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(19) **United States**(12) **Patent Application Publication****Jones et al.**(10) **Pub. No.: US 2021/0017247 A1**(43) **Pub. Date: Jan. 21, 2021**(54) **FUSION MOLECULES TARGETING
IMMUNE REGULATORY CELLS AND USES
THEREOF**(71) Applicant: **Torque Therapeutics, Inc.**, Cambridge,
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(US); **Ulrik B. Nielsen**, Cambridge,
MA (US)(21) Appl. No.: **16/628,374**(22) PCT Filed: **Jul. 3, 2018**(86) PCT No.: **PCT/US2018/040786**

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(2) Date: **Jan. 3, 2020****Related U.S. Application Data**(60) Provisional application No. 62/528,411, filed on Jul.
3, 2017, provisional application No. 62/598,433, filed
on Dec. 13, 2017, provisional application No. 62/620,
418, filed on Jan. 22, 2018, provisional application
No. 62/620,107, filed on Jan. 22, 2018, provisional
application No. 62/657,455, filed on Apr. 13, 2018.**Publication Classification**(51) **Int. Cl.****C07K 14/54** (2006.01)**C07K 16/28** (2006.01)**C07K 14/765** (2006.01)**C12N 5/0783** (2006.01)**A61K 39/395** (2006.01)**A61K 38/20** (2006.01)**A61K 35/17** (2006.01)**A61P 37/06** (2006.01)(52) **U.S. Cl.**CPC **C07K 14/5443** (2013.01); **A61K 2039/505**
(2013.01); **C07K 16/2866** (2013.01); **C07K**
16/289 (2013.01); **C07K 14/765** (2013.01);
C12N 5/0637 (2013.01); **A61K 39/39541**
(2013.01); **A61K 38/2086** (2013.01); **A61K**
35/17 (2013.01); **A61P 37/06** (2018.01); **C07K**
2317/31 (2013.01); **C07K 2319/33** (2013.01);
C07K 2319/31 (2013.01); **C07K 2317/55**
(2013.01); **C12N 2501/2315** (2013.01); **C07K**
16/2812 (2013.01)

(57)

ABSTRACTFusion proteins that include an immune (e.g., Treg) targeting
moiety and a cytokine or growth factor molecule, pharma-
ceutical and formulations thereof, and methods of using and
making the same, are disclosed.**Specification includes a Sequence Listing.**

