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(54) Title: REGULATING TRANSPLANT REJECTION OF DONOR AND EMBRYONIC STEM CELL-DERIVED TISSUES AND ORGANS

(57) Abstract: The invention provides methods and cells for improved transplantation of donor transplants to subjects in need thereof.

REGULATING TRANSPLANT REJECTION OF DONOR AND EMBRYONIC STEM CELL-DERIVED TISSUES AND ORGANS.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No: 61/807,632, filed April 02, 2013, the disclosure of which is explicitly incorporated by reference herein in its entirety.

BACKGROUND

Stem cell therapy is a rapidly advancing field where huge strides have been made in the repair of critical organs. Unfortunately, as with any other graft, stem cell transplants have a high probability of developing immune-mediated complications ultimately resulting in rejection. While strategies such as host immune suppression or use of host-derived cells address the issue of rejection, they are not ideal due to compromised patient health or the prohibitive cost of individualized treatment. Identification of key triggers and development of novel, broadly applicable strategies to specifically control the immune response is imperative for wide scale implementation of cell therapies. Alanyl (membrane) aminopeptidase (ANPEP) is a cell surface protease expressed by monocytes, macrophages, dendritic cells, among many others. The inventors have recently identified ANPEP as a regulator of receptor-mediated antigen uptake and presentation in dendritic cells, an inflammatory adhesion molecule, a regulator of innate immunity, and a regulator of endocytosis, and tested whether ANPEP was involved in immune responses elicited by stem cell transplantation.

SUMMARY OF THE INVENTION

In a first aspect the invention provides a method of limiting development of transplant rejection, comprising administering to a subject in need thereof and/or treating a donor transplant with an effective amount of an inhibitor of alanyl (membrane) aminopeptidase (ANPEP) to treat or limit development of transplant rejection. The inhibitor can prevent the expression, activity and/or function of ANPEP; any suitable ANPEP inhibitor can be used, as deemed most appropriate for an intended use. In exemplary embodiments, the inhibitor can be selected from the group consisting of anti-ANPEP antibody, anti-ANPEP aptamer, ANPEP small interfering RNA, ANPEP

small internally segmented interfering RNA, ANPEP short hairpin RNA, ANPEP micro RNA, ANPEP antisense oligonucleotides and small molecule ANPEP inhibitors. In a preferred embodiment, the inhibitor is an anti-ANPEP antibody (*i.e.*, an antibody that binds to ANPEP).

In a second aspect the invention provides a pluripotent cell population, wherein the population does not express functional ANPEP. Functional ANPEP can be knocked out or inhibited by a method selected from generation of knock-in null mutant ANPEP cell population using homologous recombination, transcription activator-like effector nucleases(TALENs), clustered regulatory interspaced short palindromic repeat (CRISPR) Cas-based RNA-guided DNA endonucleases technology, generation of ANPEP knockout cell line using homologous recombination, TALEN or CRISPR technology, generation of knock-in point mutation of ANPEP using homologous recombination, TALEN or CRISPR technology, anti-ANPEP antibody, anti-ANPEP aptamer, ANPEP small interfering RNA, ANPEP small internally segmented interfering RNA, ANPEP short hairpin RNA, ANPEP micro RNA, ANPEP antisense oligonucleotides and small molecule ANPEP inhibitors. In some embodiments, cells, tissues or organs derived from the pluripotent cell population that does not express functional ANPEP can be transplanted into a subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

The disclosed exemplary aspects have other advantages and features which will be more readily apparent from the detailed description, the appended claims, and the accompanying figures. A brief description of the figures is below.

Figure 1 shows that CD13-null skin grafts survive longer. **(A)** Gross images of wild type (WT) and CD13-null skin grafts in WT recipients at days 25 (top) and 100 (bottom). **(B)** Skin graft measurements over time demonstrate decreased diameter until complete rejection in WT grafts compared to CD13-null grafts. **(C)** H&E staining of skin graft sections demonstrate higher numbers of infiltrating cells in WT compared to CD13-null skin grafts. **(D)** Quantification of immunohistochemical staining of skin graft sections demonstrate higher levels of CD3 staining, indicative of T cell infiltration, in WT grafts. WTWT= WT donor tissue, WT recipient; KOWT= KO donor tissue, WT recipient; WTKO= WT donor tissue, KO recipient; KOKO= KO donor tissue, KO recipient.

Figure 2 shows that mast cell presence is higher in surviving grafts. **(A)** Toluidine blue staining of mast cell granules in skin at baseline. **(B)** Quantification of toluidine blue staining in skin graft tissue at days 5-7 post-transplant indicates loss of mast cell granule staining in WT graft tissue. **(C)** Quantification of toluidine blue staining in draining lymph nodes of mice receiving minor histocompatibility (MiHC) mismatch skin grafts demonstrates higher levels of mast cells that have not degranulated in recipients receiving CD13-null donor tissue.

Figure 3 shows that cultured mast cells demonstrate different reactivity. **(A)** Bone marrow derived mast cells demonstrate 80-90% positivity for c-kit expression after 4-6 weeks in culture as well as high forward and side scatter profiles by flow cytometry. **(B)** Co-culture of bone marrow derived mast cells show loss of forward and side scatter profile upon stimulation (left panels) as well as stimulation of proliferation (right panels) demonstrated by loss of CFSE dye. Interestingly, WT and KO mast cells preserved more of their original cell morphology and displayed less proliferation when stimulated by CD13-null dermal fibroblasts. In addition, CD13-null mast cells also maintained more of their original cell morphology and proliferated less than WT mast cells when stimulated with WT dermal fibroblasts.

Figure 4 shows SL13 treatment is sufficient to prolong WT graft survival. **(A)** WT skin grafts show prolonged survival in WT gender mismatched recipients treated for two weeks with SL13. Vehicle treated controls demonstrated signs of rejection as early as day 13 post-transplant while SL13 treated recipients maintained functional grafts past 3 weeks even after discontinuation of SL13 at time of surgery

Figure 5 shows that mES transplantation recapitulates skin graft studies. **(A)** Gross images of teratomas generated in immune-competent hosts at four weeks shows clear growth advantage cy CD13-null mES as well as apparent growth advantage in CD13-null hosts. **(B-C)** Proliferation is enhanced in CD13-null teratomas as seen in quantification of Ki67 expression **(B)** as well as BrDU incorporation **(C)**. **(D)** This growth advantage is not seen in immune-compromised nude mice. **(E)** There is less CD3+ T cell infiltration in CD13-null teratomas, especially in those teratomas generated in CD13-null hosts. **(F)** WT teratomas also demonstrate higher numbers (15% in WT versus 3.29% in CD13 KO) of CD11b+CD11c+ dendritic cells and **(G)** F4/80+ macrophages by flow cytometry. **(H)** Toluidine blue staining and quantification of mast cells within teratomas indicate higher levels in CD13-null teratomas.

Figure 6 shows a schematic for MiHC mismatch skin graft. Donor male skin is harvested from the dorsal side of the ear and transplanted onto the shaved exposed backs of female recipient mice.

Figure 7 shows **(A)** Flow cytometry for immune cell populations in post- transplant Day 5 spleen.

Figure 8 shows that mixed lymphocyte reactions with WT and CD13-null MiHC mismatched splenocytes show that CD13-null splenocytes demonstrated reduced proliferation upon stimulation compared to WT splenocytes.

Figure 9 shows **(A)** a SL13 treatment Scheme. **(B)** Check for SL13 antibody in serum of treated and control mice

Figure 10 shows CD13-null mES cell characterization. **(A)** CD13-null mESCs express the pluripotency marker Oct4. **(B)** WT mES generate teratomas containing cell types of all three germ layers. **(C)** CD13-null teratomas also display cell types of all three germ layers. **(D)** None of the teratomas display any significant apoptosis. **(E)** CD13-null teratomas contain more CD31 and aSMA positive staining cells, indicating higher levels of vascularity.

DETAILED DESCRIPTION OF THE INVENTION

Methods well known to those skilled in the art can be used to construct expression vectors and recombinant bacterial cells according to this invention. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, *in vivo* recombination techniques, and PCR techniques. See, for example, techniques as described in Maniatis *et al.*, 1989, MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory, New York; Ausubel *et al.*, 1989, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Associates and Wiley Interscience, New York, and *PCR Protocols: A Guide to Methods and Applications* (Innis *et al.*, 1990, Academic Press, San Diego, CA).

All publications, patents and patent applications cited herein are hereby expressly incorporated by reference for all purposes.

Before describing the present invention in detail, a number of terms will be defined. As used herein, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. For example, reference to a "nucleic acid" means one or more nucleic acids.

In a first aspect the invention provides a method of limiting development of transplant rejection, comprising administering to a subject in need of a transplant and/or treating a donor transplant with an effective amount of an inhibitor of alanyl (membrane) aminopeptidase (ANPEP) to limit development of transplant rejection in the subject.

The inventors have surprisingly discovered that the methods of the invention allow the ability of donor transplant to engraft and survive with little to no immune suppression required of the recipient. Furthermore, the cells, tissues and organs of the invention in which ANPEP is inhibited or that do not express functional ANPEP can be transplanted universally without minor histocompatibility complex (MiHC) matching, human leukocyte antigen (HLA) matching or immune suppression. Thus, the inhibition of ANPEP expression, activity or function in a donor transplant or cells, tissues or organs to be transplanted can allow more successful transplantation by mitigating or eliminating the immune reactions triggered by transplantation.

For uses in this application, CD13 and ANPEP will be used synonymously. The approved HUGO Gene Nomenclature Committee (HGNC) Symbol for human CD13 is ANPEP, and the approved HGNC name is alanyl (membrane) aminopeptidase. Previous names include: CD13, PEPN; APN; LAP1; P150; GP150. Synonyms include: aminopeptidase M, aminopeptidase N, microsomal aminopeptidase. Human ANPEP has the following identifiers: UniProtKB/Swiss-Prot, P15144 (SEQ ID NO: 01); NCBI Reference Sequence (mRNA), NM_001150.2 (SEQ ID NO: 02); and NCBI Reference Sequence (protein), NP_001141.2 (SEQ ID NO: 03) and point mutations (SEQ ID NOs: 04-06). CD13 is a type II zinc-dependent metallopeptidase that is found on the surface of all myeloid cells in addition to pericytes, activated endothelial cells, and subsets of organ-specific epithelial cells. It is a multifunctional protein with both enzyme-dependent and independent functions that contribute to adhesion, cell migration, angiogenesis, inflammatory trafficking, adhesion, antigen presentation, and endocytosis.

Table 1: ANPEP Sequences

Human Aminopeptidase N (EC=3.4.11.2) ANPEP
>sp|P15144|AMPN_HUMAN Aminopeptidase N (SEQ ID NO: 01)

MAKGFYISKSLGILGILLGVAAVCTIIALSVVYSQEKNKNANSSPVASTTPSASATTNPA
SATTLDQSKAWNRYRLPNTLKPDSYRVTLRPYLTPNDRGLYVFKGSSTVRFTCKEATDVI
IIHSKKLNYTLSQGHRVVLRGVGGSQPPDIDKTELVEPTEYLVVHLKGSLVKDSQYEMDS

EFEGELADDLAGFYRSEYMEGNVRKVVATTQMQAADARKSFPCFDEPAMKAEFNITLIHP
KDLTALSNMLPKGPSTPLPEDPNWNVTEFHTTPKMSTYLLAFIVSEFDYVEKQASNGVLI
RIWARPSAIAAGHGDYALNVTGPILNFFAGHYDTPYPLPKSDQIGLPDFNAGAMENWGLV
TYRENSLLFDPLSSSSSNKERVVTVIAHELAHQWFGNLVTIEWWNDLWLNEGFASYVEYL
GADYAEPTWNLKDLMVLNDVYRVMAVDALASSHPLSTPASEINTPAQISELFDAISYSKG
ASVLRMLSSFLSEDVFKQGLASYLHTFAYQNTIYLNLWDHLQEAVNNRSIQLPTTVRDIM
NRWTLQMGFPVITVDTSTGTLSQEHFLLDPDSNVTRPSEFNYVWIVPITSIRDGRQQQDY
WLIDVRAQNDLFSTSGNEWVLLNLNVTGYYRVNYDEENWRKIQTQLQRDHSAIPVINRAQ
IINDAFNLASAHKVPVTLALNNTLFLIEERQYMPWEAALSSLSYFKLMFDRSEVYGPMKN
YLKKQVTPLFIHFRNNTNNWREIPENLMDQYSEVNAISTACSNGVPECEEMVSGLFKQWM
ENPNNNPIHPNLRSTVYCNAIAQGGEEEWDFAWEQFRNATLVNEADKLRAALACSKELWI
LNRYLSYTLNPDLIRKQDATSTIISITNNVIGQGLVWDFVQSNWKKLFNDYGGGSFSFSN
LIQAVTRRFSTEYELQQLEQFKKDNEETGFGSGTRALEQALEKTKANIKWVKENKEVVLQ
WFTENSK

***Homo sapiens* alanyl (membrane) aminopeptidase (ANPEP), mRNA**
NCBI Reference Sequence: NM_001150.2 (SEQ ID NO: 02)

gggacggcgg cggcgcagct cggaacccgc cagggtccag ggtccaggtt ccagcgcccg
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gccccagtctg cctgttgtgg ggctcctccc ctttggggat ataagcccgg cctggggctg
ctccgttctc tgcctggcct gaggctccct gagccgcctc cccaccatca ccatggccaa
gggcttctat atttccaagt ccctgggcat cctggggatc ctcctgggcg tggcagccgt
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caataataac ccgatccacc ccaacctgcg gtccaccgtc tactgcaacg ctatcgccca
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gacgaaagcc aacatcaagt gggtgaagga gaacaaggag gtggtgctcc agtggttcac
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tgtaccctct ttcaccttc cctaaagacc ctaaatctga ggaatcaaca gggcagcaga
tctgtatatt ttttctaag agaaaatgta aataaaggat ttctagatga aaaaaaaaaa
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aaaaaaaaaa aaaaaaaaaa

aminopeptidase N precursor [*Homo sapiens*]
NCBI Reference Sequence: NP_001141.2 (SEQ ID NO: 03)

MAKGFYISKS LGILGILLGV AAVCTIIALS VVYSQEKNKN ANSSPVASTT PSASATTNPA
SATTLDQSKA WNRYRLPNTL KPDSYRVTLR PYLTPNDRGL YVFKGSSTVR FTCKEATDVI
IIHSKKLNYT LSQGHRVVLR GVGGSQPPDI DKTELVEPTE YLVVHLKGSL VKDSQYEMDS
EFEGELADDL AGFYRSEYME GNVRKVVATT QMQAADARKS FPCFDEPAMK AEFNITLIHP
KDLTALSNML PKGPSTPLPE DPNWNVTEFH TTPKMSTYLL AFIVSEFDYV EKQASNGVLI
RIWARPSAIA AGHGDYALNV TGPILNFFAG HYDTPYPLPK SDQIGLPDFN AGAMENWGLV
TYRENSLLFD PLSSSSSNKE RVVTVIAHEL AHQWFGNLVT IEWWNDLWLN EGFASYVEYL
GADYAEPTWN LKDLMVLNDV YRVMAVDALA SSHPLSTPAS EINTPAQISE LFDAISYSKG
ASVLRMLSSF LSEDVFKQGL ASYLHTFAYQ NTIYLNLWDH LQEAVNNRSI QLPTTVRDIM
NRWTLQMGFP VITVDTSTGT LSQEHFLLDP DSNVTRPSEF NYVWIVPITS IRDGRQQQDY
WLIDVRAQND LFSTSGNEWV LLNLNVTGYY RVNYDEENWR KIQTQLQRDH SAIPVINRAQ
IINDAFNLAS AHKVPVTLAL NNTLFLIEER QYMPWEAALS SLSYFKLMFD RSEVYGPMKN
YLKKQVTPLF IHFRNNTNNW REIPENLMDQ YSEVNAISTA CSNGVPECEE MVSGLFKQWM
ENPNNNPIHP NLRSTVYCNA IAQGGEEEWD FAWEQFRNAT LVNEADKLRA ALACSKELWI
LNRYLSYTLN PDLIRKQDAT STIISITNNV IGQGLVWDFV QSNWKKLFND YGGGSFSFSN
LIQAVTRRFS TEYELQQLEQ FKKDNEETGF GSGTPALEQA LEKTKANIKW VKENKEVVLQ
WFTENSK

AMPN_HUMAN Aminopeptidase N enzymatically inactive mutant H392A (SEQ ID NO: 04)

