
Molecule	Linker length (aa)	GDF15 activity (EC ₅₀ , pM)	GDF15 activity (EC ₅₀ , pM)
Native GDF15 (SEQ ID NO: 9)	No linker	10.3	8.2
Fusion protein 1 (SEQ ID NO: 50)	40	24.3	42.4
Fusion protein 2 (SEQ ID NO: 51)	80	31.2	64.9

NO: 51)			
Fusion protein 5 (SEQ ID NO: 54))	40	34.7	67.6
Fusion protein 12 (SEQ ID NO: 59))	40	56.5	78.1
Fusion protein 7 (SEQ ID NO: 56))	20	45.9	130.0
Fusion protein 8 (SEQ ID NO: 57))	8	43.3	151.2
Fusion protein 9 (SEQ ID NO: 58))	4	41.3	269.8

[00219] Conclusion:

[00220] The Fusion proteins 1, 2, 5, 7, 8, 9 and 12 respectively having the amino acid sequence of **SEQ ID NO: 50, 51, 54, 56, 57, 58 and 59**, showed comparable in vitro potency with native GDF15 in the cell based assay under HSA free conditions. However, Fusion proteins 7, 8 and 9 showed much lower potency in the assay with the supplement of 1% HSA, implying that albumin binding has significant effect on in vitro potency of Fusion proteins 7, 8 and 9. Albumin binding only have minor effect on in vitro potency of fusion proteins 1, 2, 5 and 12. Therefore, it was unexpectedly found that the linker length determines GDF15 activities in the presence of albumin.

[00221] **Example 4: In vivo activities of the fusion protein.**

[00222] Method:

[00223] 10 week old male C57BL/6 mice were injected everyday subcutaneously with 30 nmol/kg of the fusion proteins for 10 days (n=5/group), semaglutide was studied in parallel as a control. Body weight (BW) was measured daily

for each individual animal and food intake was measured daily for each entire treatment group. Day 1 and Day 10 are the first day and the last day of treatment. %BW loss= $100 \times (\text{BW on Day } n - \text{BW on Day } 1) / (\text{BW on Day } 1)$. Cumulative food intake on Day n represents sum of food intake from Day 1 to Day n. Data are indicated as mean values and standard error (SEM) or pooled values. Body weight reduction on Day 11 is calculated by $-1 \times (\% \text{BW loss of treatment group} - \% \text{BW loss of vehicle group})$; Cumulative food intake reduction on Day 11 is calculated by $-100 \times (\text{cumulative food intake of treatment group} - \text{cumulative food intake of vehicle group}) / \text{cumulative food intake of vehicle group}$.

Table 3a. Food intake and body weight reduction in C57 mice at Day11

Molecule	Cumulative food intake reduction at 30 nmol/kg (% reduction compared to vehicle)(Day11)	Body weight reduction at 30 nmol/kg (% reduction compared to vehicle)(Day11)
Semaglutide	11.8	7.27
Fusion protein 1 (SEQ ID NO: 50)	15.9	17.77
Fusion protein 2 (SEQ ID NO: 51)	18.3	17.28

Table 3b. Food intake and body weight reduction in C57 mice at Day11

Molecule	Cumulative food intake reduction at 30 nmol/kg (% reduction compared to vehicle)(Day11)	Body weight reduction at 30 nmol/kg (% reduction compared to vehicle)(Day11)
Fusion protein 5 (SEQ ID NO: 51)	7.3	14.05
Fusion protein 12 (SEQ ID NO: 60)	14.5	15.74

[00224]

Conclusion

[00225] The fusion protein demonstrated better BW reduction efficacy than Semaglutide (**Figures 1a and 1b** and **Tables 3a and 3b**) at the end of the treatment period.

[00226] **Example 5: Pharmacokinetic measurement of the fusion protein.**

[00227] Method:

[00228] 6-8 week old male C57BL/6 mice were administrated in a single subcutaneous dose of 30 nmol/kg of the fusion protein (n=2/group). Plasma samples were collected pre-dose (-5 min), 0.5 hr, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 12 hr, 24 hr, 48 hr and 72 hr after the injection. The concentrations of the fusion protein in the plasma were measured with a GDF15 ELISA assay (R&D Systems, DY957).

[00229] Conclusion:

[00230] As shown in **Figure 2**, both fusion proteins demonstrated high Cmax and long half-life in mice. Due to the short sample collection period, it is difficult to accurately calculate the T1/2 for each compound, which will be greater than 50 hours.

[00231] **Example 6: Efficacy study in disease models**

[00232] Selected molecules are assessed in disease animal models (such DIO mice) to determine body weight, food intake, glucose efficacy with dose responses in chronic studies. Some biomarkers are also measured, including fasting insulin, plasma triglyceride, cholesterol, liver triglyceride, and inflammatory biomarkers (ALT, AST and CRP).

[00233] Method:

[00234] 22 week old DIO male C57BL/6 mice (~40g) were injected once every day (QD) subcutaneously with fusion proteins (fusion proteins 5 and 12) for 12 days. Food intake and body weight were measured daily. Five animals were used for each treatment group. Body weight was monitored for each individual animal, but food intake for each group animals was measured together and was divided by animal numbers. Day 1 and Day 12 are first day and last day of molecule dosage. Data are indicated as mean values and standard error (SEM) or pooled values. Statistical analysis was performed by One-way ANOVA. Body weight reduction on Day 12 is calculated by $-1 * (\%BW \text{ loss} - \%BW \text{ loss})$

of vehicle group); Cumulative food intake reduction is calculated by $-100 \times (\text{cumulative food intake} - \text{cumulative food intake of vehicle}) / \text{cumulative food intake of vehicle}$.

[00235] Conclusion: in DIO study, as shown in Figure 3a and 3b, Fusion proteins 5 and 12 have significant effect on body weight reduction and food intake suppression.

[00236] **Example 7: PK study in non-human primates.**

[00237] Pharmacokinetics of selected molecules are assessed in monkeys. Both subcutaneous and intravenous injections are performed.

[00238] **Example 8: Immunogenicity assessment.**

[00239] Selected fusion protein conjugates are also assessed for immunogenicity by in silico (iTope and TCED methods) and ex vivo (EpiScreen) methods.