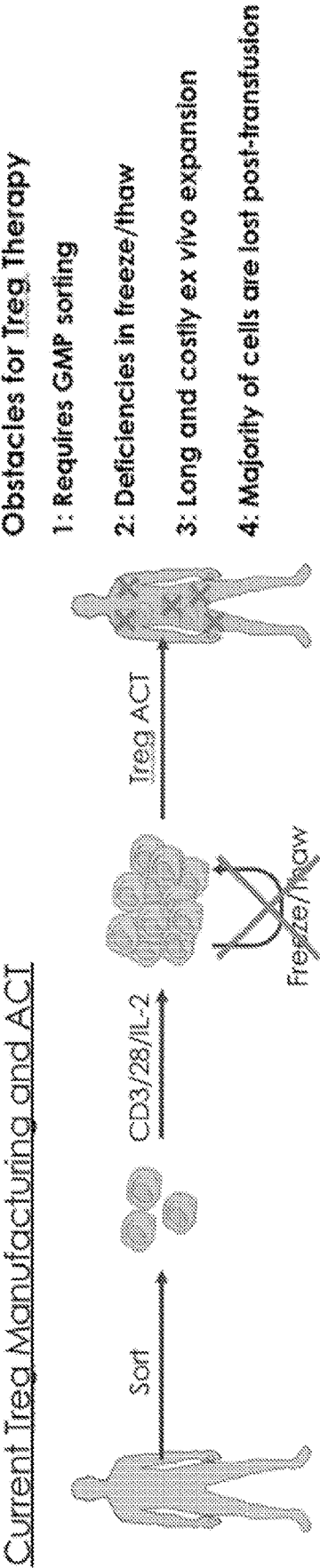


FIG. 1A

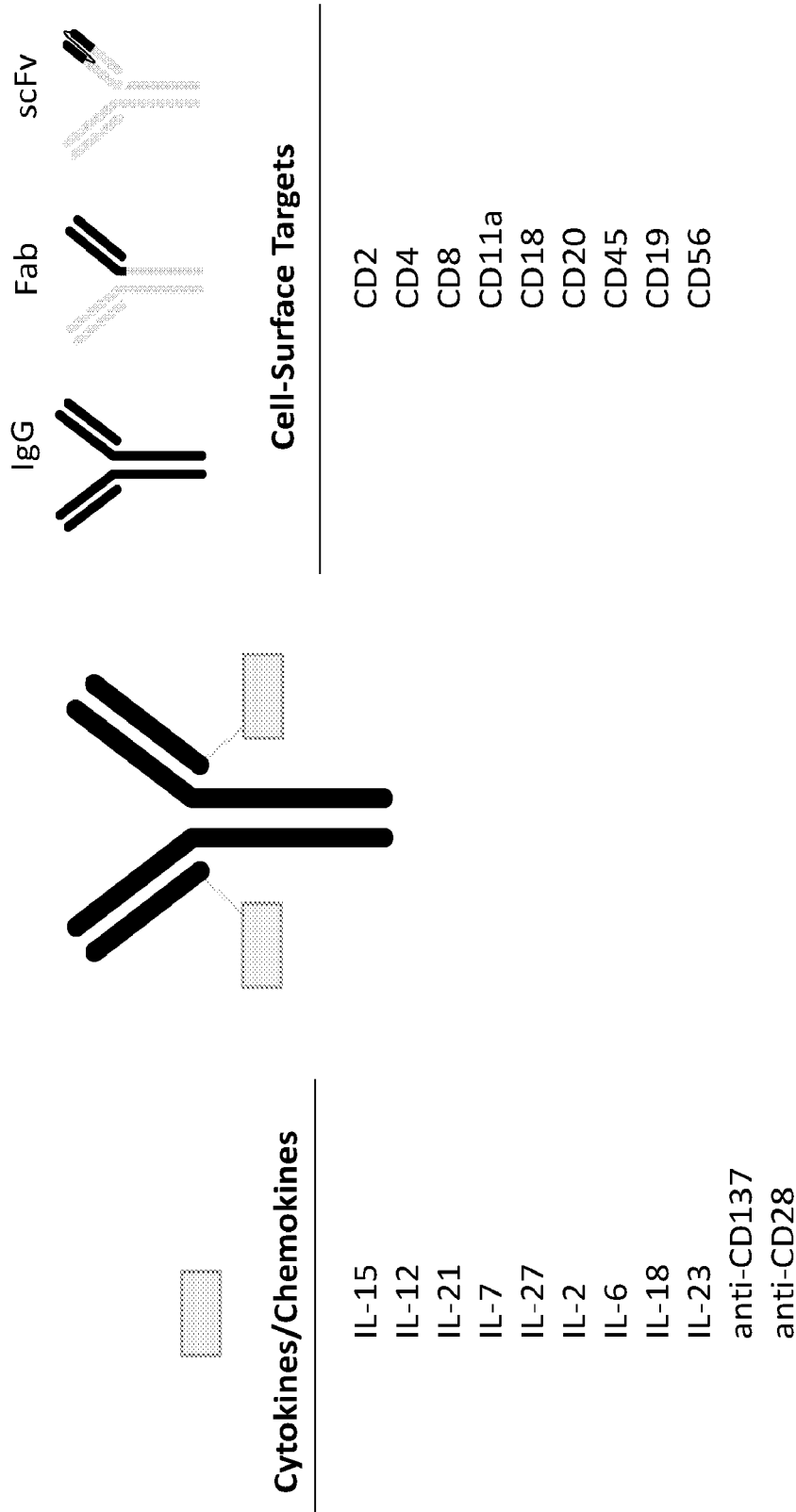


FIG. 1B