MAKGFYISKSLGILGILLGVAAVCTIIALSVVYSQEKNKNANSSPVASTTPSASATTNPA
SATTLDQSKAWNRYRLPNTLKPDSYRVTLRPYLTPNDRGLYVFKGSSTVRFTCKEATDVI
IIHSKKLNYTLSQGHRVVLRGVGGSQPPDIDKTELVEPTEYLVVHLKGSLVKDSQYEMDS
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RIWARPSAIAAGHGDYALNVTGPILNFFAGHYDTPYPLPKSDQIGLPDFNAGAMENWGLV
TYRENSLLFDPLSSSSSNKERVVTVIAHELA**A**QWFGNLVTIEWWNDLWLNEGFASYVEYL
GADYAEPTWNLKDLMVLNDVYRVMAVDALASSHPLSTPASEINTPAQISELFDAISYSKG
ASVLRMLSSFLSELVFKQGLASYLHTFAYQNTIYLNLWDHLQEAVNNRSIQLPTTVRDIM
NRWTLQMGFPVITVDTSTGTLSQEHFLLDPDSNVTRPSEFNYVWIVPITSIRDGRQQQDY
WLIDVRAQNDLFSTSGNEWVLLNLNVTGYYRVNYDEENWRKIQTQLQRDHSAIPVINRAQ
IINDAFNLASAHKVPVTLALNNTLFLIEERQYMPWEAALSSLSYFKLMFDRSEVYGPMKN
YLKKQVTPLFIHFRNNTNNWREIPENLMDQYSEVNAISTACSNGVPECEEMVSGLFKQWM
ENPNNNPIHPNLRSTVYCNAIAQGGEEEWDFAWEQFRNATLVNEADKLRAALACSKELWI
LNRYLSYTLNPDLIRKQDATSTIISITNNVIGQGLVWDFVQSNWKKLFNDYGGGSFSFSN
LIQAVTRRFSTEYELQQLEQFKKDNEETGFGSGTRALEQALEKTKANIKWVKENKEVVLQ
WFTENSK

AMPN_HUMAN Aminopeptidase N enzymatically inactive mutant H388A (SEQ ID NO: 05)

MAKGFYISKSLGILGILLGVAAVCTIIALSVVYSQEKNKNANSSPVASTTPSASATTNPA
SATTLDQSKAWNRYRLPNTLKPDSYRVTLRPYLTPNDRGLYVFKGSSTVRFTCKEATDVI
IIHSKKLNYTLSQGHRVVLRGVGGSQPPDIDKTELVEPTEYLVVHLKGSLVKDSQYEMDS
EFEGELADDLAGFYRSEYMEGNVRKVVATTQMQAADARKSFPCFDEPAMKAEFNITLIHP
KDLTALSNMLPKGPSTPLPEDPNWNVTEFHTTPKMSTYLLAFIVSEFDYVEKQASNGVLI
RIWARPSAIAAGHGDYALNVTGPILNFFAGHYDTPYPLPKSDQIGLPDFNAGAMENWGLV
TYRENSLLFDPLSSSSSNKERVVTVIA**A**ELAHQWFGNLVTIEWWNDLWLNEGFASYVEYL
GADYAEPTWNLKDLMVLNDVYRVMAVDALASSHPLSTPASEINTPAQISELFDAISYSKG
ASVLRMLSSFLSEDVFKQGLASYLHTFAYQNTIYLNLWDHLQEAVNNRSIQLPTTVRDIM
NRWTLQMGFPVITVDTSTGTLSQEHFLLDPDSNVTRPSEFNYVWIVPITSIRDGRQQQDY
WLIDVRAQNDLFSTSGNEWVLLNLNVTGYYRVNYDEENWRKIQTQLQRDHSAIPVINRAQ
IINDAFNLASAHKVPVTLALNNTLFLIEERQYMPWEAALSSLSYFKLMFDRSEVYGPMKN
YLKKQVTPLFIHFRNNTNNWREIPENLMDQYSEVNAISTACSNGVPECEEMVSGLFKQWM
ENPNNNPIHPNLRSTVYCNAIAQGGEEEWDFAWEQFRNATLVNEADKLRAALACSKELWI
LNRYLSYTLNPDLIRKQDATSTIISITNNVIGQGLVWDFVQSNWKKLFNDYGGGSFSFSN
LIQAVTRRFSTEYELQQLEQFKKDNEETGFGSGTRALEQALEKTKANIKWVKENKEVVLQ
WFTENSK

AMPN_HUMAN Aminopeptidase N non-phosphorylatable mutant Y6F (SEQ ID NO: 06)

MAKGF**F**ISKSLGILGILLGVAAVCTIIALSVVYSQEKNKNANSSPVASTTPSASATTNPA
SATTLDQSKAWNRYRLPNTLKPDSYRVTLRPYLTPNDRGLYVFKGSSTVRFTCKEATDVI
IIHSKKLNYTLSQGHRVVLRGVGGSQPPDIDKTELVEPTEYLVVHLKGSLVKDSQYEMDS
EFEGELADDLAGFYRSEYMEGNVRKVVATTQMQAADARKSFPCFDEPAMKAEFNITLIHP
KDLTALSNMLPKGPSTPLPEDPNWNVTEFHTTPKMSTYLLAFIVSEFDYVEKQASNGVLI
RIWARPSAIAAGHGDYALNVTGPILNFFAGHYDTPYPLPKSDQIGLPDFNAGAMENWGLV
TYRENSLLFDPLSSSSSNKERVVTVIHELAHQWFGNLVTIEWWNDLWLNEGFASYVEYL
GADYAEPTWNLKDLMVLNDVYRVMAVDALASSHPLSTPASEINTPAQISELFDAISYSKG
ASVLRMLSSFLSEDVFKQGLASYLHTFAYQNTIYLNLWDHLQEAVNNRSIQLPTTVRDIM
NRWTLQMGFPVITVDTSTGTLSQEHFLLDPDSNVTRPSEFNYVWIVPITSIRDGRQQQDY
WLIDVRAQNDLFSTSGNEWVLLNLNVTGYYRVNYDEENWRKIQTQLQRDHSAIPVINRAQ
IINDAFNLASAHKVPVTLALNNTLFLIEERQYMPWEAALSSLSYFKLMFDRSEVYGPMKN
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ENPNNNPIHPNLRSTVYCNAIAQGGEEEWDFAWEQFRNATLVNEADKLRAALACSKELWI
LNRYLSYTLNPDLIRKQDATSTIISITNNVIGQGLVWDFVQSNWKKLFNDYGGGSFSFSN
LIQAVTRRFSTEYELQQLEQFKKDNEETGFGSGTRALEQALEKTKANIKWVKENKEVVLQ
WFTENSK

***Mus musculus* alanyl (membrane) aminopeptidase (ANPEP), mRNA NCBI Reference Sequence: NM_008486.2 (SEQ ID NO: 07)**

ggcagtgggg ctccaccccc tgtgaggata taagctggcc ccgggctgc tgttctttcc
tcttggcctg agctattccg agctccctgt ccaccggcat catggccaag gggttctaca
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cacctaacat tgacaaaacg gaactggtag agcgtactga gtacctggtg gtgcacctgc
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tcgactgggt gaaggagaac aaagatgcgg tattcaagtg gttcacagag aacagcagtt
agttcctggt tctgagaacc acttgtccca gtatgacacc tcttactatc tcagcagcct
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tccagcctgt ggagccagcc tagctcctaa ctgtcaggct gacggacacc tcccaggtct
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cagctctgaa gctagatttа ctggacaaag ggcagcctgg aaagagactc cctgaatgct
ttactatccc tgccccctac ccccaccccc acccccacg agatccagaa ccaaagaatc
aacagggcac aagatctata tatattttta agagaaaatg taaataaaga atttctaaaa
tgaaaaaaaa aaaaaaaaa

aminopeptidase N [*Mus musculus*]
NCBI Reference Sequence: NP_032512.2 (SEQ ID NO: 08)

MAKGFYISKT LGILGILLGV AAVCTIIALS VVYAQEKNRN AENSATAPTL PGSTSATTAT
TTPAVDESKP WNQYRLPKTL IPDSYRVILR PYLTPNNQGL YIFQGNSTVR FTCNQTTDVI
IIHSKKLNYT LKGNHRVVLR TLIGTPAPNI DKTELVEPTE YLVVHLQGSL VEGRQYEMDS
QFQGELADDL AGFYRSEYME GDVKKVVATT QMQAADARKS FPCFDEPAMK AMFNITLIYP
NNLIALSNML PKESKPYPED PSCTMTEFHS TPKMSTYLLA YIVSEFKNIS SVSANGVQIG
IWARPSAIDE GQGDYALNVT GPILNFFAQH YNTSYPLPKS DQIALPDFNA GAMENWGLVT
YRESSLVFDS QSSSISNKER VVTVIAHELA HQWFGNLVTV AWWNDLWLNE GFASYVEYLG
ADYAEPTWNL KDLMVLNDVY RVMAVDALAS SHPLSSPADE INTPDQIMEL FDSITYSKGA
SVIRMLSSFL TEDLFKKGLS SYLHTYQYSN TVYLDLWEHL QKAVNQQTAV QPPATVRTIM
DRWILQMGFP VITVNTNTGE ISQKHFLLDS KSNVTRPSEF NYIWIAPIPF LKSGQEDHYW
LDVEKNQSAK FQTSSNEWIL LNINVTGYYL VNYDENNWKK LQNQLQTDLS VIPVINRAQI
IHDSFNLASA KMIPITLALD NTLFLVKEAE YMPWQAALSS LNYFTLMFDR SEVYGPMKRY
LKKQVTPLFF YFQNRTNNWV NRPPTLMEQY NEINAISTAC SSGLKECRDL VVELYSQWMK
NPNNNTIHPN LRSTVYCNAI AFGGEEEWNF AWEQFRNATL VNEADKLRSA LACSKDVWIL

NRYLSYTLNP DYIRKQDTTS TIISIASNVA GHPLVWDFVR SNWKKLFENY GGGSFSFANL IQGVTPRFSS EFELQQLEQF KALNSATGFG TGTRALEQAL ENTPANIDWV KENKLAVFKW FTENSS

The inhibitor can prevent the expression, activity and/or function of ANPEP; any suitable ANPEP inhibitor can be used, as deemed most appropriate for an intended use. In exemplary embodiments, the inhibitor can be selected from the group consisting of anti-ANPEP antibody, anti-ANPEP aptamer, ANPEP small interfering RNA, ANPEP small internally segmented interfering RNA, ANPEP short hairpin RNA, ANPEP micro RNA, ANPEP antisense oligonucleotides and small molecule ANPEP inhibitors. In a preferred embodiment, the inhibitor is an anti-ANPEP antibody (*i.e.*, an antibody that binds to ANPEP).

As used herein, the term "subject" or "patient" is meant to include any subject for which a donor transplant or donor or treatment may be required. "Subjects" or "patients" can comprise both humans and non-humans and includes, but is not limited to, humans, monkeys, cattle, dogs, cats, guinea pigs, rabbits, rats, mice, insects, horses, chickens, and so on. Most preferably, the subject is human. In some embodiments, the subject or patient may require a graft or transplant due to a congenital defect (*e.g.*, congenital anomaly of the heart, limbs or kidneys), traumatic injury, acquired allergic reactions or disease sequelae (*e.g.*, chronic kidney disease can be a sequel of diabetes). In an exemplary embodiment, a patient born with a congenital heart defect can receive a new heart or heart tissue with no defect created with the pluripotent cells that lack ANPEP expression. In another embodiment, tissues such as skin, muscle or nerve can be created and stored in hospitals for the immediate transplant into trauma patients. In yet another embodiment, therapeutic cells that produce insulin or mast cell stabilizers can be created from pluripotent cells lacking ANPEP expression and transplanted into diabetes patients or patients suffering from allergic airway disease and asthma. In embodiments where the immune system should be enhanced instead of suppressed, such as in cancer patients, CD13 inhibition would not be used; rather, CD13 function will be enhanced to exert the corresponding effects on immunity.

Transplant rejection occurs when transplanted tissue is rejected by the recipient's immune system, which destroys the transplanted tissue. Transplant rejection can be lessened by determining the molecular similarity between donor and recipient and by use of immunosuppressant drugs before and after the transplant procedure. Immune cells from the subject receiving the donor transplant or donor graft recognize the

donor transplant or donor graft as "foreign". The recipient's immune cells then attack the transplanted donor transplant or donor graft which can result in transplant rejection. In addition, residual immune cells in the donor transplant or donor graft can mobilize to a recipient's peripheral lymphoid organs and initiate graft versus host disease, attacking host cells and causing extensive damage. The methods and compositions of the invention can prevent transplant rejection by mitigating or eliminating the immune reactions of the recipient to the donor transplant or donor graft.

As used herein, "treating transplant rejection" or "limiting development of transplant rejection" means accomplishing one or more of the following: 1) reducing the severity of an immune response to a donor transplant or donor graft; 2) inhibiting or preventing development of an immune response at the donor transplant or graft location; 3) inhibiting or preventing the worsening of an immune response at the donor transplant or graft location; or 4) inhibiting or preventing the development of immune-mediated complications ultimately resulting in rejection of the donor transplant or graft.

In an embodiment, the subject receiving a donor transplant is treated with an ANPEP inhibitor before the transplant procedure occurs. The inhibitor can be administered to the subject one day, two days, three days, four days five days, six days, one week, two weeks, three weeks, four week or more before the transplant procedure; and continued to be administered for one week, two weeks, three weeks, one month, two months, three months or more after the transplant procedure as necessary in order to prevent transplant rejection. In another embodiment, the donor transplant is treated with an ANPEP inhibitor for the transplant procedure. The inhibitor can be administered to the donor transplant one day, two days, three days, four days five days, six days, one week, two weeks, three weeks, four week or more before the transplant procedure; and continued to be administered for one week, two weeks, three weeks, one month, two months, three months or more after the transplant procedure as necessary in order to prevent transplant rejection. In some embodiments, both the subject receiving the donor transplant and the donor transplant are treated with an ANPEP inhibitor. In such cases, the inhibitor can be administered to the subject and donor transplant one day, two days, three days, four days five days, six days, one week, two weeks, three weeks, four week or more before the transplant procedure; and continued to be administered for one week, two weeks, three weeks, one month, two months, three months or more after the transplant procedure as necessary in order to prevent transplant rejection.

As used herein, the term "donor transplant" or "donor graft" refers to a population of cells, or a tissue or an organ that is to be moved from one body to another or from a donor site to another location on the subject's own body, for the purpose of replacing the recipient's damaged or absent tissue or organ. In some embodiments, the donor transplant can be re-grown from the patient's own cells (*e.g.*, pluripotent cells or stem cells, or cells extracted from the failing organs). In other embodiments, the donor transplant or graft can be grown from a pluripotent cell population lacking ANPEP, as described herein.

The donor transplant or graft may be a cell, tissue, or organ, as is suitable for an intended use. Exemplary donor transplants or grafts can be selected from, but are not limited to: skin cells, beta cells (*i.e.*, cells in the pancreas located in the islets of Langerhans), cardiac cells, brain cells, kidney cells, liver cells, cells of the digestive tract and accessory digestive organs, salivary gland cells, adrenal gland cells, prostate cells, lung cells, pancreatic cells, bone cells, immune cells, hematopoietic cells, vascular cells, cells of the eye, connective tissue cells, musculoskeletal cells, bone tissue, musculoskeletal tissue, cornea tissue, skin tissue, heart valves, blood vessels, immune cells, connective tissue, lung tissue, skin, a cornea, a kidney, a liver, a lung, a pancreas, a heart, and intestine. In preferred embodiments, the donor graft is comprised of skin cells, skin tissue or beta-cells.

The cell, tissue and/or organ to be transplanted can be syngeneic, allogenic or xenogenic to the subject receiving the transplant. As used herein, the term "syngenic" or "syngeneic" refers to cells, tissues or organs that are genetically identical or are derived from a genetically identical source to the transplant recipient (*e.g.*, an identical twin), especially with respect to antigens or immunological reactions. Such cells, tissues or organs are called isografts. As used herein, the term "allogenic" or "allogeneic" refers to cells, tissues or organs that are not genetically identical or are derived from a non-genetically identical source to the transplant recipient (*e.g.*, a non-related donor), especially with respect to antigens or immunological reactions. Such cells, tissues or organs are called allografts, allogeneic transplants, homografts or allotransplants As used herein, the term "xenogenic" or "xenogeneic" refers to cells, tissues or organs that are from a different species to the transplant recipient (*e.g.*, a pig donor to a human recipient), especially with respect to antigens or immunological reactions. Such cells, tissues or organs are called xenografts or xenotransplants.