[00240] **Example 9: Stability assessment.**

[00241] To test the stability, the different conjugated fusion proteins are formulated in buffers with different compositions (pH6, 7, 7.4 and 8.0) and stored at different temperatures (such as 25 °C and 37 °C) for 2-4 weeks. The %HMWP and %LMW are analyzed by size exclusion chromatography (SEC)-HPLC. The concentration and modifications were analyzed by reverse phase (RP)-UPLC and LC/MS.

Claims

1. A fusion polypeptide comprising:
 - a) a first polypeptide fragment comprising a serum albumin binding single domain antibody; and
 - b) a second polypeptide fragment comprising a GFRAL receptor agonist;wherein the first polypeptide fragment and the second polypeptide fragment are linked to one another directly or via a linker.
2. The fusion polypeptide of claim 1, wherein the single domain antibody comprises a VHH domain.
3. The fusion polypeptide of claim 2, wherein the VHH domain is humanized.
4. The fusion polypeptide of claim 2 or 3, wherein the VHH domain comprises a complementarity determining region 1 (CDR1), CDR2 and CDR3, wherein the CDR1 comprises the sequence of SEQ ID NO: 1 or a variant thereof having up to 3, 2, or 1 amino acid mutation, a CDR2 comprising the sequence of SEQ ID NO: 2 or a variant thereof having up to 3, 2, or 1 amino acid mutation, and/or a CDR3 comprising the sequence of SEQ ID NO: 3 or a variant thereof having up to 3, 2, or 1 amino acid mutation, wherein the VHH domain retains the binding specificity to serum albumin, optionally to human serum albumin.
5. The fusion polypeptide of claim 2 or 3, wherein the VHH domain comprises a complementarity determining region 1 (CDR1) comprising the sequence of SEQ ID NO: 1, a CDR2 comprising the sequence of SEQ ID NO: 2, and a CDR3 comprising the sequence of SEQ ID NO: 3.
6. The fusion polypeptide of any of claims 2-5 wherein the VHH domain comprises the sequence of SEQ ID NO: 4, or a variant thereof having at least 70% (e.g. at least 75%, 80%, 85%, 90%, 95%, 99% identity to SEQ ID NO: 4, wherein the variant retains the binding specificity and/or affinity to serum albumin.
7. The fusion polypeptide of claim 6, wherein the variant of SEQ ID NO: 4 has up to 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid mutation relative to SEQ ID NO: 4.
8. The fusion polypeptide of any one of claims 2-7, wherein the serum albumin binding single domain antibody further comprises an N-terminal extension attached to the VHH domain.

9. The fusion polypeptide of claim 8, wherein the N-terminal extension comprises amino acid residues of SG, AG, S or A.
10. The fusion polypeptide of claim 1, wherein the serum albumin binding single domain antibody comprises a sequence selected from SEQ ID NOs: 4-8.
11. The fusion polypeptide of any of claims 1-10, the GFRAL receptor agonist comprises GDF15.
12. The fusion polypeptide of claim 11, wherein the GDF15 comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 9 while retaining substantial biological activity of SEQ ID NO: 9.
13. The fusion polypeptide of claim 11, wherein the GDF15 comprises no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 mutations relative to SEQ ID NO: 9 while retaining substantial biological activity of SEQ ID NO: 9.
14. The fusion polypeptide of any of claims 11-13, wherein the GDF15 comprises one or more mutations at a position selected from the group consisting of: A1, R2, N3, H6, P11, H18, T19, V20, R21, A30, M43, A47, R53, A54, M57, H66, R67, L68, A75, A81, P85, M86, Q90, T92, and L105, or any combination thereof, relative to SEQ ID NO: 9.
15. The fusion polypeptide of claim 14, wherein the one or more mutations in GDF15 comprises:
 - 1) mutation of N3 selected from the group consisting of: N3Q, N3S, N3E, N3A, N3T, N3P, N3G, N3V, N3H, N3Y and deletion of N3; and/or
 - 2) substitution of M57 selected from the group consisting of: M57A, M57E and M57L; and/or
 - 3) substitution of M86L or M86A.
16. The fusion polypeptide of claim 14, wherein the one or more mutations in GDF15 are selected from the group consisting of: R2S, R2A, R2E, N3S, N3E, N3A, N3T, N3P, N3G, N3V, N3H, N3Y, N3Q, H6D, P11E, H18E, H18Q, T19S, V20L, R21E, A30E, M43L, M43E, A47E, R53E, A54E, M57A, M57E, M57L, H66E, R67E, L68E, A75E, A81E, P85E, M86F, M86A, M86L, Q90E, T92E, L105E, deletion of N3, and deletion of N-terminal 1-3 residues, or any combination thereof, relative to SEQ ID NO: 9.
17. The fusion polypeptide of any of claims 11-16, wherein the GDF15 comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-12 and 14.
18. The fusion polypeptide of any one of the claims 11-17, wherein the fusion polypeptide

comprises from N terminus to C terminus, the serum albumin binding single domain antibody, the linker and the GDF15.

19. The fusion polypeptide of any of claims 1-18, wherein the linker comprises a polypeptide linker.
20. The fusion polypeptide of claim 19, wherein the polypeptide linker has a length of at least 4 amino acid residues (e.g. at least 4, 12, 24, 40, 50, 60, 70, 80, 90, 100, 110, or 120 amino acid residues).
21. The fusion polypeptide of claim 19, wherein the polypeptide linker has a length of 4-80 or 20-80 or 30-80 or 40-80 amino acid residues.
22. The fusion polypeptide of claim 19, wherein the polypeptide linker has a length of about 4, 8, 20, 40, 50, 60, 70 or 80 amino acid residues.
23. The fusion polypeptide of any of claims 19-22, wherein the polypeptide linker comprises at least one acidic amino acid residue.
24. The fusion polypeptide of claim 23, wherein the at least one acidic amino acid residue comprises glutamic acid (E).
25. The fusion polypeptide of any of claims 19-24, wherein the polypeptide linker comprises one or more repeats of a repeating sequence.
26. The fusion polypeptide of claim 25, wherein the repeating sequence consists of no more than 4, 5 or 6 types of amino acid residues selected from the group consisting of: G, Q, A, E, P, T and S.
27. The fusion polypeptide of claim 25 or 26, wherein the repeating sequence comprises Q.
28. The fusion polypeptide of any of claims 25-27, wherein the repeating sequence consists of G, Q, A, E, and P.
29. The fusion polypeptide of claim 26, wherein the repeating sequence consists of a sequence selected from the group consisting of: SEQ ID NO: 15 (GQEPGAQP), SEQ ID NO: 16 (GAQPGAQP), SEQ ID NO: 17 (GQEP), SEQ ID NO: 18 (GAQP), SEQ ID NO: 40 (SEPATSGSETPGTSESATPESGPGTSTEPSEG), SEQ ID NO: 19 (GAQPGQEPGAQP), SEQ ID NO: 20 (GAQPGQEP), SEQ ID NO: 21 (GEQP), SEQ ID NO: 22 (GPQE), SEQ ID NO: 23 (GPEQ), SEQ ID NO: 24 (GSEP), SEQ ID NO: 25 (GESP), SEQ ID NO: 26 (GPSE), SEQ ID NO: 27 (GPES), SEQ ID NO: 28 (GQAP), SEQ ID NO: 29 (GPAQ), SEQ ID NO: 30 (GPQA), SEQ ID NO: 31 (GSQP), SEQ ID NO: 32 (GASP), SEQ ID NO: 33 (GPAS), SEQ ID NO: 34 (GPSA), SEQ ID NO: 35 (GGGS), SEQ ID NO: 36 (GSGS), SEQ ID NO: 37 (GGGS), SEQ ID NO: 38