Manufacturing: Ex vivo expansion

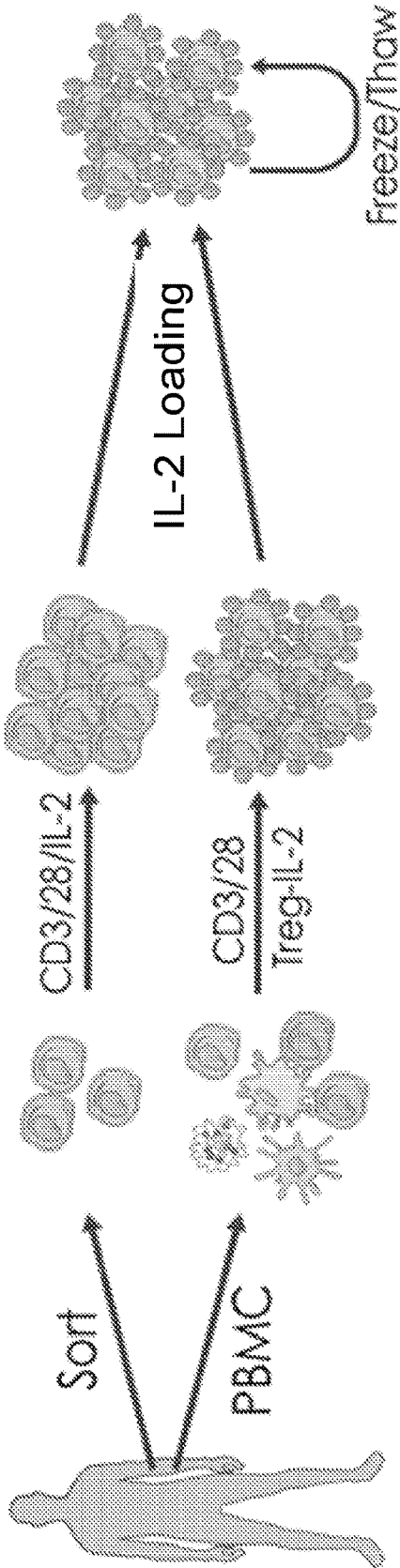


FIG. 1C