In another embodiment, the invention provides a method of reducing an immune response comprising administering to a subject in need thereof with an effective

amount of an inhibitor of alanyl (membrane) aminopeptidase (ANPEP) to reduce an immune response. In an embodiment, the subject in need thereof is a subject with an autoimmune condition, an immune hyper-reactive condition, a chronic inflammatory condition, or is in need of a transplant. An autoimmune condition can include, but is not limited to, alopecia areata, autoimmune hemolytic anemia, autoimmune hepatitis, dermatomyositis, diabetes (type 1), some forms of juvenile idiopathic arthritis, glomerulonephritis, Graves' disease, Guillain-Barré syndrome, idiopathic thrombocytopenic purpura, myasthenia gravis, some forms of myocarditis, multiple sclerosis, pemphigus/pemphigoid, pernicious anemia, polyarteritis nodosa, polymyositis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, scleroderma/systemic sclerosis, Sjögren's syndrome, systemic lupus erythematosus, some forms of thyroiditis, some forms of uveitis, vitiligo or granulomatosis with polyangiitis (Wegener's). Immune hyper-reactive conditions can include, but are not limited to, allergies, athma, eczema or chronic fatigue syndrome. Chronic inflammatory conditions can include, but are not limited to appendicitis, bursitis, colitis, cystitis, dermatitis, phlebitis, rhinitis, tendonitis, tonsillitis or vasculitis.

The subject or the donor transplant or graft may be treated with the inhibitor in any suitable manner (*i.e., in vitro; ex vivo; in vivo)* to inhibit expression, activity and/or function of ANPEP. As used herein, an "inhibitor" of expression, activity and/or function of ANPEP includes compounds that block the function, peptidase activity and/or signaling of ANPEP, compounds that reduce or prevent the transcription of ANPEP DNA into RNA, compounds that reduce or prevent the translation of ANPEP RNA into protein, and compounds that reduce or prevent the function of ANPEP protein. Such inhibiting can be complete inhibition or partial inhibition, such that the expression and/or activity of ANPEP is reduced, resulting in a reduced protease activity, adhesion or signaling, and prevention of limitation of receptor-mediated antigen uptake and presentation in dendritic cells and regulation of endocytosis and innate immune regulation in any cell type. Such inhibitors are selected from the group consisting of: antibodies that bind to ANPEP; aptamers that can interfere with ANPEP; antisense oligonucleotides directed against the ANPEP DNA or mRNA; small interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), microRNAs (miRNA) or small internally segmented interfering RNAs (sisiRNA) directed against ANPEP protein, DNA, or mRNA, small molecule ANPEP inhibitors and any other chemical or biological compound that can interfere with ANPEP activity. The inhibitor can be used alone or together with other agents as an immunosuppressant to decrease the

activity of the immune system, and may prevent transplant rejection or graft-versus-host disease.

When the inhibitor comprises an antibody, such antibodies can be polyclonal or monoclonal. The antibodies can be humanized, fully human, or murine forms of the antibodies. Such antibodies can be made by well-known methods, such as described in Harlow and Lane, Antibodies; A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., (1988). In some embodiments, additional amino acid residues may be added to either the N- or C-terminus of the antibody or antibody fragment. When the inhibitor comprises an aptamer, such aptamers can be oligonucleic acid or peptide molecules that bind to a specific target molecule. Methods of constructing and determining the binding characteristics of aptamers are well known in the art, and the aptamers can be isolated from random libraries or they can be previously identified peptides. When the inhibitor comprises antisense oligonucleotides, such antisense oligonucleotides can be small interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), microRNAs (miRNA) or small internally segmented interfering RNAs (sisiRNA). Methods of constructing and determining the binding characteristics of antisense oligonucleotides are well known in the art, and the antisense oligonucleotides can be designed using known programs or they can be previously identified antisense oligonucleotides.

Administering of ANPEP inhibitors to a subject in need can be via any suitable route as deemed appropriate for an intended use. In certain embodiments, the inhibitor, as described herein, can be administered alone. In certain embodiments, the inhibitor can be administered prior to the administration of at least one other therapeutic agent. In certain embodiments, the inhibitor can be administered concurrent with the administration of at least one other therapeutic agent. In certain embodiments, the inhibitor can be administered subsequent to the administration of at least one other therapeutic agent. In other embodiments, the inhibitor can be administered prior to the administration of at least one other therapeutic agent. As will be appreciated by one of skill in the art, in some embodiments, the inhibitor can be combined with the other agent/compound. In some embodiments, the inhibitor and other agent can be administered concurrently. In some embodiments, the inhibitor and other agent are not administered simultaneously, with inhibitor being administered before or after the agent is administered. In some embodiments, the subject receives both the inhibitor and the other agent during a same period of prevention, occurrence of a disorder, and/or period of treatment.

The methods and cells of the disclosure can be used for a wide variety of pharmaceutical, cosmetic, and medicinal purposes that are known in the art.

In some embodiments, an inhibitor of ANPEP or pharmaceutical compositions comprising an inhibitor of ANPEP can be administered in combination therapy, *i.e.*, combined with other agents. In certain embodiments, the combination therapy comprises the inhibitor, in combination with at least one other agent. Agents include other immunosuppressive agents, but are not limited to corticosteroids and glucocorticoids (*e.g.*, cortisol, hydrocortisone, cortisone, prednisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone, beclometasone, fludrocortisone acetate, deoxycorticosterone acetate or aldosterone), calcineurin inhibitors and drugs acting on immunophilins (*e.g.*, cyclosporine or tacrolimus), mTOR inhibitors (*e.g.*, everolimus or sirlumus), antiproliferative drugs (*e.g.*, azathioprine, cyclophosphamide, mycophenolic acid, mycophenolate mofetil, mizoribine. Additional agents may also include, but are not limited to antibodies or biologics, such as anti-CD3 antibodies, anti-CD20 antibodies, anti-IL2antibodies, anti-PD-1 antibodies, anti-CTLA4 antibodies or other immunosuppressive agents.

In certain embodiments, the invention provides for pharmaceutical compositions comprising the inhibitor and a therapeutically effective amount of at least one additional therapeutic agent, together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant.

In certain embodiments, acceptable formulation materials preferably are nontoxic to recipients at the dosages and concentrations employed. In some embodiments, the formulation material(s) are for sub-cutaneous (s.c.) and/or intravenous (I.V.) administration. In certain embodiments, the inhibitor of ANPEP or pharmaceutical composition comprising an inhibitor of ANPEP can contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. In certain embodiments, suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides;

disaccharides; and other carbohydrates (such as glucose, mannose or dextrins); proteins (such as serum albumin, gelatin or immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. (Remington's Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, ed., Mack Publishing Company (1995). In some embodiments, the formulation comprises PBS; 20 mM NaOAC, pH 5.2, 50 mM NaCl; and/or 10 mM NAOAC, pH 5.2, 9% Sucrose.

In certain embodiments, the optimal pharmaceutical composition comprising an ANPEP inhibitor will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format and desired dosage. See, for example, Remington's Pharmaceutical Sciences, supra. In certain embodiments, such compositions may influence the physical state, stability, rate of *in vivo* release and rate of *in vivo* clearance of the antibodies of the invention.

In certain embodiments, the primary vehicle or carrier of an inhibitor of ANPEP or pharmaceutical compositions comprising an inhibitor of ANPEP can be either aqueous or non-aqueous in nature. For example, in certain embodiments, a suitable vehicle or carrier can be water for injection, physiological saline solution or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. In some embodiments, the saline comprises isotonic phosphate-buffered saline. In certain embodiments, neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. In certain embodiments, pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which can further include sorbitol or a suitable substitute therefore.

In certain embodiments, the effective amount of an inhibitor of ANPEP or pharmaceutical compositions comprising an inhibitor of ANPEP as described herein,

with or without at least one additional therapeutic agent, to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment, according to certain embodiments, will thus vary depending, in part, upon the inhibitor delivered, the indication for which an inhibitor described herein, with or without at least one additional therapeutic agent, is being used, the route of administration, and the size (body weight, body surface or organ size) and/or condition (the age and general health) of the patient. In certain embodiments, the clinician can titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. In certain embodiments, a typical dosage comprising an antibody that binds to and inhibits ANPEP can range from about 0.1 µg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In certain embodiments, the dosage can range from 0.1 µg/kg up to about 100 mg/kg; or 1 µg/kg up to about 100 mg/kg; or 5 µg/kg up to about 100 mg/kg.

In certain embodiments, the frequency of dosing will take into account the pharmacokinetic parameters of the inhibitor described herein and/or any additional therapeutic agents in the formulation used. In certain embodiments, a clinician will administer the composition until a dosage is reached that achieves the desired effect. In certain embodiments, the composition can therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of the desired fusion protein) over time, or as a continuous infusion via an implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. In certain embodiments, appropriate dosages can be ascertained through use of appropriate dose-response data.

In certain embodiments, the route of administration of the inhibitor of ANPEP or pharmaceutical compositions comprising an inhibitor of ANPEP is in accord with known methods, *e.g.* orally, through injection by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, subcutaneously, intra-ocular, intraarterial, intraportal, or intralesional routes; by sustained release systems or by implantation devices. In certain embodiments, the compositions can be administered by bolus injection or continuously by infusion, or by implantation device.

In certain embodiments, the inhibitor of ANPEP or pharmaceutical compositions comprising an inhibitor of ANPEP can be administered locally via implantation of a membrane, sponge or another appropriate material onto which the desired inhibitor

has been absorbed or encapsulated. In certain embodiments, where an implantation device is used, the device can be implanted into any suitable tissue or organ, and delivery of the desired fusion protein can be via diffusion, timed-release bolus, or continuous administration.

In a second aspect the invention provides a pluripotent cell population, wherein the population does not express functional ANPEP. Functional ANPEP can be knocked out or inhibited by a method selected from the group consisting of generation of knock-in null mutant ANPEP cell population using homologous recombination, generation of knock-in null mutant ANPEP cell population using transcription activator-like effector nucleases (TALENs), generation of knock-in null mutant ANPEP cell population using clustered regularly interspaced short palindromic repeats (CRISPR) technology generation of ANPEP knockout cell line using homologous recombination, generation of ANPEP knockout cell lines using TALEN, generation of ANPEP knockout cell lines using CRISPR technology, generation of ANPEP mutant cell lines using homologous recombination, generation of ANPEP mutant cell lines using TALEN, generation of ANPEP mutant cell lines using CRISPR technology,anti-ANPEP antibody, anti-ANPEP aptamer, ANPEP small interfering RNA, ANPEP small internally segmented interfering RNA, ANPEP short hairpin RNA, ANPEP micro RNA, ANPEP antisense oligonucleotides and small molecule ANPEP inhibitors.

As used herein, the term "pluripotent cell population" refers to animal, especially mammalian, preferably human, pluripotent cells. Pluripotent cells refer to unspecialized cells that have the ability to self-renew for long periods of time and differentiate into specialized cells with specific functions. Pluripotent cells can refer to stem cells that have the potential to differentiate into any of the three germ layers: endoderm (*e.g.*, interior stomach lining, gastrointestinal tract, the lungs), mesoderm (*e.g.*, muscle, bone, blood, urogenital), or ectoderm (*e.g.*, epidermal tissues and nervous system). Cell pluripotency can be a continuum, ranging from the pluripotent cell that can form every cell of the embryo proper, *e.g.*, embyronic stem cells and induced pluripotent stem cells, to the incompletely or partially pluripotent cell that can form cells of all three germ layers, but that may not exhibit all the characteristics of completely pluripotent cells. Pluripotent cells can refer to progenitor cells which have the gene activation potential to differentiate into multiple, but limited cell types. For example, a pluripotent blood stem cell is a hematopoietic cell and this cell type can differentiate itself into several types of blood cell types like lymphocytes, monocytes, or neutrophils, but cannot differentiate into brain cells, bone cells or other non-blood

cell types. Pluripotent cells can be found in many, but not all human cell types (for example, pluripotent cells have been found in adipose tissue, cardiac cells, bone marrow, and mesenchymal stromal cells).

As used herein, the term "functional ANPEP" refers to ANPEP protein with both enzyme-dependent and independent functions that contribute to adhesion, cell migration, angiogenesis, inflammatory trafficking, adhesion, antigen presentation, and endocytosis. In an embodiment, the cells, tissues and organs of the invention lacking functional ANPEP can have endogenous ANPEP knocked-out of the cell, tissue or organ or expression can be disrupted at transcription or translation step and therefore the cell, tissue or organ therefore does not express ANPEP. In another embodiment, the cells, tissues or organs of the invention lacking functional ANPEP can have endogenous ANPEP replaced by knock-in of a mutant ANPEP that does not function properly and therefore ANPEP cannot contribute to adhesion, cell migration, angiogenesis, inflammatory trafficking, adhesion, antigen presentation, and endocytosis. In yet another embodiment, the cells, tissues or organs of the invention lacking functional ANPEP can have endogenous ANPEP inhibited and therefore ANPEP cannot contribute to adhesion, cell migration, angiogenesis, inflammatory trafficking, adhesion, antigen presentation, and endocytosis.

In an embodiment, the invention provides a pluripotent cell population in which functional ANPEP has been knocked out or inhibited by a method selected from the group consisting of: generation of knock-in null mutant ANPEP cell population using homologous recombination, generation of knock-in null mutant ANPEP cell population using TALEN, generation of knock-in null mutant ANPEP cell population using CRISPR technology, generation of ANPEP knockout cell lines using homologous recombination, generation of ANPEP knockout cell lines using TALEN, generation of ANPEP knockout cell lines using CRISPR technology, generation of ANPEP mutant cell lines using homologous recombination, generation of ANPEP mutant cell lines using TALEN, generation of ANPEP mutant cell lines using CRISPR technology, anti-ANPEP antibody, anti-ANPEP aptamer, ANPEP small interfering RNA, ANPEP small internally segmented interfering RNA, ANPEP short hairpin RNA, ANPEP micro RNA, small molecule inhibitors of ANPEP and ANPEP antisense oligonucleotides. CRISPR/Cas-based RNA-guided DNA endonucleases are genome editing tools (Wang *et al.*, 2013 "One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering." *Cell* 153(4):910-18). Transcription activator-like effector nucleases (TALENs) comprise chimeric nucleases that are composed of programmable, sequence-specific DNA-binding

modules linked to a nonspecific DNA cleavage domain. TALENs enable a broad range of genetic modifications by inducing DNA double-strand breaks that stimulate error-prone nonhomologous end joining or homology-directed repair at specific genomic locations (Gaj *et al.*, 2013 "ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering." *Trends Biotechnol.* 31(7):397-405).

In another embodiment, the invention provides a differentiated population of cells for transplanting into a subject in need thereof, wherein the cells are derived from the pluripotent cells lacking functional ANPEP. The differentiated population of cells can be selected from the group consisting of: skin cells, beta cells, cardiac cells, brain cells, kidney cells, liver cells, cells of the digestive tract and accessory digestive organs, salivary gland cells, adrenal gland cells, prostate cells, lung cells, pancreatic cells, bone cells, immune cells, hematopoietic cells, vascular cells, cells of the eye, connective tissue cells and musculoskeletal cells. In a preferred embodiment, the cells are skin cells or beta cells lacking functional ANPEP.

The differentiated population of cells can be derived from the pluripotent cells lacking functional ANPEP by a number of protocols that are already developed or are currently being optimized by others in the field. These methods can include, but are not limited to, systematic induction of differentiation using special cell culture matrices, media and growth factor or small molecule supplements administered in specific time windows that mimic normal developmental timepoints, introduction of certain genes and growth factors or cytokines into cells to promote immediate switching of cell types, or the *in vivo* introduction of certain genes, growth factors or cytokines into cells to promote endogenous differentiation of cells.

In yet another embodiment, the invention provides a tissue for transplanting into a subject in need thereof, wherein the tissue is derived from the pluripotent cell population lacking functional ANPEP. The tissue can be selected from the group consisting of: bone tissue, musculoskeletal tissue, cornea tissue, skin tissue, heart valves, and blood vessels, immune cells, connective tissue, lung tissue. In a preferred embodiment, the tissue is skin tissue lacking functional ANPEP.

The differentiated tissue can be derived from the pluripotent cells lacking functional ANPEP by a number of protocols that are already developed or are currently being optimized by others in the field. These methods can include, but are not limited to, systematic induction of differentiation using special cell culture matrices, media and growth factor or small molecule supplements administered in specific time windows that mimic normal developmental timepoints, introduction of certain genes and

growth factors or cytokines into cells to promote immediate switching of cell types, or the *in vivo* introduction of certain genes, growth factors or cytokines into cells to promote endogenous differentiation of cells. Cells generated *in vitro* can be seeded onto bioengineered scaffolds that can provide the three dimensional structure of the tissue of interest. These scaffolds can be created by three dimensional printing with biomaterials specifically developed for these purposes, including but not limited to, synthetic materials, protein based materials and polysaccharide based materials, such as polyglycolic acid, polylactic acid, fibrin, glycosaminoglycans.