(GQEPGQAP), SEQ ID NO: 39: (GQAPGQEP), SEQ ID NO: 41 (SEPATS), SEQ ID NO: 42 (GSETPG), SEQ ID NO: 43 (TSESAT), SEQ ID NO: 44 (PESGPG), SEQ ID NO: 45 (TSTEPS) and GS.

30. The fusion polypeptide of claim 29, the polypeptide linker comprises (Repeat1)_r(Repeat2)_s(Repeat3)_x(Repeat4)_y, wherein:

Repeat1, Repeat2, Repeat3 and Repeat4 are linked via a peptide bond or via one or more amino acid residues,

Repeat1, Repeat2, Repeat3 and Repeat4 independently, comprise or consist of a sequence selected from the group consisting of: SEQ ID NO: 15 (GQEPGAQP), SEQ ID NO: 16 (GAQPGAQP), SEQ ID NO: 17(GQEP), SEQ ID NO: 18 (GAQP), SEQ ID NO: 40 (SEPATSGSETPGTSESATPESGPGTSTEPSEG), SEQ ID NO: 19 (GAQPGQEPGAQP), SEQ ID NO: 219 (GAQPGQEP), SEQ ID NO: 21 (GEQP), SEQ ID NO: 22 (GPQE), SEQ ID NO: 23 (GPEQ), SEQ ID NO: 24 (GSEP), SEQ ID NO: 25 (GESP), SEQ ID NO: 26 (GPSE), SEQ ID NO: 27 (GPES), SEQ ID NO: 28 (GQAP), SEQ ID NO: 29 (GPAQ), SEQ ID NO: 30 (GPQA), SEQ ID NO: 31 (GSQP), SEQ ID NO: 32 (GASP), SEQ ID NO: 33 (GPAS), SEQ ID NO: 34 (GPSA), SEQ ID NO: 35 (GGGS), SEQ ID NO: 36 (GSGS), SEQ ID NO: 37 (GGGS), SEQ ID NO: 38 (GQEPGQAP), SEQ ID NO: 39 (GQAPGQEP), SEQ ID NO: 41 (SEPATS), SEQ ID NO: 42 (GSETPG), SEQ ID NO: 43 (TSESAT), SEQ ID NO: 44 (PESGPG), SEQ ID NO: 45 (TSTEPS) and GS, and

r, s, x and y are independently an integer selected from 0 to 30, provided that r, s, x and y are not 0 at the same time.

31. The fusion polypeptide of claim 30, wherein x and y are 0, r, and s are independently an integer selected from 1 to 30, and Repeat1 and Repeat2 are a combination selected from the group consisting of:

- a) Repeat1 comprises or consists of a sequence of SEQ ID NO: 15, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18;
- b) Repeat1 comprises or consists of a sequence of SEQ ID NO: 17, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18;
- c) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 17;
- d) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15;

- e) Repeat1 comprises or consists of a sequence of SEQ ID NO: 19, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18;
 - f) Repeat1 comprises or consists of a sequence of SEQ ID NO: 40, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18;
 - g) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 38 and
 - h) Repeat1 comprises or consists of a sequence of SEQ ID NO: 40, Repeat2 comprises or consists of a sequence of SEQ ID NO: 17.
32. The fusion polypeptide of claim 30, wherein r, x and y are 0, s is an integer selected from 1 to 30, and Repeat1 comprises or consists of a sequence of SEQ ID NO: 17.
33. The fusion polypeptide of claim 30, wherein y is 0, r, s and x are independently an integer selected from 1 to 30, and Repeat1, Repeat2, and Repeat 3 are a combination selected from the group consisting of:
- (a) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 19, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
 - (b) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 17, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
 - (c) Repeat1 comprises or consists of a sequence of SEQ ID NO: 40, Repeat2 comprises or consists of a sequence of SEQ ID NO: 17, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
 - (d) Repeat1 comprises or consists of a sequence of SEQ ID NO: 40, Repeat2 comprises or consists of a sequence of SEQ ID NO: 18, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 17;
 - (e) Repeat1 comprises or consists of a sequence of SEQ ID NO: 15, Repeat2 comprises or consists of a sequence of SEQ ID NO: 28, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
 - (f) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 38, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 15;

- (g) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 18;
- (h) Repeat1 comprises or consists of a sequence of SEQ ID NO: 17, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 17; and
- (i) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat3 comprises or consists of a sequence of SEQ ID NO: 38.
34. The fusion polypeptide of claim 30, wherein r, s, x and y are independently an integer selected from 1 to 30, and Repeat1, Repeat2, Repeat 3 and Repeat 4 are a combination selected from the group consisting of:
- (a) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 20, Repeat3 comprises or consists of a sequence of SEQ ID NO: 17, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 18;
- (b) Repeat1 comprises or consists of a sequence of SEQ ID NO: 15, Repeat2 comprises or consists of a sequence of SEQ ID NO: 38, Repeat3 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 18;
- (c) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 38, Repeat3 comprises or consists of a sequence of SEQ ID NO: 15, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 18;
- (d) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 28, Repeat3 comprises or consists of a sequence of SEQ ID NO: 18, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 15; and
- (e) Repeat1 comprises or consists of a sequence of SEQ ID NO: 18, Repeat2 comprises or consists of a sequence of SEQ ID NO: 15, Repeat3 comprises or consists of a sequence of SEQ ID NO: 39, and Repeat4 comprises or consists of a sequence of SEQ ID NO: 38.
35. The fusion polypeptide of any of claims 19-34, wherein the polypeptide linker

comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 17, 46-49 and 61.