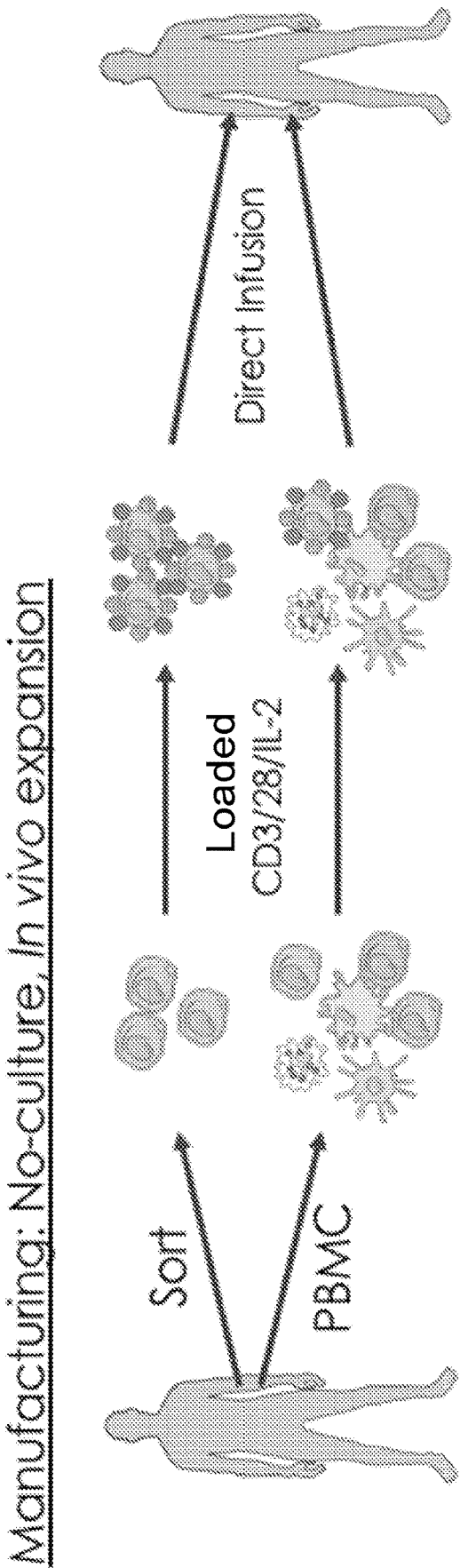


FIG. 1D

IL-2 Loading-Driven, Expansion, Survival and Engraftment