In a further embodiment, the invention provides an organ for transplanting into a subject in need thereof, wherein the organ is derived from the pluripotent cell population lacking functional ANPEP. The organ can be selected from, but is not limited to, the group consisting of: cornea, skin, kidney, liver, lung, heart, pancreas and intestine. In a preferred embodiment, the organ is skin lacking functional ANPEP.

The differentiated organ can be derived from the pluripotent cells lacking functional ANPEP by a number of protocols that are already developed or are currently being optimized by others in the field. These methods can include, but are not limited to, systematic induction of differentiation using special cell culture matrices, media and growth factor or small molecule supplements administered in specific time windows that mimic normal developmental timepoints, introduction of certain genes and growth factors or cytokines into cells to promote immediate switching of cell types, or the *in vivo* introduction of certain genes, growth factors or cytokines into cells to promote endogenous differentiation of cells. Differentiated cells or stem cells can be seeded onto bioengineered scaffolds to promote further development of a complete organ with the various different cell types and organization that is associated with the particular organ of interest. Bioengineered scaffolds can be created through three dimensional printing with biomaterials or decellularization of existing organs according to protocols optimized by experts in that field. In an alternative method, pluripotent stem cells can be used to generate large multidimensional organoids according to culture methods currently being developed by other experts in the field.

In an another embodiment, the invention provides cells, tissues or organs for transplanting into a subject in need thereof, wherein the cells, tissues or organs are derived from the pluripotent cell population lacking functional ANPEP and also comprise at least one additional modification. In some embodiments, an additional modification can be the genetic engineering of the cells, tissues or organs lacking functional ANPEP to express a gene or peptide. In such an embodiment, the donor

transplant can also serve as a means for administering therapeutic proteins and peptides. The peptide is not limited to any particular peptide, but can include any peptide that can be used to treat any number of diseases, disorders or conditions. For example, the therapeutic peptide can include, but is not limited to, nesiritide, ceruletide, bentiromide, exenatide, gonadorelin, enfuvirtide, vancomycin, icatibant, secretin, leuprolide, glucagon recombinant, oxytocin, bivalirudin, sermorelin, gramicidin D, insulin, capreomycin, calcitonin, vasopressin, cosyntropin, bacitracin, octreotide, abarelix, vapreotide, thymalfasin, mecasermin, cetrorelix, teriparatide, corticotropin or pramlintide.

In an additional embodiment, the invention provides a method of transplanting cells, tissues and/or organs into a subject in need thereof comprising: pre-treating the cells, tissues and/or organs with ANPEP inhibitors prior to transplanting the cells, tissues and/or organs lacking or blocking functional ANPEP into a subject in need thereof or treating the subject with ANPEP inhibitors prior to transplant. The cell, tissue and/or organ to be transplanted can be syngeneic, allogeneic or xenogeneic to the subject receiving the transplant.

As used herein, the term "syngenic" or "syngeneic" refers to cells, tissues or organs that are genetically identical or are derived from a genetically identical source to the graft recipient (*e.g.*, an identical twin), especially with respect to antigens or immunological reactions. Such cells, tissues or organs are called isografts.
As used herein, the term "allogenic" or "allogeneic" refers to cells, tissues or organs that are not genetically identical or are derived from a non-genetically identical source to the graft recipient (*e.g.*, a non-related donor), especially with respect to antigens or immunological reactions. Such cells, tissues or organs are called allografts, allogeneic transplants, homografts or allotransplants
As used herein, the term "xenogenic" or "xenogeneic" refers to cells, tissues or organs that are from a different species to the graft recipient (*e.g.*, a pig donor to a human recipient), especially with respect to antigens or immunological reactions. Such cells, tissues or organs are called xenografts or xenotransplants.

EXAMPLES

The Examples that follow are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

Example 1: ANPEP expression and the immune response

To determine the role of ANPEP in immune responses elicited by stem cell transplantation, mouse embryonic stem (mES) cells were derived from ANPEP WT and ANPEP KO mice. These cells proliferate at similar rates and express characteristic pluripotency markers *in vitro*. When implanted into syngeneic mice, mES of both genotypes form teratomas that contain cell types representing all three embryonic germ layers, demonstrating pluripotency *in vivo* as well. Surprisingly, teratomas generated from ANPEP KO mES grow more rapidly, achieve a larger size, and show a higher degree of differentiation than those generated from ANPEP WT mES cells. Analysis of potential underlying causes showed that ANPEP KO teratomas contained fewer infiltrating T-lymphocytes and dendritic cells, indicating that lack of ANPEP expression may permit stem cells to escape immune detection and allow implants to thrive. In agreement with this hypothesis, growth and differentiation of WT and ANPEP KO mES implanted into immunocompromised mice were comparable, consistent with an underlying immune mechanism. Consequently, modulation of ANPEP expression may alter immune responses toward implanted stem cells and enhance their engraftment to ultimately facilitate repair.

Example 2: Assessment of ANPEP expression and immunomodulation

To study the immunomodulatory effects of ANPEP further, the inventors have been studying skin grafts between ANPEP WT and ANPEP KO mice with gender or major histocompatibility-mismatches. Surprisingly, the skin graft studies confirm the observations with the teratomas. ANPEP KO female skin engrafts successfully on both ANPEP WT and ANPEP KO male mice, while ANPEP WT female skin is rejected from both ANPEP WT and ANPEP KO male mice after 14 days. Comparison of histological sections at sites of engraftment shows that there is significant inflammation and disruption of normal skin architecture in ANPEP WT skin grafts. There is only mild inflammation in ANPEP KO skin engrafted in ANPEP WT mice, while there is virtually no disruption of ANPEP KO skin engrafted in ANPEP KO mice. These studies provide surprising and important evidence that immunological tolerance can be engendered through ANPEP blockade.

Example 3: Skin grafts lacking CD13 survive longer with diminished inflammation.

Initial characterization of mice lacking CD13 expression on a global level demonstrated no notable developmental deficiencies or defects in homeostatic processes (Winnicka *et al.* 2010). However, while site-specific mechanisms

influenced specific outcomes, the inflammatory responses of CD13-null animals were universally compromised upon challenge in various injury models (Pereira *et al.* 2013, Rahman *et al.* 2013, Subramani *et al.* 2013, Ghosh *et al.* 2014). Based on these observations, it was determined if the diminished inflammatory responses seen in CD13-null mice could potentially extend to the inflammatory reactions mediating transplant rejection. Using wild type and global CD13-null mice in a standard model of minor histocompatibility (MiHC) mismatched allograft rejection (Figure 6), long-term survival of full-thickness CD13-null male donor skin grafts was observed on female recipients that survived for over 100 days (Figure 1A). The CD13-null grafts maintained their original size as compared to WT skin grafts that wither and gradually shrink in diameter until fully rejected between two to three weeks post-transplant (Figure 1B). Furthermore, the CD13-null grafts retained the characteristic thin, vascularized skin of the donor dorsal ear and did not adopt the thick fur of the host dorsal skin, suggesting that CD13 can be a potential instigator of allograft rejection. Indeed, while there is evidence of inflammatory cell infiltration into CD13-null graft tissue after one week, the number of cells and the extent of tissue damage was significantly less than that observed in WT skin grafts (Figure 1C). Immunohistochemical quantification of CD3 positive cells in grafts at five days post-transplant indicated that significantly more T cells infiltrated into WT grafts as compared in CD13-null grafts in both WT and CD13-null recipients (Figure 1D). Flow cytometric analyses of host spleens receiving grafts of either genotype at days 5 and 100 post-transplant indicated that there were no significant differences in various splenic immune populations (Figure 7) implying that the differences in graft acceptance observed were most likely due to local events in the graft and draining lymph nodes.

Example 4. Mast cell-mediated inflammation is reduced in CD13-null grafts

Mast cells are traditionally considered pro-inflammatory mediators of the acute immune response; however, recent studies have elucidated novel anti-inflammatory roles for these cells particularly in the setting of allograft survival (Galli *et al.* 2005, Lu *et al.* 2006), where mast cells have been shown to be critical for graft survival and their degranulation reverses tolerance to skin allografts (Lu *et al.* 2006, de Vries *et al.* 2009). Pertinent to this study, mast cells are known to express high levels of CD13, but the functional role of CD13 in mast cell activity has yet to be demonstrated. To determine the contribution of mast cells to this model of allograft survival, WT and CD13-null skin grafts were examined for the presence of mast cells and their degranulation status using toluidine blue to stain mast cell-specific granular contents.

Interestingly, higher levels of toluidine blue staining was observed in CD13-null graft tissue, potentially due to reduced mast cell degranulation in the absence of CD13 (Figure 2A and 2B). This increase in granule staining was also seen in the draining lymph nodes, but not the spleens of mice that received CD13-null skin grafts (Figure 2C). Orthotopic applications of gender mismatched dermal fibroblasts survive longer when mixed with CD13-null mast cells. These results confirm the histological observations that inflammation is reduced in the absence of CD13. Furthermore, given that mast cells are known to produce IL-4, a known stimulator of reparative M2 macrophages, and IL-10, a potent immunosuppressor, it is possible that the diminished inflammation and elevated number of alternatively activated macrophages in CD13-null grafts (Figure 1F) can be attributed to a degranulation-independent anti-inflammatory function of mast cells.

Example 5. Absence of CD13 on mast cells corresponds to diminished mast cell activation and degranulation *in vitro*

To confirm the *in vivo* observations of effects of CD13 on mast cell activity, primary mast cells were derived from bone marrow of WT and CD13-null mice according to previously published protocols (Kalesnikoff and Jalli 2011). After 4-6 weeks of culture, cultures consisted of 80-90% ckit+FcεR1α+ mast cells with a high degree of forward and side scatter, indicating these cells are large and complex (Figure 3A). To test the response of mast cells to MiHC determinants, isolated WT or CD13-null mast cells were co-cultured with gender-mismatched dermal fibroblasts and changes were assessed by flow cytometry. The forward and side scatter profiles of both WT and CD13-null mast cells were decreased indicating loss of granularity and size, presumably the result of degranulation; however, the changes in CD13-null mast cell size and granularity were significantly less upon stimulation with either WT or CD13-null dermal fibroblasts than WT mast cells (Figure 3B). This is in accord with results from mixed lymphocyte reactions between gender mismatched splenocytes where the response of CD13-null splenocytes was considerably weaker (Figure 8). However, MiHC mismatched CD13-null stimulators invoked more robust responses from WT splenocytes than WT mast cells, suggesting a lack of allorecognition.

Additionally, the supernatant collected from CD13-null mast cells cultured with dermal fibroblasts contained higher levels of IL-4. To determine if CD13-null mast cells could be exerting their anti-inflammatory role at least in part by skewing macrophage differentiation towards the reparative M2 phenotype at the expense of a pro-inflammatory M1 phenotype, WT and CD13-null mast cells are co-cultured with WT and CD13-null immature macrophages for 1-3 days before determining relative

numbers of M1 and M2 macrophages by flow cytometry. As a whole, these data show that CD13-null mast cell degranulation and pro-inflammatory responses are impaired while a more anti-inflammatory or immunosuppressive microenvironment is enhanced, thus promoting transplant survival.

Example 6. Transient blockade of CD13 prolongs graft survival

To confirm the effects on transplant acceptance were due to CD13, a CD13 blocking antibody, SL13, was used in transient treatment studies (Figure 9A). Following two weeks of intraperitoneal SL13-mAb administration, SL13 was detectable in the serum of treated mice, but not in the serum of mice treated with vehicle alone or isotype control (Figure 9B). All of the mice tolerated the treatment and after two weeks there were no significant pathological changes in any major organ indicating that SL13 can be used safely *in vivo*. After the two week treatment period, SL13 treatment was halted and WT female recipients of both groups received gender-mismatched skin grafts from WT and CD13-null male donors. Similar to studies in the global CD13-null animals, CD13-null grafts universally survived until time of harvest in both groups (Figure 4A left panels; KO male skin graft). Importantly, rejection of WT male skin was delayed by 3 weeks in treated WT recipients, well after WT skin grafts had been rejected by the untreated mice (Figure 4A right panels; WT male skin graft). Reversal of graft acceptance following the withdrawal of SL13 treatment indicated that CD13 blockade using a monoclonal antibody prolongs graft survival. SL13 treatment for 1 week prior to skin transplant can prolong graft survival when SL13 is routinely given. Once SL13 treatment is discontinued, WT skin grafts show signs of failing. Toluidine blue staining of WT skin that survives in SL13 treated mice indicates an increase in mast cell staining as compared to that seen in CD13-null grafts, suggesting that a similar mechanism of locally induced immune acceptance.

Example 7. Tissues derived from mESC lacking CD13 are accepted by immune-competent mice.

Pluripotent stem cell derived therapies are the ultimate goal of regenerative medicine; however, their utility in the clinic is hampered by the same immunologic reactions that limit current transplantation therapies (Tang and Drukker 2011). To determine if CD13 blockade could be applied in a stem cell-derived therapeutic setting, mouse embryonic stem cells (mES) were generated from WT and CD13-null mice. These cells displayed characteristic mES cell morphology and expressed traditional pluripotency markers Oct4 and Nanog (Figure 10A). Both WT and CD13-null mESCs proliferated at similar rates *in vitro* and generated all three germ layers

when differentiated in teratoma assays (Figure 10B and 10C). Interestingly, CD13-null mES generated substantially larger teratomas than did WT mES even though starting cell numbers, host mice and duration of growth are matched (Figure 5A). These CD13-null teratomas also proliferated at higher levels as indicated by increased Ki67 staining and BrDU incorporation (Figure 5B and 5C), but did not display any significant apoptosis by Tunel staining (Figure 10D, arrows indicate apoptotic cells). Furthermore, CD13-null teratomas are also more vascularized as detected by endothelial CD31 and pericyte αSMA staining (Figure 10E).

Overall, similar to the observations with CD13-null skin grafts these data indicate that CD13-null mES possess a survival advantage over WT mES. To ascertain the potential immunological processes underlying this apparent growth advantage, WT and CD13-null mES were injected subcutaneously into immunocompromised mice lacking T, B and NK cells. Both WT and CD13-null mES generated teratomas of equal size and at similar rates (Figure 5D) confirming that the difference in growth was not attributable to inherent differences in the embryonic stem cells themselves, but rather to events occurring at the donor-host interface. Analysis of infiltrating immune cells in teratomas generated in immunocompromised mice shows no significant differences; while numbers of infiltrating CD3+ T cells in immune competent mice are higher in WT in CD13-null teratomas. Furthermore, highly significant differences in numbers of infiltrating T cells were observed in teratomas generated in CD13-null hosts (Figure 5E). This would imply that WT mES are more capable of stimulating an immune reaction against the transplanted cells and that the response of CD13-null mice to immunogenic donor cells is impaired and is in agreement with the immune cell analyses in the skin graft studies discussed above. Additionally, higher levels of dendritic cells and macrophages were detected in WT teratomas (Figure 5F). Toluidine blue staining of teratoma sections also indicated higher levels of mast cell staining within and around CD13-null teratomas, consistent with reduced mast cell degranulation in the skin graft model (Figure 5G).

Taken together, these data demonstrate that the CD13 blockade-dependent transplant of cells and tissues is most likely due to the absence CD13-mediated immunological rejection. To test the applicability of CD13-blockade in promoting cell therapy, skin and lung cells were generated from male WT and CD13-null mES and transplanted into WT female recipients.

The invention would significantly increase the success of organ and skin transplant as well as eliminate the time transplant recipients must wait for a suitable donor transplant or graft to become available. Furthermore, this invention can minimize the necessity of systemic immune suppression, thereby significantly improving quality of life in terms of avoiding infection and taking medication with deleterious side effects. The method would provide universal transplants or grafts and obviate the need and cost of creating personalized pluripotent cell lines and differentiated tissues that would only be applicable to one or a few patients.

Furthermore, the invention could potentially be used by transplant and reconstructive surgeons for patients who require any type of graft or organ. For example, skin grafts could be used for severely burned patients without the current limitations of time, access to viable skin, or immunogenicity. Neurons or neuronal support cells such as oligodendrocytes could also be produced for transplant into patients suffering from neuromuscular diseases. Cardiac cells could be transplanted into patients who suffered myocardial infarction or congestive heart failure.

Having described the invention in detail and by reference to specific embodiments thereof, it will be apparent that modifications and variations are possible without departing from the scope of the invention defined in the appended claims. More specifically, although some aspects of the present invention are identified herein as particularly advantageous, it is contemplated that the present invention is not necessarily limited to these particular aspects of the invention.