36. The fusion polypeptide of any one of claims 1-35, comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 50-60 and 13.
37. A polypeptide complex comprises a dimer of the fusion polypeptide of any of claims 1-36.
38. The polypeptide complex of claim 37, which is a homo-dimer.
39. The polypeptide complex of claim 37 or 38, wherein the dimer is associated with a disulfide bond.
40. The polypeptide complex of claim 39, wherein the disulfide bond is formed between the second polypeptide fragments comprising the GDF15.
41. A polynucleotide encoding the fusion polypeptide of any claims 1-36, or the polypeptide complex of any of claims 37-40.
42. A vector comprising the polynucleotide of claim 41.
43. A host cell comprising the vector of claim 42.
44. The host cell of claim 43, wherein the host cell is a prokaryotic cell or eukaryotic cell.
45. A method of producing the polypeptide complex of any of claims 1-36, comprising culturing the host cell of claim 43 under a condition that allows expression of the polynucleotide of claim 41.
46. The method of claim 45, wherein the host cell is prokaryotic cell or a eukaryotic cell.
47. The method of claim 46, wherein the polypeptide complex is expressed as inclusion bodies.
48. The method of claim 47, wherein the method further comprises renaturing the polypeptide complex from the inclusion bodies.
49. A pharmaceutical composition comprising the fusion polypeptide of any of claims 1-36, or the polypeptide complex of any claims 37-40 and a pharmaceutically acceptable carrier.
50. A method of preventing or treating a metabolic disorder in a subject in need thereof, comprising administering a therapeutically effective amount of the fusion polypeptide of any of claims 1-36, or the polypeptide complex of any claims 37-40.
51. The method of claim 50, wherein the metabolic disorder is diabetes, obesity, non-alcoholic steatohepatitis (NASH), cardiovascular like dyslipidemia, arteriosclerosis, alcoholic steatohepatitis (ASH), diabetic nephropathy, gestational diabetes, metabolic

syndrome such as metabolic syndrome X, nonalcoholic fatty liver disease (NAFLD), end-stage liver disease, hepatic steatosis (fatty liver), liver cirrhosis, or primary biliary cirrhosis (PBC).