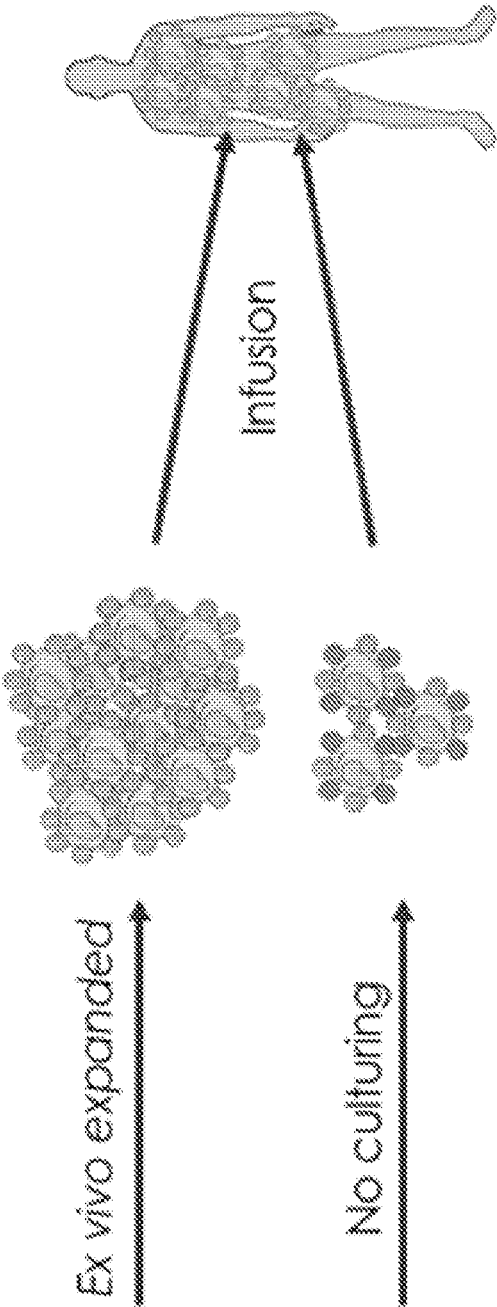


FIG. 1E

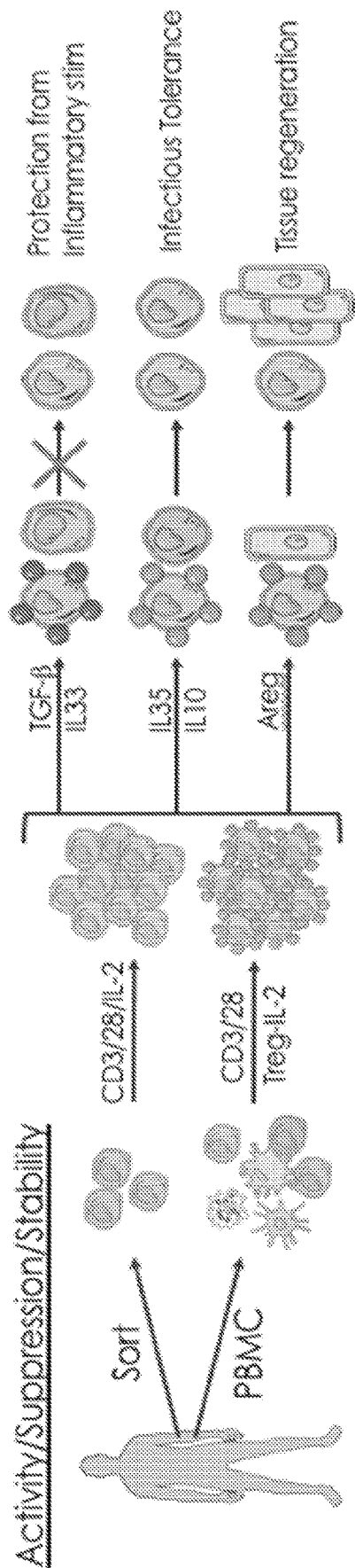


FIG. 1F