WHAT IS CLAIMED IS:

1. An isolated pluripotent cell population, wherein the population does not express functional ANPEP.

2. The isolated pluripotent cell population of claim 1, wherein functional ANPEP has been knocked out or inhibited by a method selected from the group consisting of: generation of knock-in null mutant ANPEP cell population using homologous recombination, generation of knock-in null mutant ANPEP cell population using TALEN, generation of knock-in null mutant ANPEP cell population using CRISPR technology, generation of ANPEP knockout cell lines using homologous recombination, generation of ANPEP knockout cell lines using TALEN, generation of ANPEP knockout cell lines using CRISPR technology, generation of ANPEP mutant cell lines using homologous recombination, generation of ANPEP mutant cell lines using TALEN, generation of ANPEP mutant cell lines using CRISPR technology, anti-ANPEP antibody, anti-ANPEP aptamer, ANPEP small interfering RNA, ANPEP small internally segmented interfering RNA, ANPEP short hairpin RNA, ANPEP micro RNA, small molecule inhibitors of ANPEP and ANPEP antisense oligonucleotides.

3. The isolated pluripotent cell population of any of claims 1-2, wherein the cells are cultured into a differentiated population of cells.

4. The differentiated population of cells of claim 3, wherein the cells are selected from the group consisting of: skin cells, beta cells, cardiac cells, brain cells, kidney cells, liver cells, cells of the digestive tract and accessory digestive organs, salivary gland cells, adrenal gland cells, prostate cells, lung cells, pancreatic cells, bone cells, immune cells, hematopoietic cells, vascular cells, cells of the eye, connective tissue cells and musculoskeletal cells.

5. The differentiated population of cells of claim 4, wherein cells comprise a tissue selected from the group consisting of: bone tissue, musculoskeletal tissue, cornea tissue, skin tissue, kidney tissue, liver tissue, pancreatic tissue, heart valves, blood vessels, immune cells, connective tissue and lung tissue.

6. The differentiated population of cells of claim 4, wherein cells comprise an organ selected from the group consisting of: cornea, skin, kidney, liver, lung, pancreas and intestine.

7. The differentiated population of cells of any of claims 4-6, wherein the cells are genetically engineered to express a therapeutic peptide.

8. A method of transplantation comprising: transplanting the cells, tissues and/or organs of any of claims 1-7 into a subject in need thereof.

9. The method of claim 8, wherein the subject in need thereof is pre-treated with an effective amount of an inhibitor of alanyl (membrane) aminopeptidase (ANPEP).

10. The method of claim 9, wherein the inhibitor is selected from the group consisting of: anti-ANPEP antibody, anti-ANPEP aptamer, ANPEP small interfering RNA, ANPEP small internally segmented interfering RNA, ANPEP short hairpin RNA, ANPEP micro RNA, ANPEP antisense oligonucleotides, and small molecule inhibitors of ANPEP.

11. The method of claim 10, wherein the inhibitor is an anti-ANPEP antibody.

12. The method of claim 8, wherein the cell, tissue and/or organ is selected from the group consisting of: skin cells and skin tissue.

13. The method of any of claims 8-12, wherein the cell, tissue and/or organ is syngeneic, allogeneic or xenogeneic to the subject.

14. The use of the cells, tissues and/or organs of any of claims 1-7 for transplanting into a subject in need thereof.

15. A method of limiting development of transplant rejection, comprising:

a) administering to a subject in need of a transplant, and/or

b) treating a donor transplant with,

an effective amount of an inhibitor of alanyl (membrane) aminopeptidase (ANPEP) to limit development of transplant rejection in the subject.

16. The method of claim 15, wherein the subject receives a donor transplant and wherein the method includes treating the donor transplant; wherein treating the donor transplant comprises pre-treatment with an inhibitor of alanyl (membrane) aminopeptidase (ANPEP).

17. A method of any of claims 15 or 16, wherein the subject's immune system is rejecting the donor transplant, comprising administering to the subject an effective amount of an inhibitor of alanyl (membrane) aminopeptidase (ANPEP).

18. The method of any of claims 15-17, wherein the subject is receiving a donor transplant or has received a donor transplant and the donor transplant is selected from the group consisting of: skin cells, beta cells, cardiac cells, brain cells, kidney cells, liver cells, cells of the digestive tract and accessory digestive organs, salivary gland cells, adrenal gland cells, prostate cells, lung cells, pancreatic cells, bone cells, immune cells, hematopoietic cells, vascular cells, cells of the eye, connective tissue cells, musculoskeletal cells, bone tissue, musculoskeletal tissue, cornea tissue, skin tissue, heart valves, blood vessels, immune cells, connective tissue, lung tissue, skin, a cornea, a kidney, a liver, a lung, a pancreas, a heart, and intestine.

19. The method of claim 18, wherein the donor transplant is selected from the group consisting of: beta cells, skin cells and skin tissue.

20. The method of any of claims 15-19, wherein the subject is receiving a donor transplant or has received a donor transplant and the donor transplant is syngeneic, allogeneic or xenogeneic to the subject.

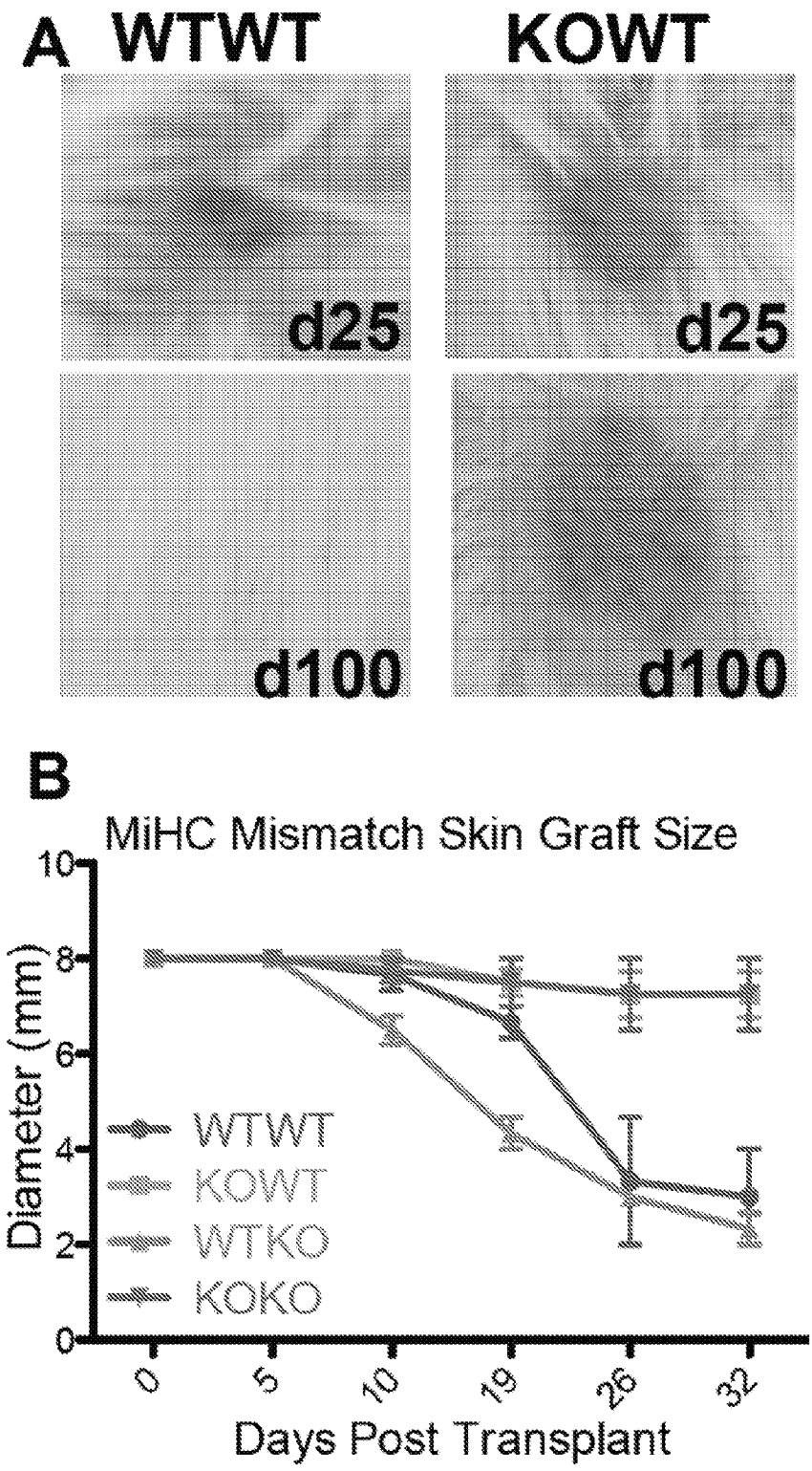
21. A method of reducing an immune response comprising administering to a subject in need thereof with an effective amount of an inhibitor of alanyl (membrane) aminopeptidase (ANPEP) to reduce an immune response.

22. The method of claim 21, wherein the subject in need thereof is a subject with an autoimmune condition, an immune hyper-reactive condition, a chronic inflammatory condition, or is in need of a transplant.

23. The method of any of claims 15-22, wherein the inhibitor is selected from the group consisting of anti-ANPEP antibody, anti-ANPEP aptamer, ANPEP small interfering RNA, ANPEP small internally segmented interfering RNA, ANPEP short hairpin RNA, ANPEP micro RNA, ANPEP antisense oligonucleotides, and small molecule inhibitors of ANPEP.

24. The method of any of claims 15-23, wherein the inhibitor comprises an anti-ANPEP antibody.

Figure 1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26
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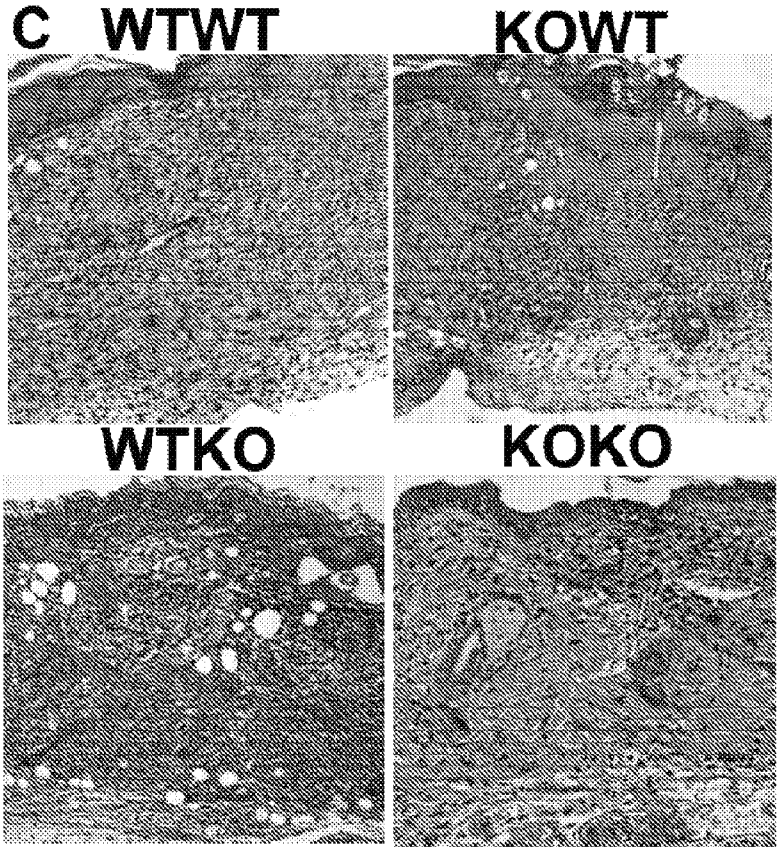
Days Post Transplant

Figure 1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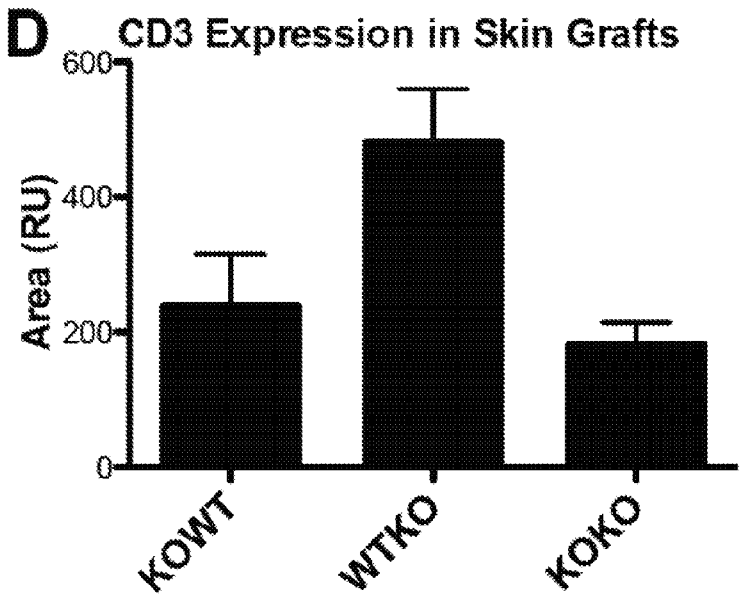
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CD3 Expression in Skin Grafts
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200
0
KOWT
WTKO
KOKO

Figure 2

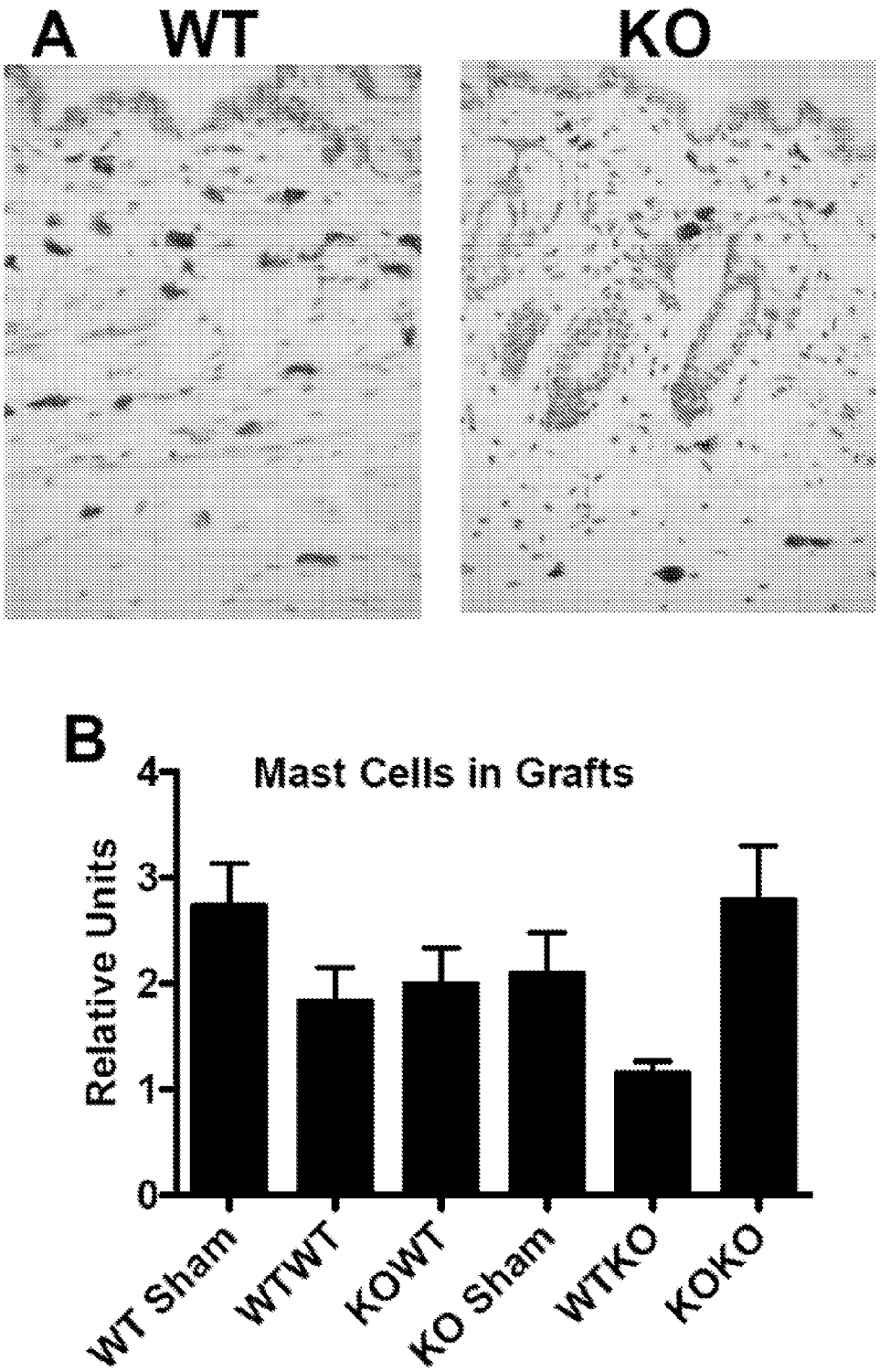

A
WT
KO
B
Mast Cells in Grafts
Relative Units
0
1
2
3
4
WT Sham
WTWT
KOWT
KO Sham
WTKO
KOKO