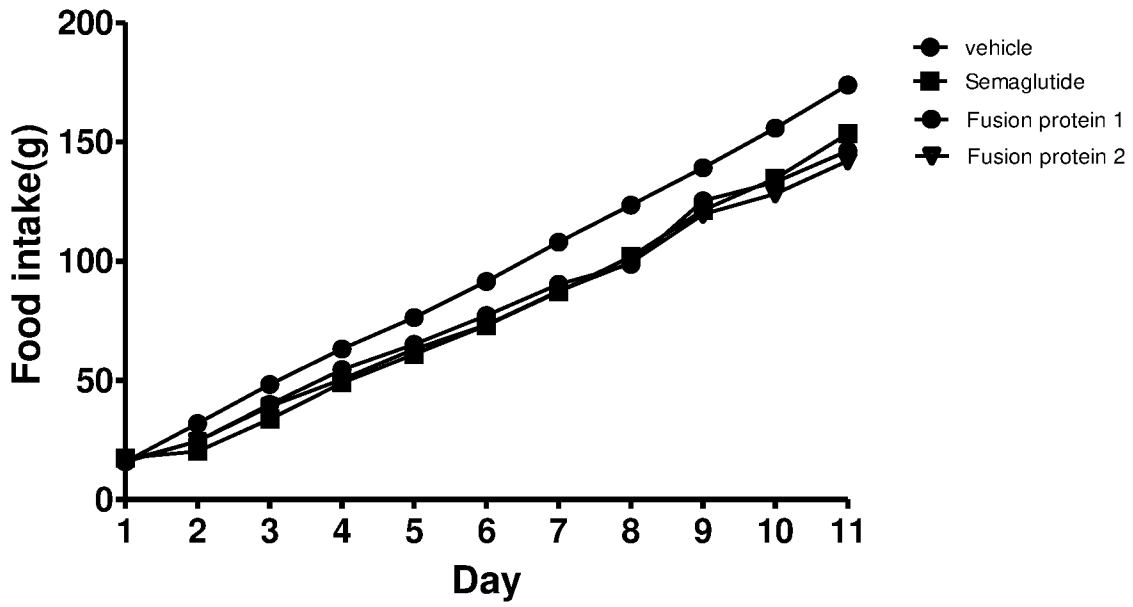


Figure 1a

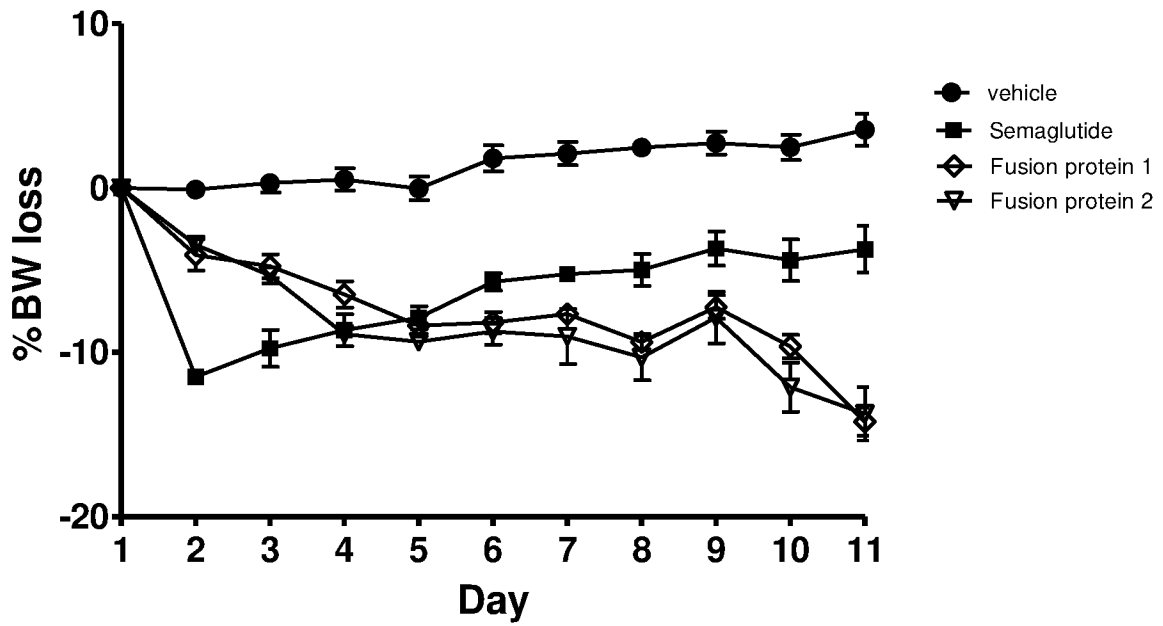


Figure 1b

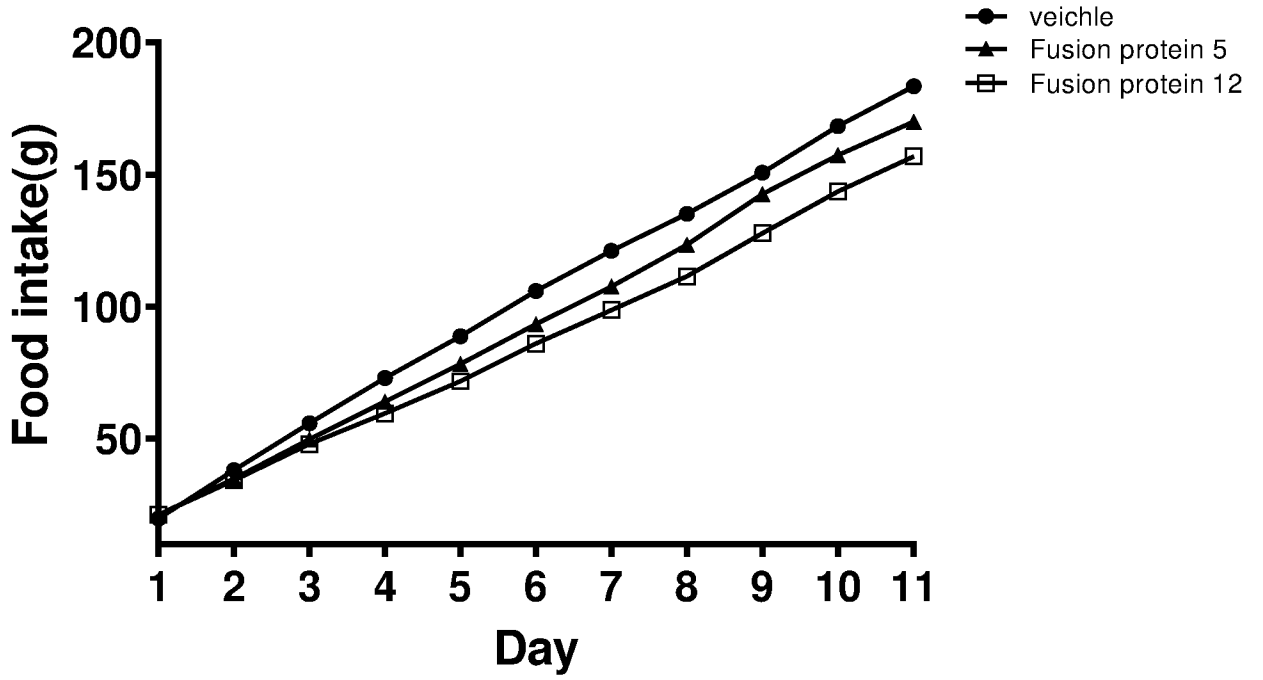


Figure 1c

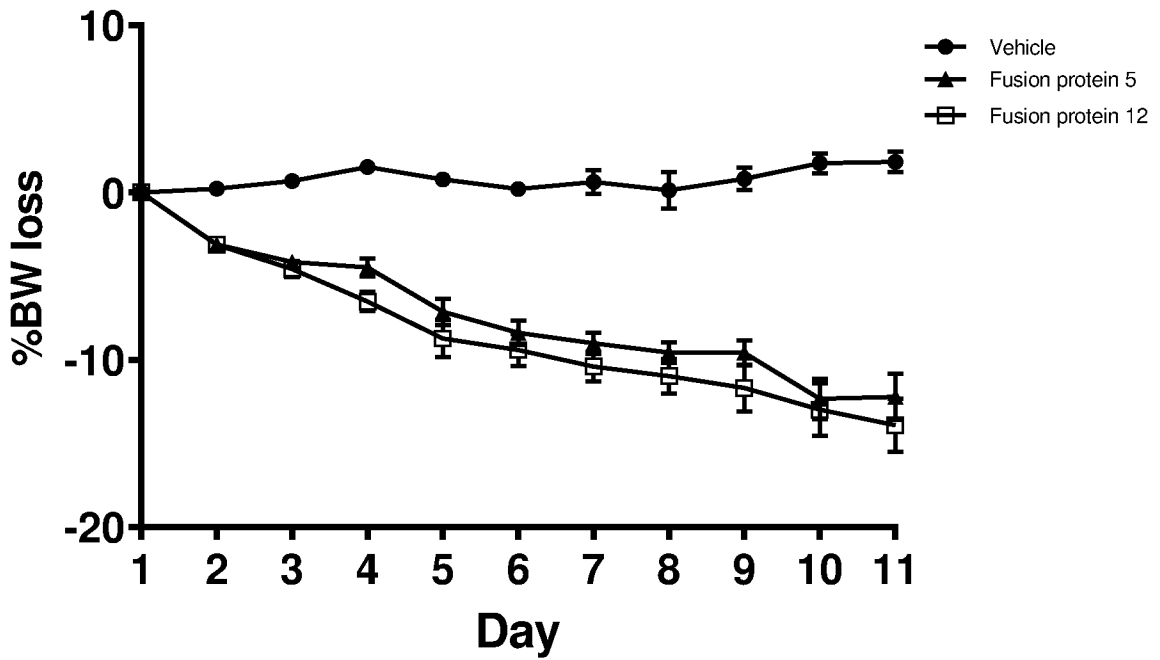


Figure 1d

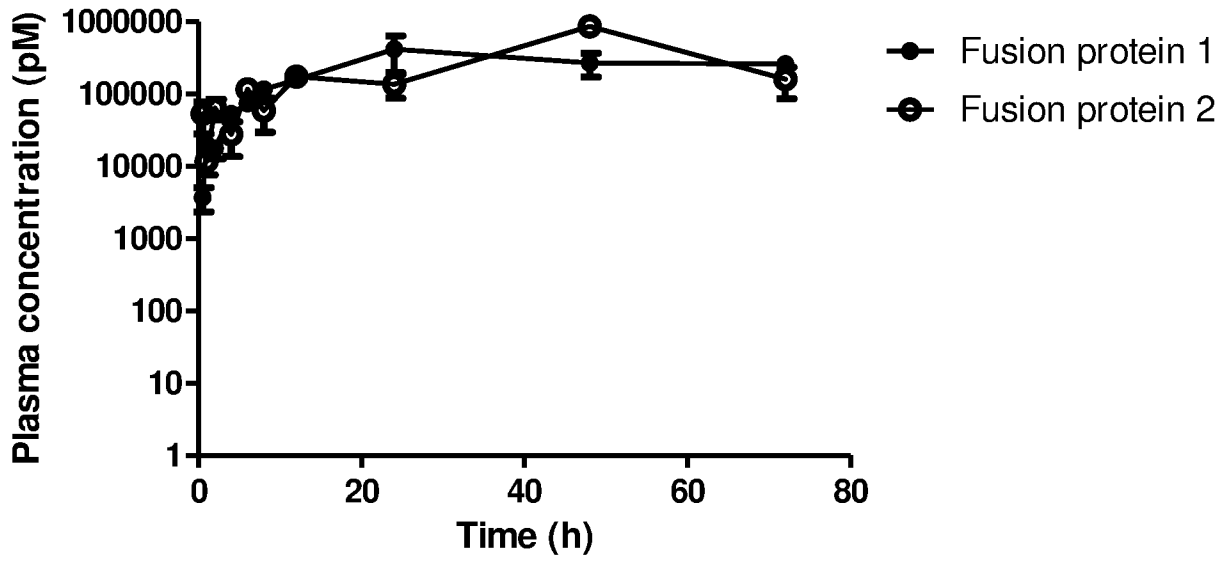


Figure 2

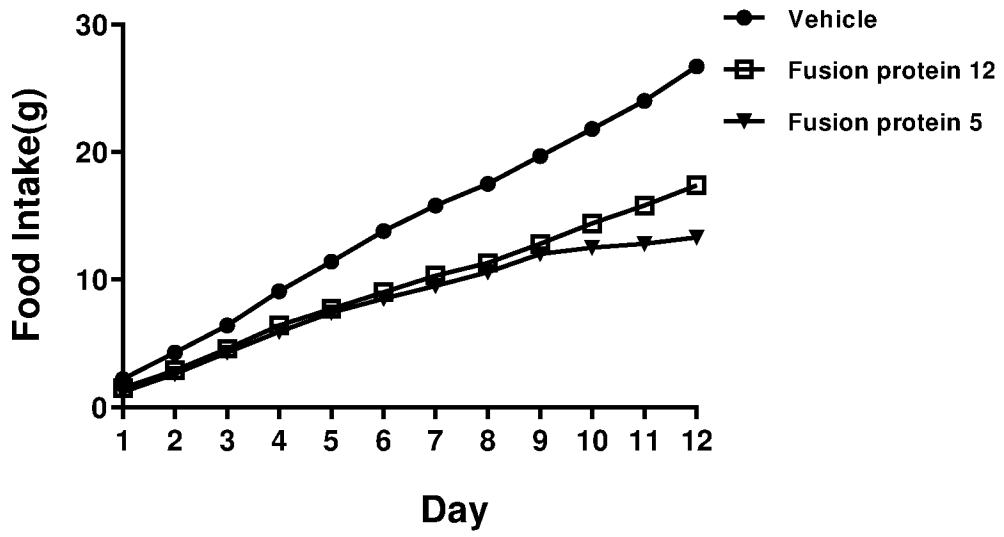


Figure 3a

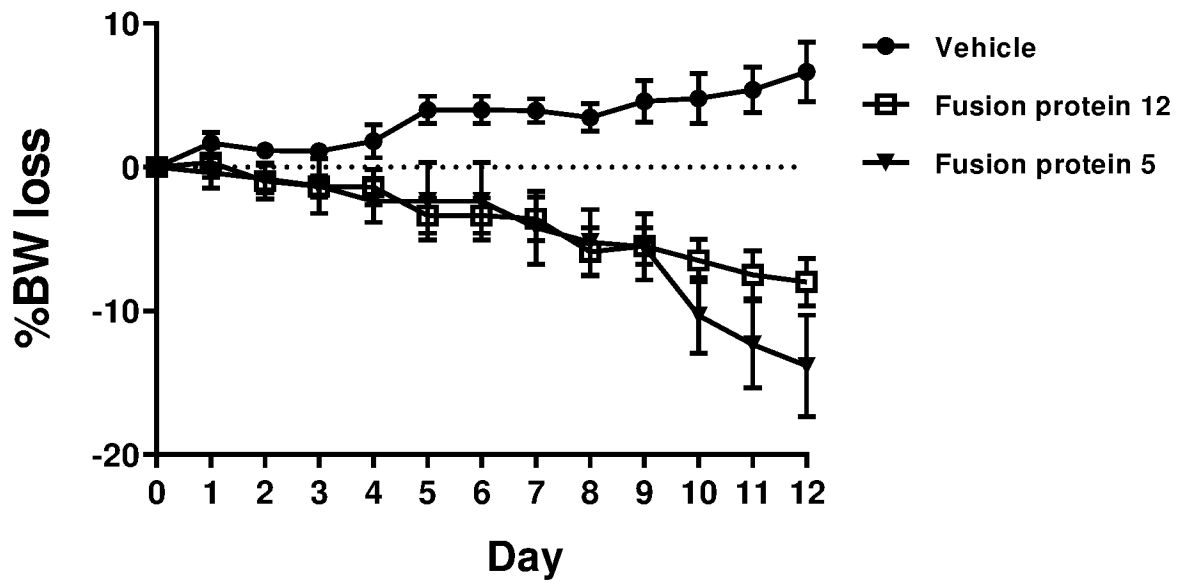


Figure 3b

Serum Albumin Binding Single Domain Antibody

SEQ ID NO	Sequence of Serum Albumin Binding Single Domain Antibody
4	EVQLVESGGGLVQPGNSLRRLSCAASGFTFSS FGMS WVRQAPGKG LEWVSS SISGSGSD TYADSVKGRFTISRDNAKTTLYLQMNSLRPED TAVYYCTIGGSLSRSSQGTLVTVSS
5	A EVQLVESGGGLVQPGNSLRRLSCAASGFTFSSFGMSWVRQAPGK GLEWVSSISGSGSDTYADSVKGRFTISRDNAKTTLYLQMNSLRPE DTAVYYCTIGGSLSRSSQGTLVTVSS
6	S EVQLVESGGGLVQPGNSLRRLSCAASGFTFSSFGMSWVRQAPGK GLEWVSSISGSGSDTYADSVKGRFTISRDNAKTTLYLQMNSLRPE DTAVYYCTIGGSLSRSSQGTLVTVSS
7	AG EVQLVESGGGLVQPGNSLRRLSCAASGFTFSSFGMSWVRQAPG KGLEWVSSISGSGSDTYADSVKGRFTISRDNAKTTLYLQMNSLRP EDTAVYYCTIGGSLSRSSQGTLVTVSS
8	SG EVQLVESGGGLVQPGNSLRRLSCAASGFTFSSFGMSWVRQAPG KGLEWVSSISGSGSDTYADSVKGRFTISRDNAKTTLYLQMNSLRP EDTAVYYCTIGGSLSRSSQGTLVTVSS

GDF15

SEQ ID NO	GDF15 amino acid sequence
9	ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMC IGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQ KTDTGVSLQTYDDLAKDCHCI
10	AR Q GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTM CIGACPSQFRAAN L HAQIKTSLHRLKPDTVPAPCCVPASYNPMVLI QKTDTGVSLQTYDDLAKDCHCI