Treg-targeting of Tethered Fusions

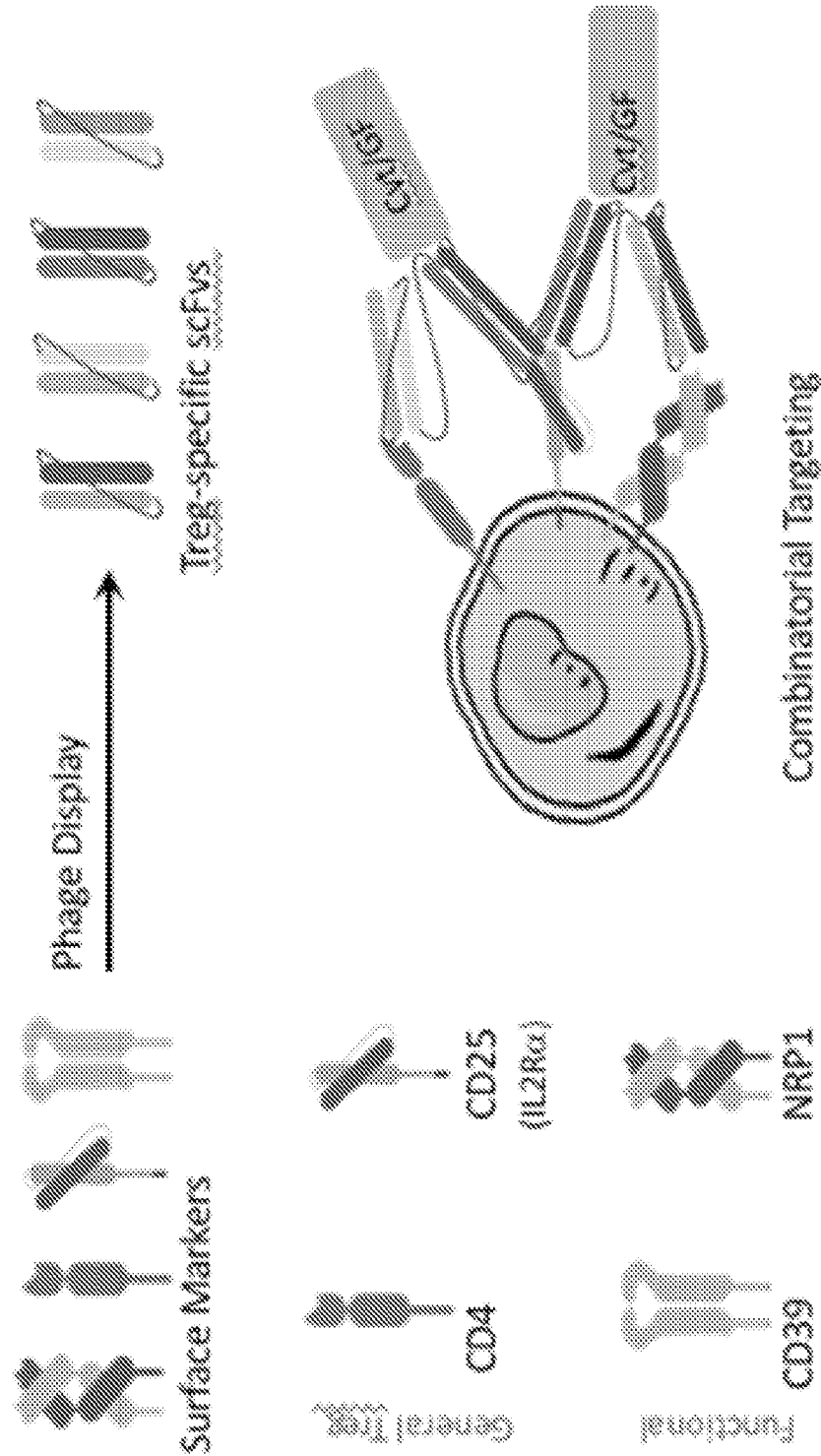


FIG. 1G