Figure 2

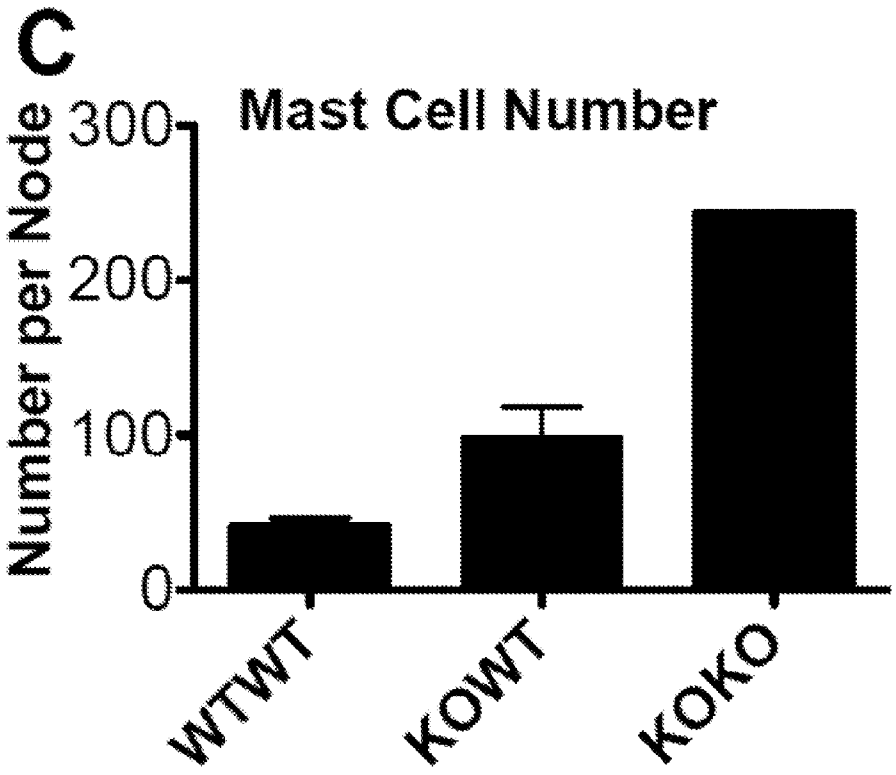

C
Mast Cell Number
Number per Node
300
200
100
0
WTWT
KOWT
KOKO

Figure 3

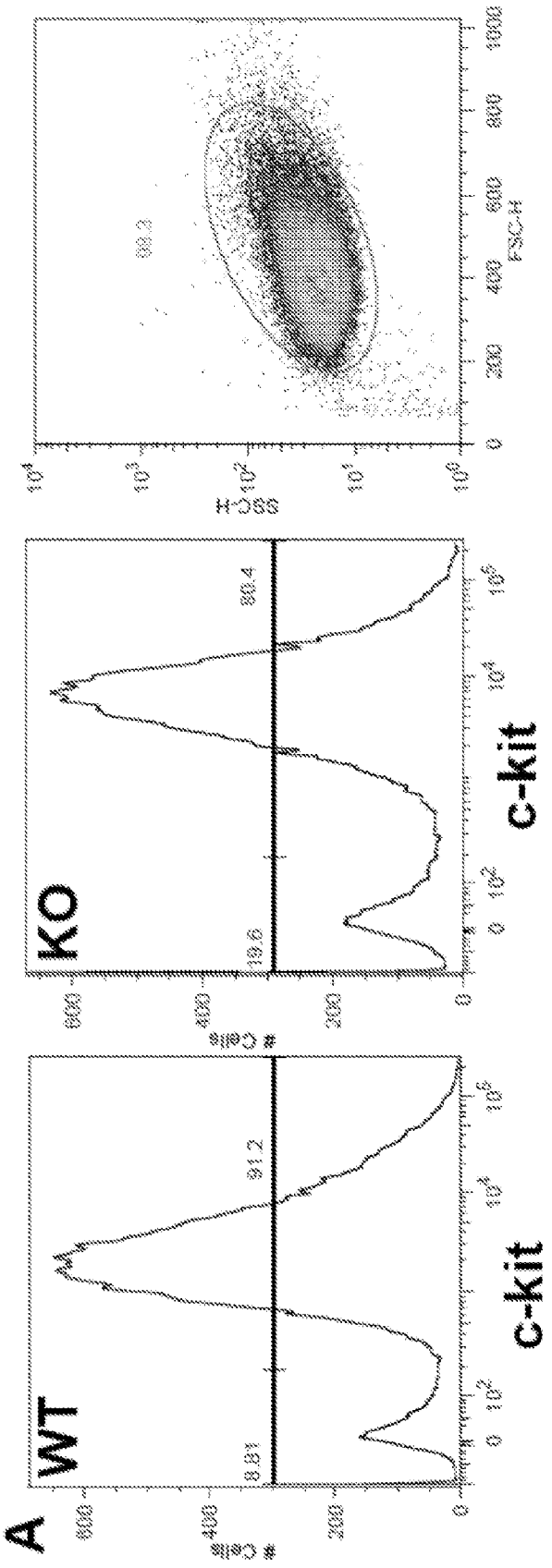
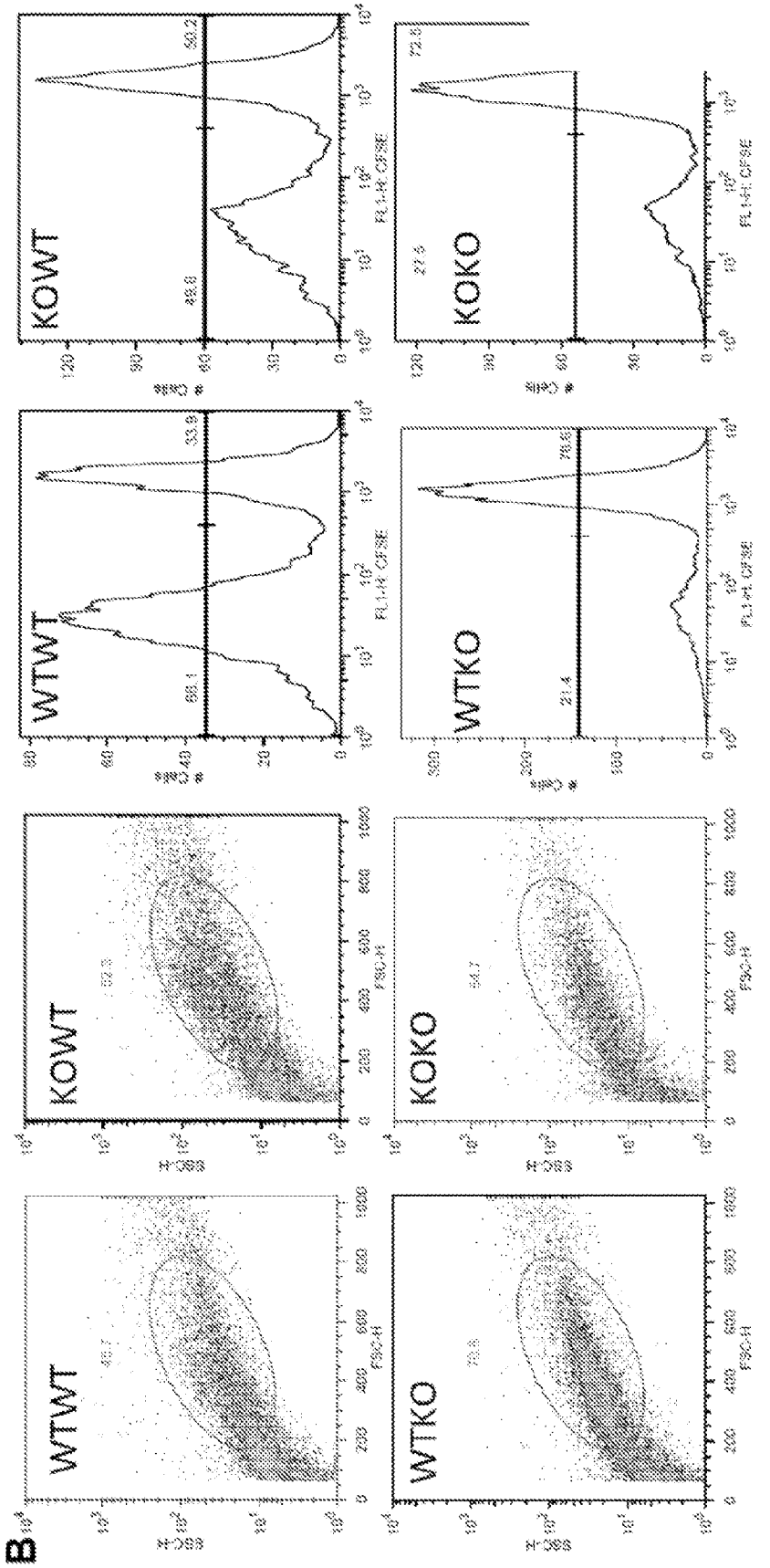


Figure 3


B
WTWT
KOWT
WTKO
KOKO
SSC-H
FSC-H
Cells
FL1-H: CFSE
46.8
50.2
68.1
33.9
21.4
78.6
27.5
72.5

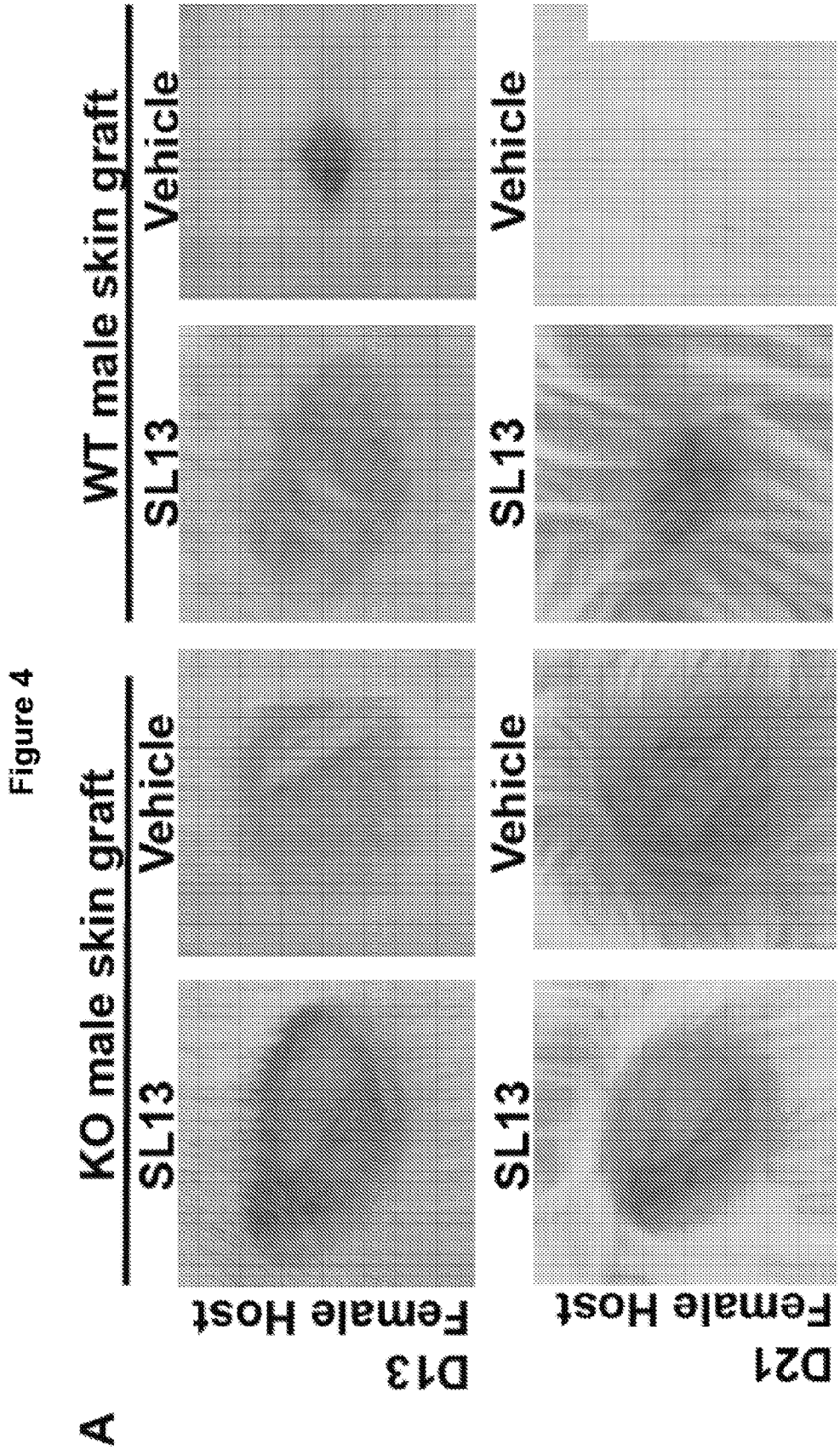

A
D13
Female Host
D21
Female Host
KO male skin graft
WT male skin graft
SL13
Vehicle
SL13
Vehicle
SL13
Vehicle
SL13
Vehicle

Figure 4

Figure 5

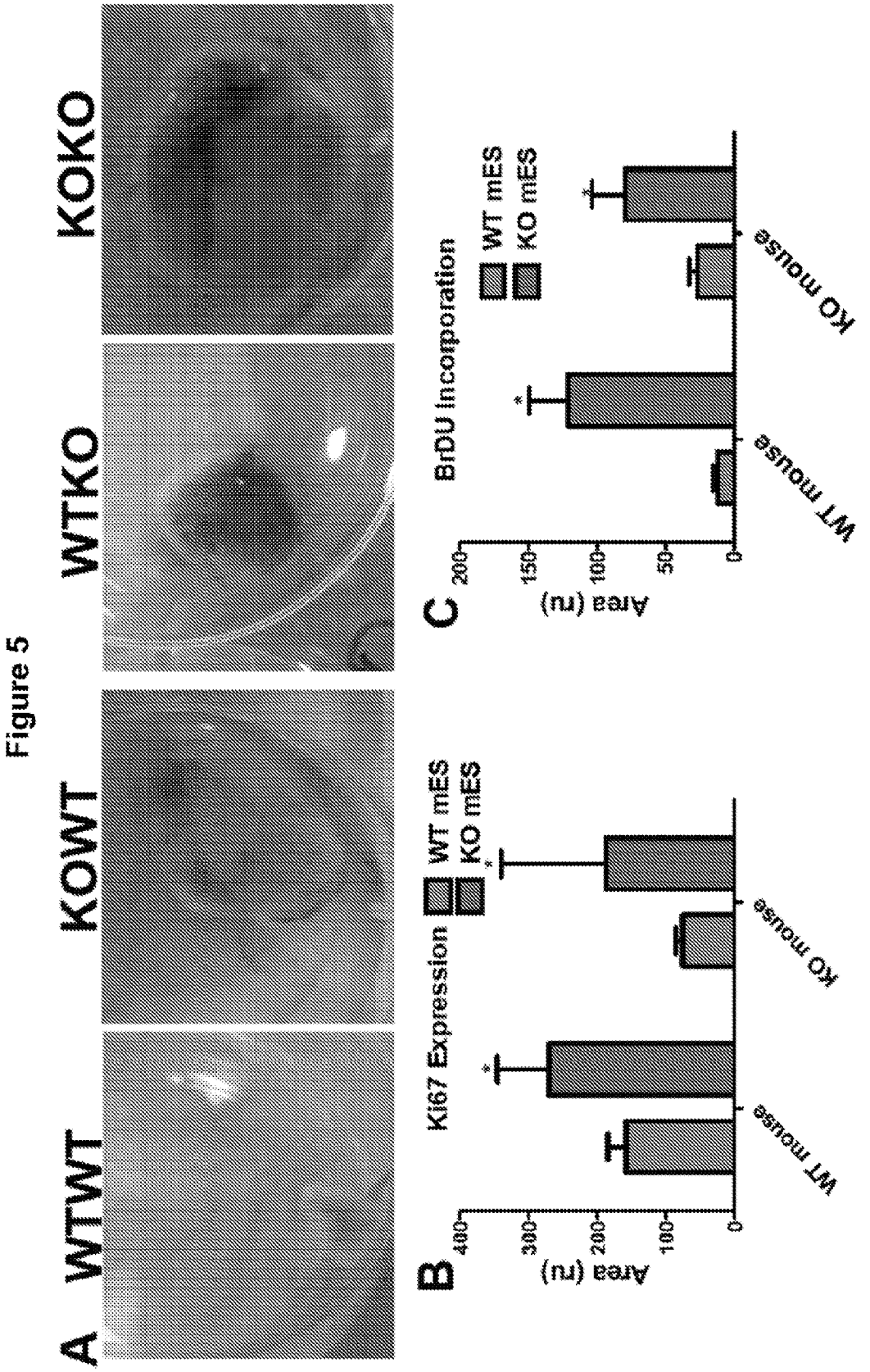
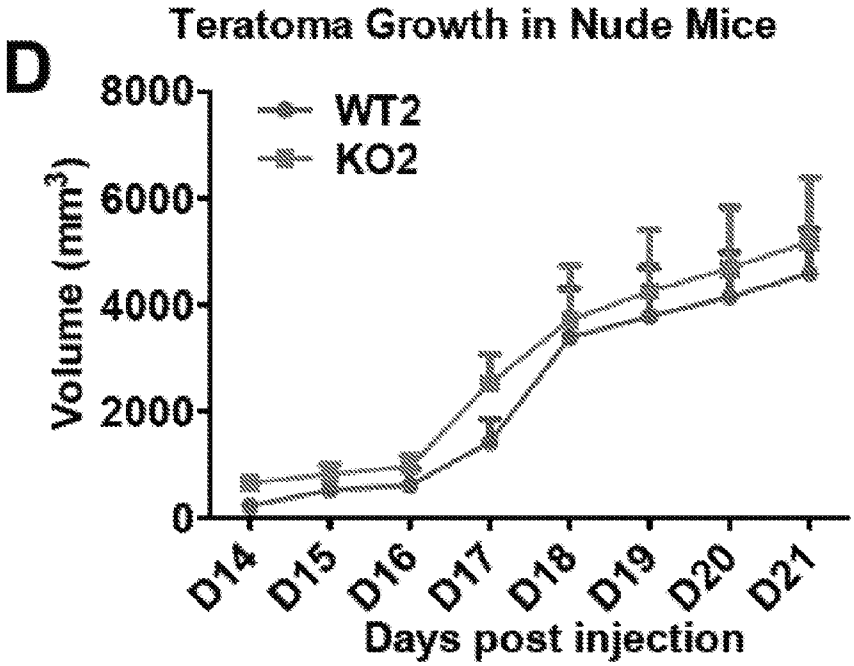