11	GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMCIGA CPSQFRAAN L HAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQKTD TGVSLQTYDDLAKDCHCI
12	AR Q GDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTM CIGACPSQFRAANMHAQIKTSLHRLKPDTVPAPCCVPASYNPMVLI QKTDTGVSLQTYDDLAKDCHCI
14	ARNGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQVTMC IGACPSQFRAAN L HAQIKTSLHRLKPDTVPAPCCVPASYNPMVLIQK TDTGVSLQTYDDLAKDCHCI

Figure 4b

Fusion polypeptide

SEQ ID NO	Fusion polypeptide sequence
50	AEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGK GLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPE DTAVYYCTIGGSLSRSSQGT LVTVSSGAQPGAQPGAQPGAQPGAQ EPGQEPGQEPGQEPGQEPGQEPARQGDHCPLGPGRCCRLHTVR ASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANLHAQIKTSLH RLKPDTVPAPCCVPASYNPMVLIQKTD TGVSLQTYDDLAKDCHCI
51	AEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGK GLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPE DTAVYYCTIGGSLSRSSQGT LVTVSSGAQPGAQPGAQPGAQPGA QPGAQPGAQPGAQPGAQPGAQPGAQPGAQPGAQPGAQPGAQ GAQPGAQPGAQPGAQPGAQPARQGDHCPLGPGRCCRLHTVRAS LEDLGWADWVLSPREVQVTMCIGACPSQFRAANLHAQIKTSLHRL KPDTVPAPCCVPASYNPMVLIQKTD TGVSLQTYDDLAKDCHCI
52	SEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGK GLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPE DTAVYYCTIGGSLSRSSQGT LVTVSSGAQPGAQPGAQPGAQPGA EPGQEPGQEPGQEPGQEPGQEPARQGDHCPLGPGRCCRLHTVR ASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANLHAQIKTSLH RLKPDTVPAPCCVPASYNPMVLIQKTD TGVSLQTYDDLAKDCHCI