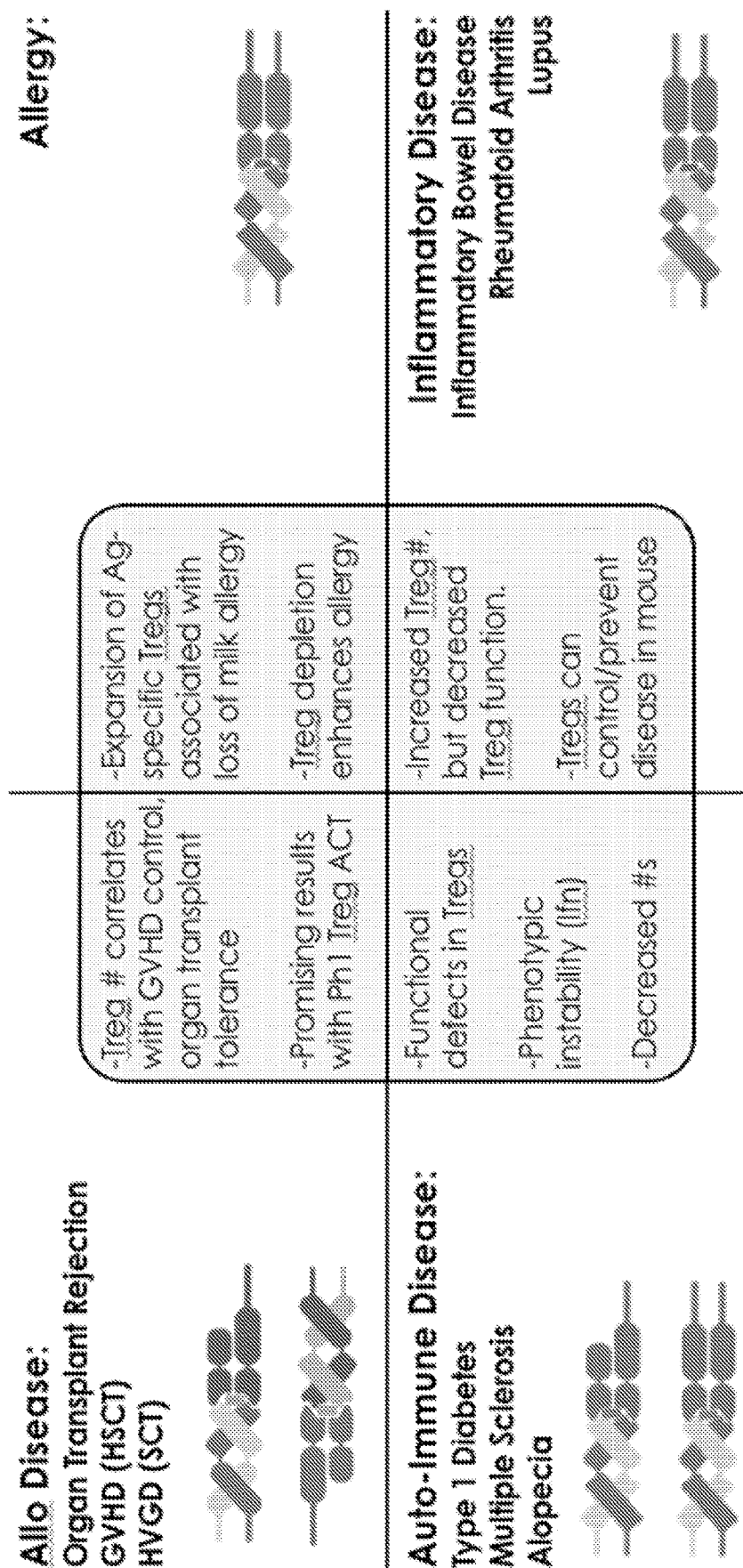


FIG. 1H

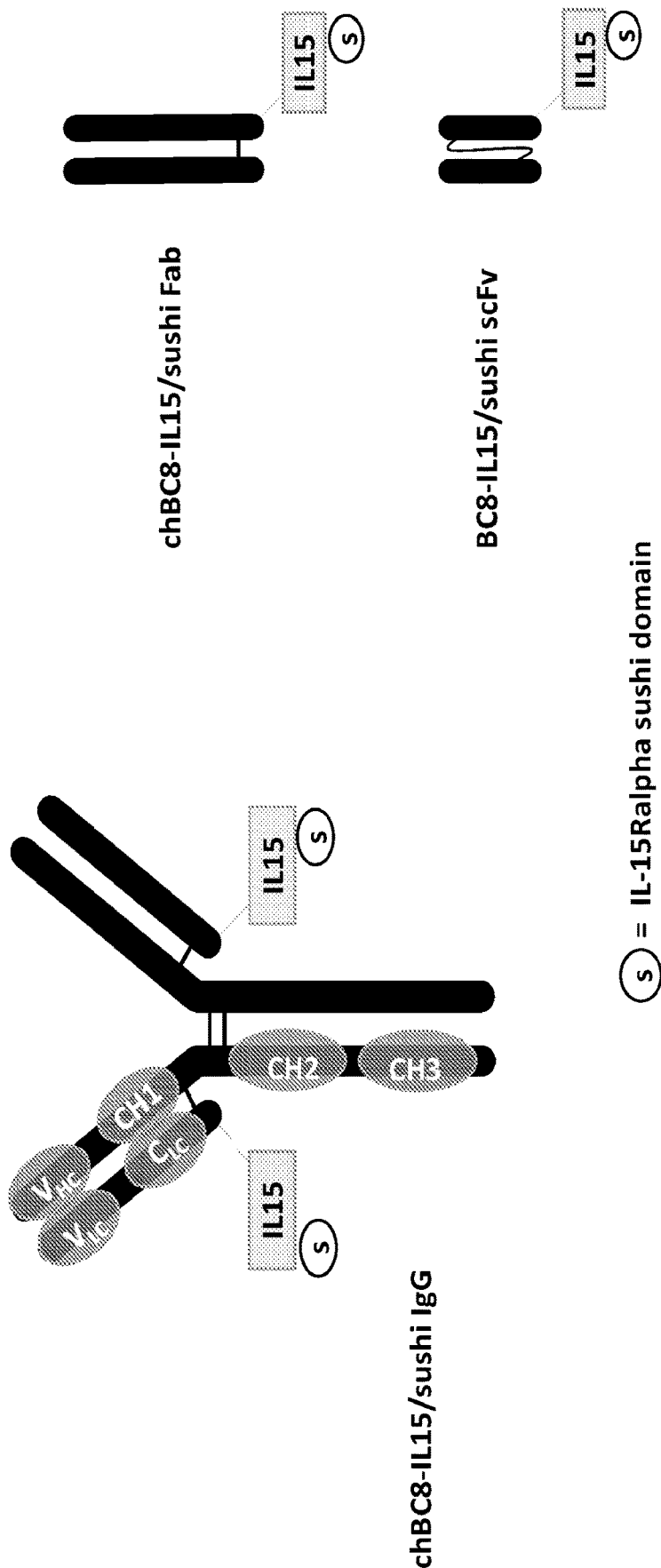


FIG. 2A