A
WTWT
KOWT
WTKO
KOKO
B
Ki67 Expression
WT mES
KO mES
Area (π)
400
300
200
100
0
WT mouse
KO mouse
C
BrDU Incorporation
WT mES
KO mES
Area (π)
200
150
100
50
0
WT mouse
KO mouse

Figure 5


D
Teratoma Growth in Nude Mice
8000
6000
4000
2000
0
Volume (mm3)
WT2
KO2
D14
D15
D16
D17
D18
D19
D20
D21
Days post injection

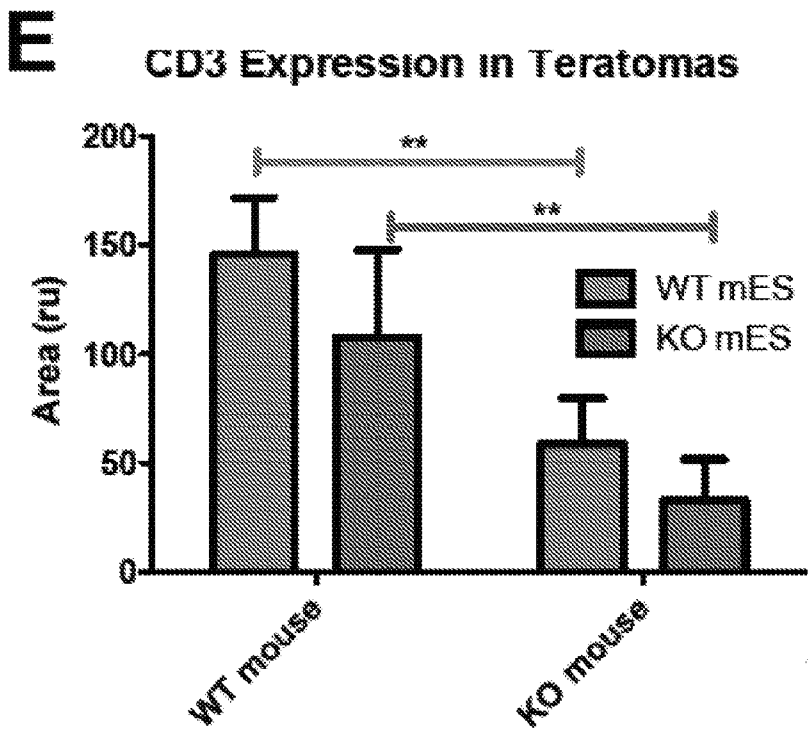

E
CD3 Expression in Teratomas
200
150
100
50
0
Area (ru)
**
**
WT mES
KO mES
WT mouse
KO mouse

Figure 5

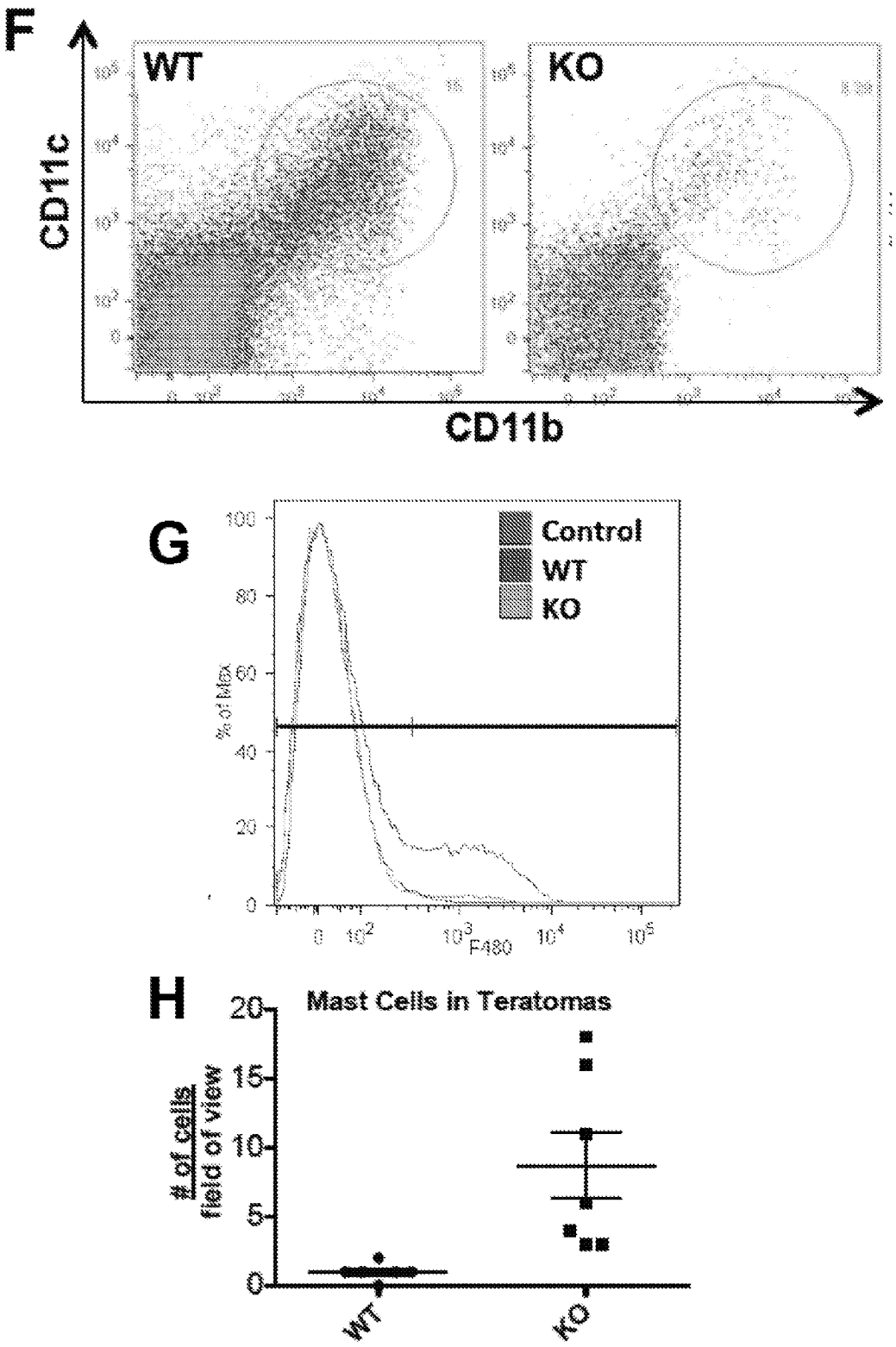

F
WT
KO
CD11c
CD11b
G
Control
WT
KO
% of Max
F480
H
Mast Cells in Teratomas
# of cells
field of view
WT
KO

Figure 6

Murine Skin Graft Model
CD13 WT and KO Male Donor
CD13 WT and KO Female Recipient
Harvest skin from dorsal side of ear and graft onto shaved, exposed back of recipients

Figure 7

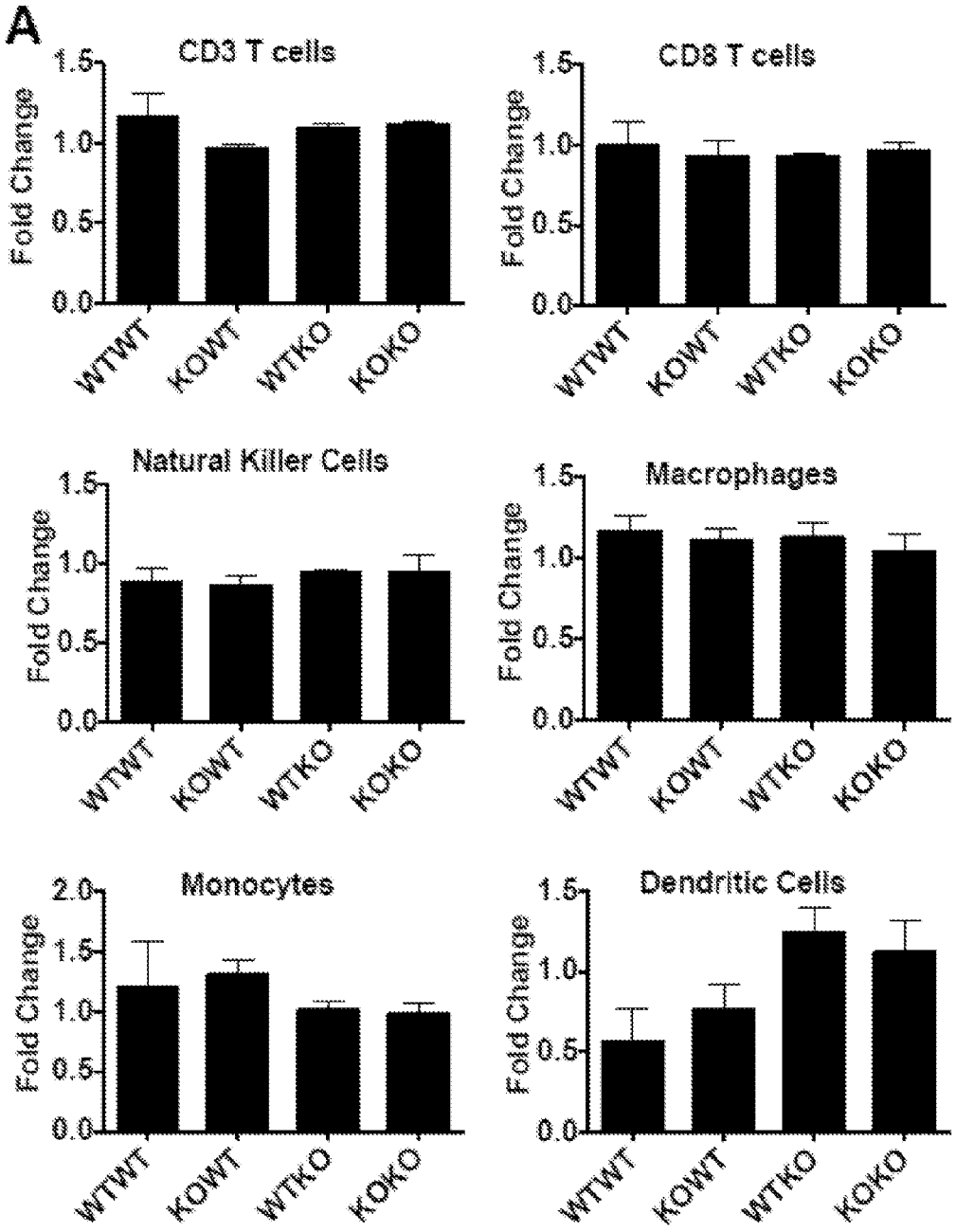

A
CD3 T cells
CD8 T cells
Natural Killer Cells
Macrophages
Monocytes
Dendritic Cells
Fold Change
WTWT
KOWT
WTKO
KOKO

Figure 8

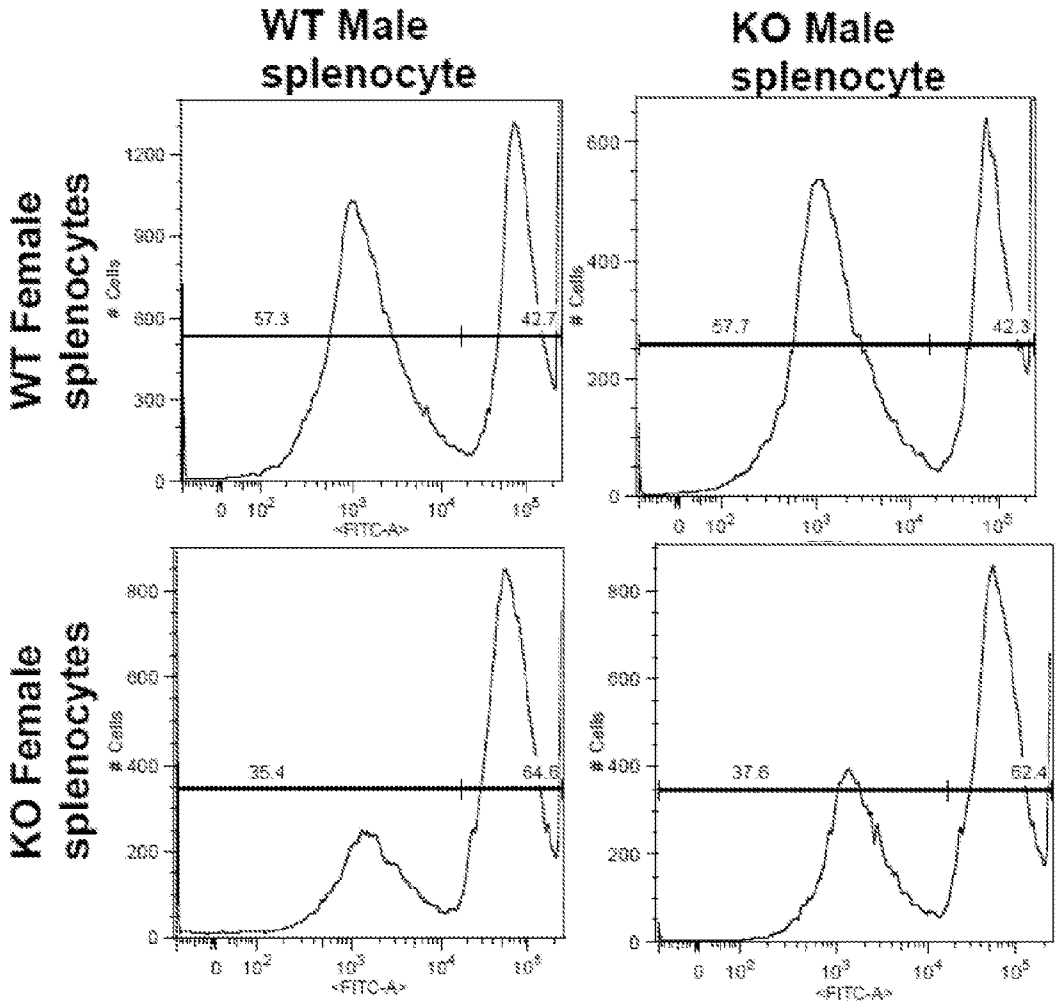

WT Male splenocyte
KO Male splenocyte
WT Female splenocytes
KO Female splenocytes
Cells
<FITC-A>
57.3
42.7
57.7
42.3
35.4
64.6
37.6
62.4