53	AGEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPG KGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRP EDTAVYYCTIGGSLSRSSQGT LVTVSSGAQPGAQPGAQPGAQPGA QEPGQEPGQEPGQEPGQEPGQEPARQGDHCPLGPGRCCRLHTV RASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANLHAQIKTSL HRLKPDTVPAPCCVPASYNPMVLIQKTD TGVSLQTYDDLAKDCHC I
54	SGEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPG KGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRP EDTAVYYCTIGGSLSRSSQGT LVTVSSGAQPGAQPGAQPGAQPGA QEPGQEPGQEPGQEPGQEPGQEPARQGDHCPLGPGRCCRLHTV RASLEDLGWADWVLSPREVQVTMCIGACPSQFRAANLHAQIKTSL HRLKPDTVPAPCCVPASYNPMVLIQKTD TGVSLQTYDDLAKDCHC I
55	SGEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPG KGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRP EDTAVYYCTIGGSLSRSSQGT LVTVSSGAQPGAQPGAQPGAQPGA QPGAQPGAQPGAQPGAQPGAQPGAQPGAQPGAQPGAQPGA GAQPGAQPGAQPGAQPGAQPARQGDHCPLGPGRCCRLHTVRAS LEDLGWADWVLSPREVQVTMCIGACPSQFRAANLHAQIKTSLHRL KPDTVPAPCCVPASYNPMVLIQKTD TGVSLQTYDDLAKDCHCI
56	SGEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPG KGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRP EDTAVYYCTIGGSLSRSSQGT LVTVSSGQEPGQEPGQEPGQEPG QEPARQGDHCPLGPGRCCRLHTVRASLEDLGWADWVLSPREVQV TMCIGACPSQFRAANLHAQIKTSLHRLKPDTVPAPCCVPASYNPMV LIQKTD TGVSLQTYDDLAKDCHCI

Figure 4c

INTERNATIONAL SEARCH REPORT

PCT/CN2021/126453

A. CLASSIFICATION OF SUBJECT MATTER		
C07K 14/76(2006.01)i; C07K 14/475(2006.01)i; C07K 14/00(2006.01)i; C07K 16/46(2006.01)i; C12N 15/14(2006.01)i; C12N 15/11(2006.01)i; A61K 38/38(2006.01)i; A61K 38/00(2006.01)i; A61K 47/64(2017.01)i		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) C07K; C12N; A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Data bases: CNABS, SIPOABS, DWPI, CNTXT, WOTXT, USTXT, EPTXT, GBTXT, CATXT, SGTXT, ATTXT, LEXTXT, CHTXT, CNKI, WANFANG, PubMed, ISI_Web of Science, Baidu Scholar, STN, GenBank, EMBL, Chinese Patent Biological Sequence Retrieval System; Search terms: MIC-1, GDF-15, GDF15, MIC1, GFRAL, GFRAL receptor agonist, serum albumin, HSA, metabolic, VHH, antibody, fusion, GS, linker, linked, homo-dimer, N-terminal extension, obesity, diabetes, SEQ ID NOS 1-60.		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CN 106046168 A (ABLYNX NV) 26 October 2016 (2016-10-26) see claims 1-7, paragraphs 4-137	1-51
Y	CN 110461870 A (ABLYNX NV) 15 November 2019 (2019-11-15) see claims 1-18, paragraphs 15-236	1-51
Y	WO 2015198199 A1 (NOVARTIS AGet al.) 30 December 2015 (2015-12-30) see claims 1-24, paragraphs 6-133	1-51
Y	CN 109414471 A (NOVO NORDISK AS) 01 March 2019 (2019-03-01) see claims 1-15, paragraphs 40-283	1-51
Y	CN 103254309 A (ABLYNX NV) 21 August 2013 (2013-08-21) see claims 1-43	1-51
Y	CN 108367053 A (NOVARTIS AG) 03 August 2018 (2018-08-03) see claims 1-24, paragraphs 15-360	1-51
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 10 January 2022		Date of mailing of the international search report 28 January 2022
Name and mailing address of the ISA/CN National Intellectual Property Administration, PRC 6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088, China Facsimile No. (86-10)62019451		Authorized officer LIANG, Yaru Telephone No. 010-62411056

INTERNATIONAL SEARCH REPORT

PCT/CN2021/126453

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CN 109451726 A (JANSSEN BIOTECH INC) 08 March 2019 (2019-03-08) see claims 1-45, paragraphs 7-241	1-51
Y	CN 111163795 A (NOVO NORDISK AS) 15 May 2020 (2020-05-15) see claims 1-14, paragraphs 10-190	1-51
Y	CN 106459222 A (NOVO NORDISK AS) 22 February 2017 (2017-02-22) see claims 1-15, paragraphs 22-369	1-51
A	Seung Joon Baek, et al. "Growth differentiation factor 15 (GDF15): A survival protein with therapeutic potential in metabolic diseases" <i>Pharmacol Ther.</i> , Vol. 198, 18 February 2019 (2019-02-18), ISSN: 0163-7258, see the whole document	1-51
A	Anthony P Coll, et al. "GDF15 mediates the effects of metformin on body weight and energy balance" <i>Nature</i> , Vol. 578, No. 7795, 25 December 2019 (2019-12-25), ISSN: 1476-4687, see the whole document	1-51

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. forming part of the international application as filed:
 - in the form of an Annex C/ST.25 text file.
 - on paper or in the form of an image file.
 - b. furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. furnished subsequent to the international filing date for the purposes of international search only:
 - in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: **50,51**
because they relate to subject matter not required to be searched by this Authority, namely:
 - [1] PCT Rule 39.1 (iv)-Method for treatment of the human or animal body by surgery or therapy.
 - [2] The search of claims 50 and 51 is based on the following subject-matter that could reasonably be expected: use of the fusion polypeptide of any of claims 1-36 or the polypeptide complex of any of claims 37-40 in manufacturing a medicament for preventing or treating a metabolic disorder in a subject in need thereof.
2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

PCT/CN2021/126453

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	106046168	A	26 October 2016	CA	2839779	C	06 October 2020
				EP	3466972	A1	10 April 2019
				CA	2839779	A1	27 December 2012
				LT	2723771	T	10 December 2019
				HR	P20192160	T1	21 February 2020
				DK	2723771	T3	02 December 2019
				AU	2012271974	B2	12 January 2017
				EP	2723771	B1	11 September 2019
				WO	2012175400	A1	27 December 2012
				US	9573992	B2	21 February 2017
				JP	2014520129	A	21 August 2014
				US	2017210789	A1	27 July 2017
				US	2014228546	A1	14 August 2014
				JP	6324887	B2	16 May 2018
				PL	2723771	T3	30 April 2020
				PT	2723771	T	11 December 2019
				ES	2759936	T3	12 May 2020
				JP	2021088585	A	10 June 2021
				JP	6843090	B2	17 March 2021
				EP	2723771	A1	30 April 2014
				JP	2018127478	A	16 August 2018
				IN	414CHN2014	A	03 April 2015
				CN	103619878	A	05 March 2014
				HU	E047238	T2	28 April 2020
				SI	2723771	T1	31 December 2019
				HR	P20170535	T1	16 June 2017
				CN	103619878	B	26 October 2016
AU	2012271974	A1	12 December 2013				
CN	110461870	A	15 November 2019	US	2019367598	A1	05 December 2019
				MX	2019008536	A	10 September 2019
				KR	20190104609	A	10 September 2019
				JP	2020506898	A	05 March 2020
				AU	2018209150	A1	25 July 2019
				WO	2018134234	A1	26 July 2018
				EP	3571224	A1	27 November 2019
				BR	112019014600	A2	18 February 2020
				IL	267897	D0	26 September 2019
				RU	2019125949	A	19 February 2021
				SG	11201906341X	A	27 August 2019
				CA	3050574	A1	26 July 2018
				RU	2019125949	A3	09 March 2021
WO	2015198199	A1	30 December 2015	EP	3157947	A1	26 April 2017
				US	2017204149	A1	20 July 2017
				US	2020181216	A1	11 June 2020
CN	109414471	A	01 March 2019	WO	2017202936	A1	30 November 2017
				US	2021198331	A1	01 July 2021
				AU	2017269496	B2	25 March 2021
				BR	112018072034	A2	12 February 2019
				MX	2018014023	A	04 April 2019
				PH	12018502465	A1	08 April 2019

INTERNATIONAL SEARCH REPORT
Information on patent family members

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				RU 2018141829 A3	07 October 2020
				RU 2018141829 A	25 June 2020
				AU 2017269496 A1	15 November 2018
				CA 3025251 A1	30 November 2017
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				TW 201741333 A	01 December 2017
				CL 2018003319 A1	01 February 2019
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				MX 342271 B	21 September 2016
				RU 2007142444 A	27 June 2009
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