Figure 9

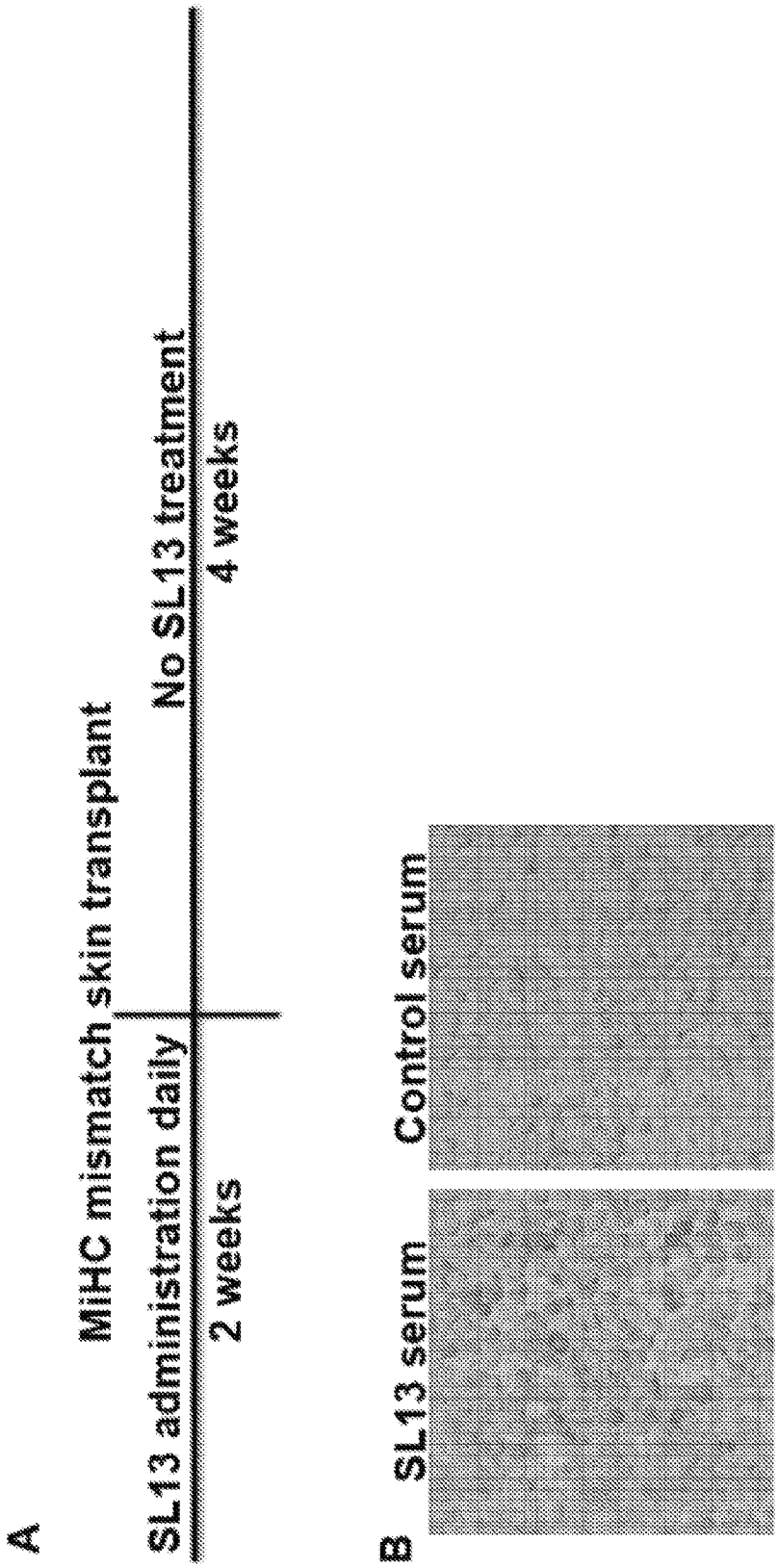

A
MiHC mismatch skin transplant
SL13 administration daily
2 weeks
No SL13 treatment
4 weeks
B
SL13 serum
Control serum

Figure 10

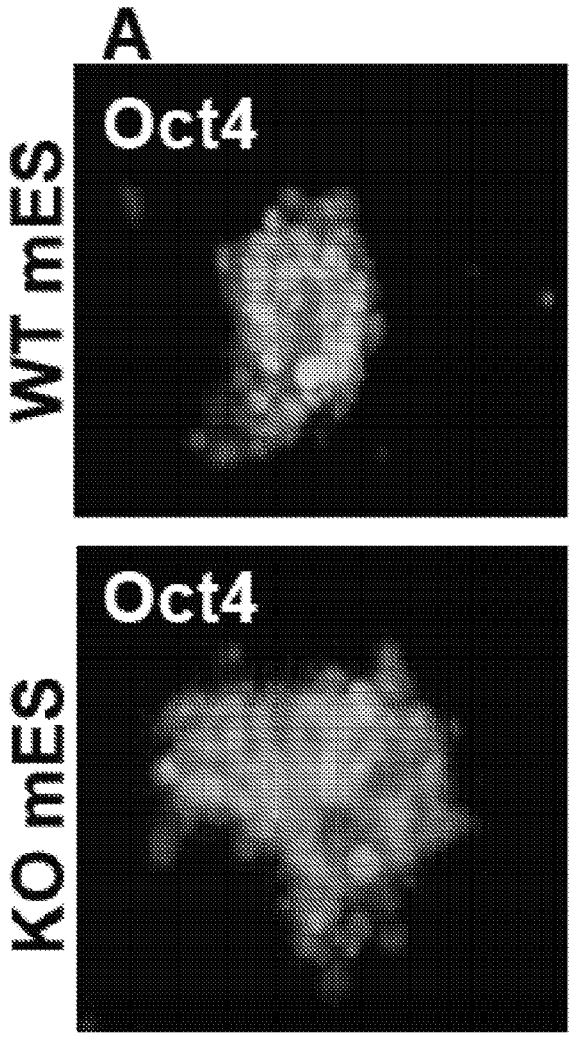
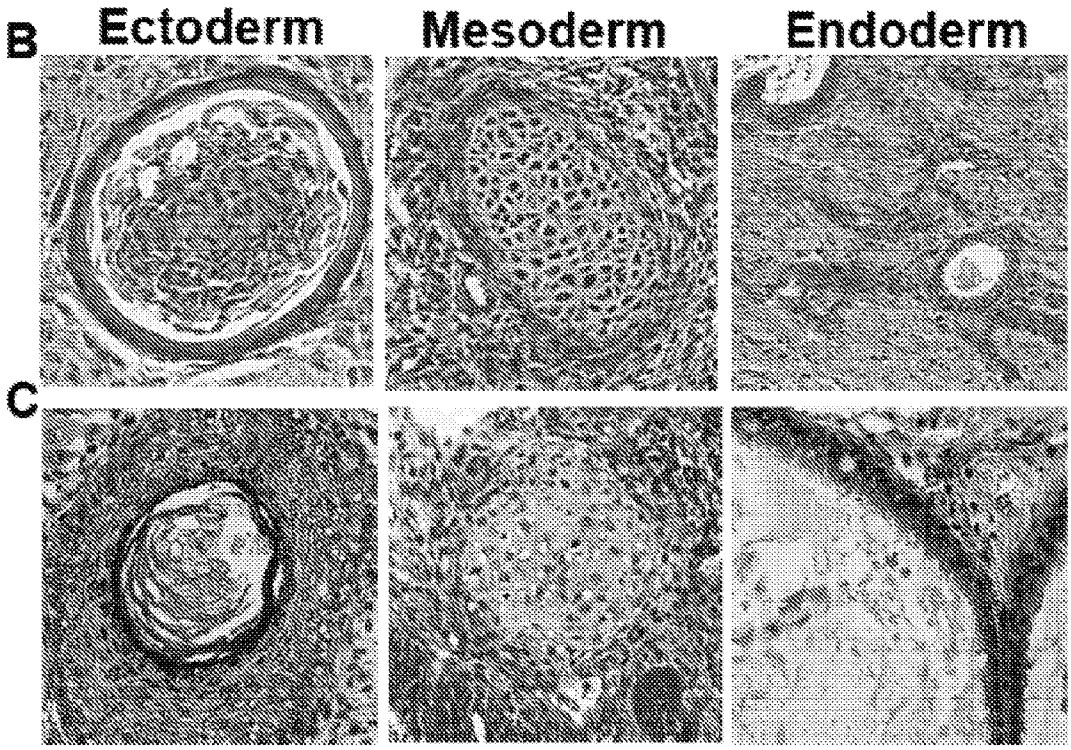


Figure 10



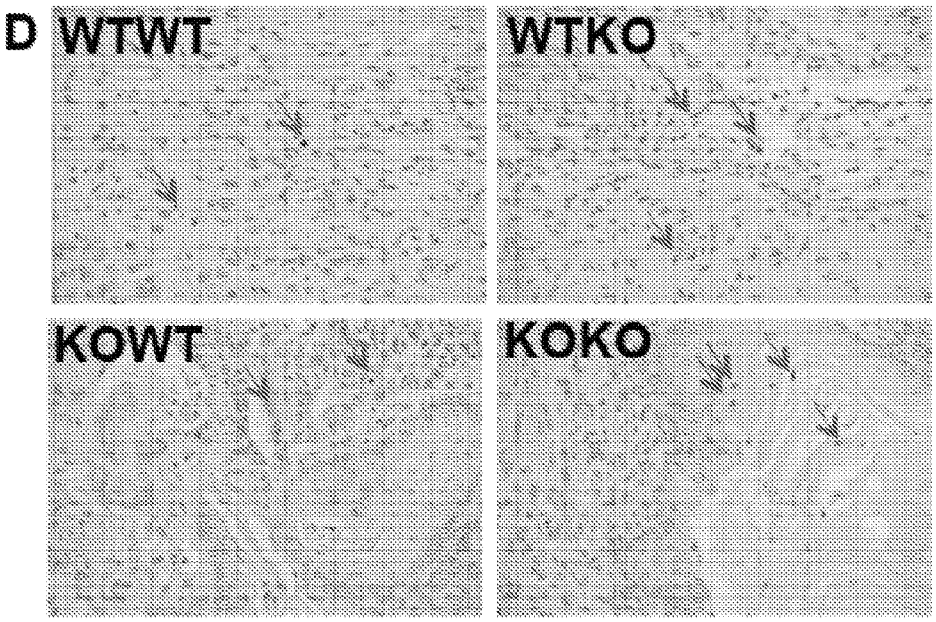
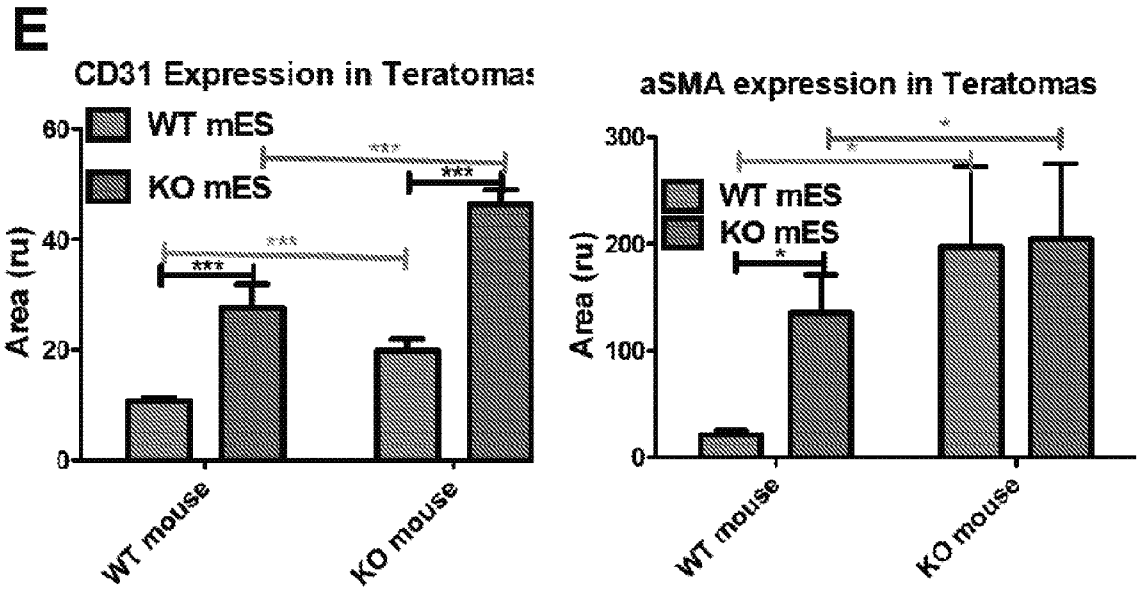


Figure 10


E
CD31 Expression in Teratomas
WT mES
KO mES
Area (ru)
60
40
20
0
***
***
***
***
WT mouse
KO mouse
aSMA expression in Teratomas
WT mES
KO mES
Area (ru)
300
200
100
0
*
*
WT mouse
KO mouse

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/032641

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/0735
ADD.

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)
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)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ELAYNE M CHAN1 ET AL: "Live cell imaging distinguishes bona fide human iPS cells from partially reprogrammed cells", NATURE BIOTECHNOLOGY, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol. 27, no. 11, 1 November 2009 (2009-11-01), pages 1033-1037, XP002621305, ISSN: 1087-0156, DOI: 10.1038/NBT.1580 [retrieved on 2009-10-11] the whole document ----- -/--	1

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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Date of the actual completion of the international search	Date of mailing of the international search report
21 August 2014	29/08/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Armandola, Elena

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	B. WINNICKA ET AL: "CD13 is dispensable for normal hematopoiesis and myeloid cell functions in the mouse", JOURNAL OF LEUKOCYTE BIOLOGY, vol. 88, no. 2, 29 April 2010 (2010-04-29) , pages 347-359, XP055081313, ISSN: 0741-5400, DOI: 10.1189/jlb.0210065 the whole document	4,5
X	WO 01/54707 A2 (MELTEC MULTI EPITOPE LIGAND TE [DE]; SCHUBERT WALTER [DE]) 2 August 2001 (2001-08-02) claim 3	15-17, 21-23
A	BAUVOIS BRIGITTE ET AL: "Aminopeptidase-N/CD13 (EC 3.4.11.2) inhibitors: chemistry, biological evaluations, and therapeutic prospects", MEDICINAL RESEARCH REVIEWS, NEW YORK, NY, US, vol. 26, no. 1, 1 January 2006 (2006-01-01), pages 88-130, XP007906395, ISSN: 0198-6325, DOI: 10.1002/MED.20044	1-25
A	MINA-OSORIO ET AL: "The moonlighting enzyme CD13: old and new functions to target", TRENDS IN MOLECULAR MEDICINE, ELSEVIER CURRENT TRENDS, GB, vol. 14, no. 8, 1 August 2008 (2008-08-01) , pages 361-371, XP023439839, ISSN: 1471-4914, DOI: 10.1016/J.MOLMED.2008.06.003 [retrieved on 2008-07-05] the whole document	1-24
A	INO K ET AL: "MONOCYTE ACTIVATION BY AN ORAL IMMUNOMODULATOR (BESTATIN) IN LYMPHOMA PATIENTS FOLLOWING AUTOLOGOUS BONE MARROW TRANSPLANTATION", CANCER IMMUNOLOGY AND IMMUNOTHERAPY, SPRINGER-VERLAG, BERLIN, DE, vol. 43, no. 4, 1 January 1996 (1996-01-01), pages 206-212, XP001204027, ISSN: 0340-7004, DOI: 10.1007/S002620050323	1-25

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MAGDALENA LIS ET AL: "Modulatory effects of bestatin on T and B lymphocyte subsets and the concentration of cytokines released by Th1/Th2 lymphocytes in cyclophosphamide-treated mice", CENTRAL EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 1, 1 January 2013 (2013-01-01), pages 42-53, XP055135803, ISSN: 1426-3912, DOI: 10.5114/ceji.2013.34357 -----	1-25
T	M. MAMUNUR RAHMAN ET AL: "CD13 promotes mesenchymal stem cell-mediated regeneration of ischemic muscle", FRONTIERS IN PHYSIOLOGY, vol. 4, 1 January 2014 (2014-01-01), XP055135805, DOI: 10.3389/fphys.2013.00402 -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/032641

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		JP 2003520821 A	08-07-2003
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		US 2003130180 A1	10-07-2003
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		WO 0154707 A2	02-08-2001