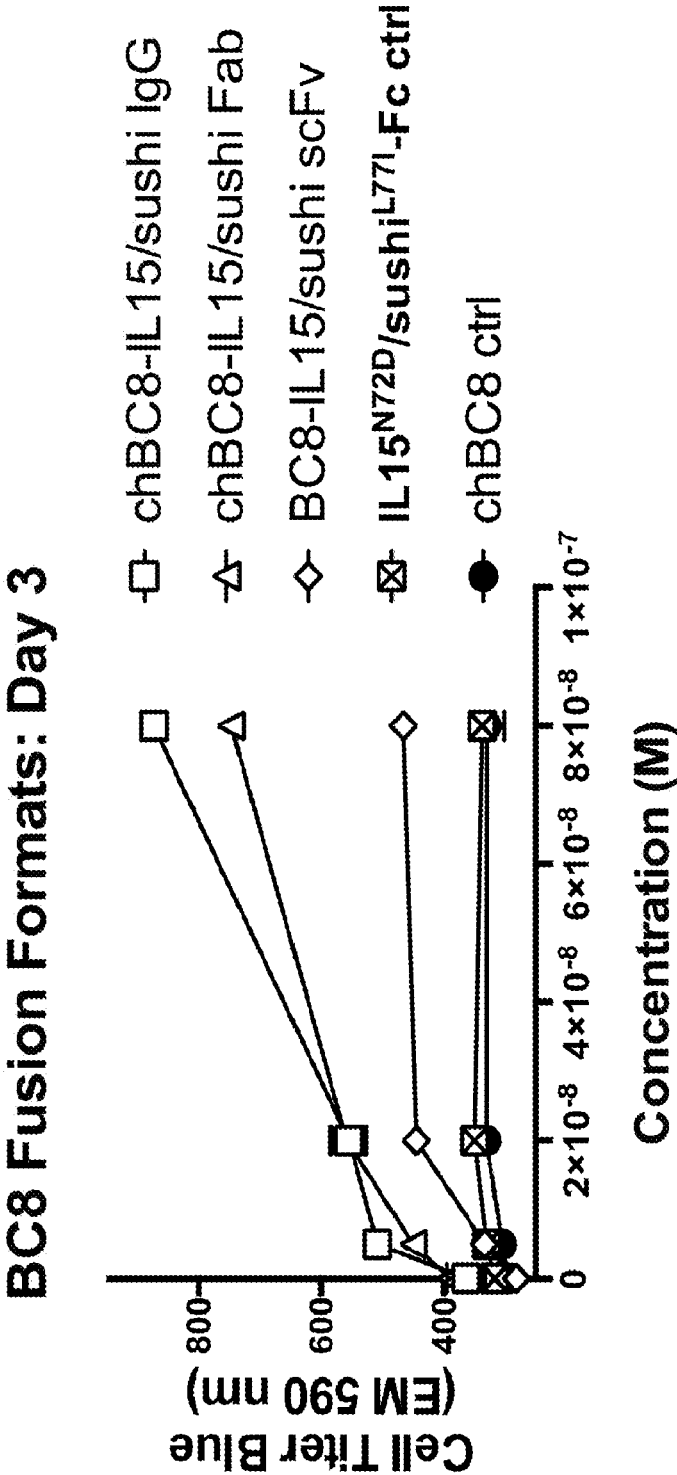


FIG. 2B

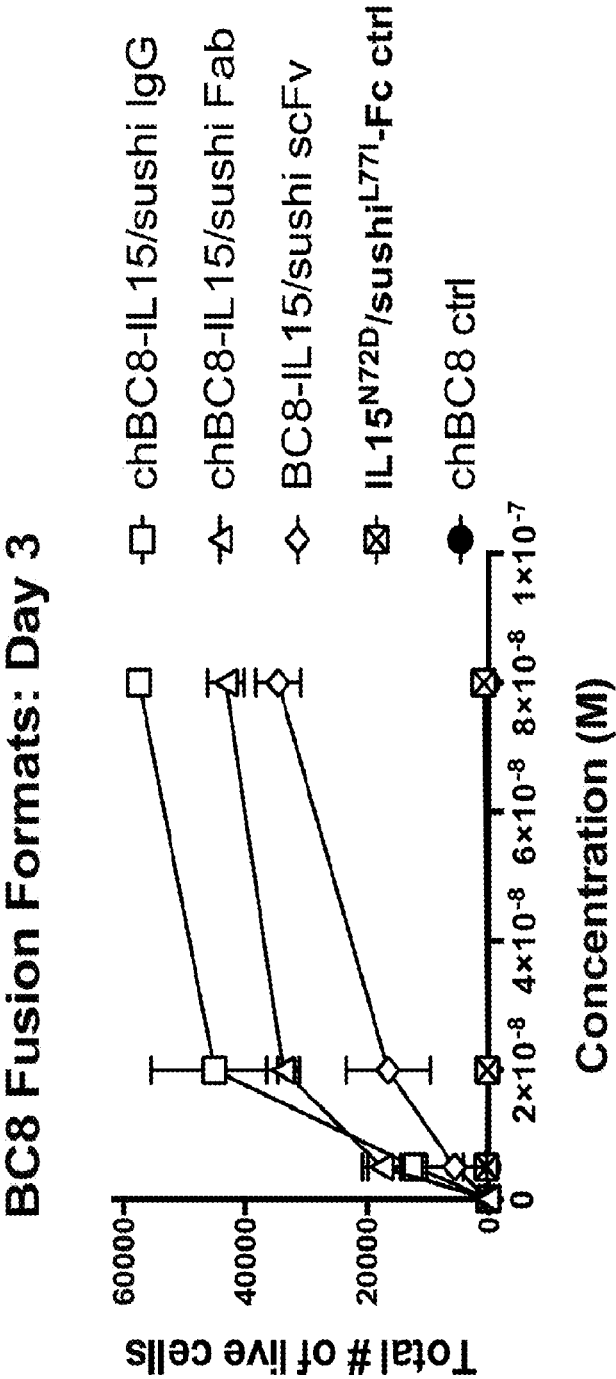


FIG. 2C

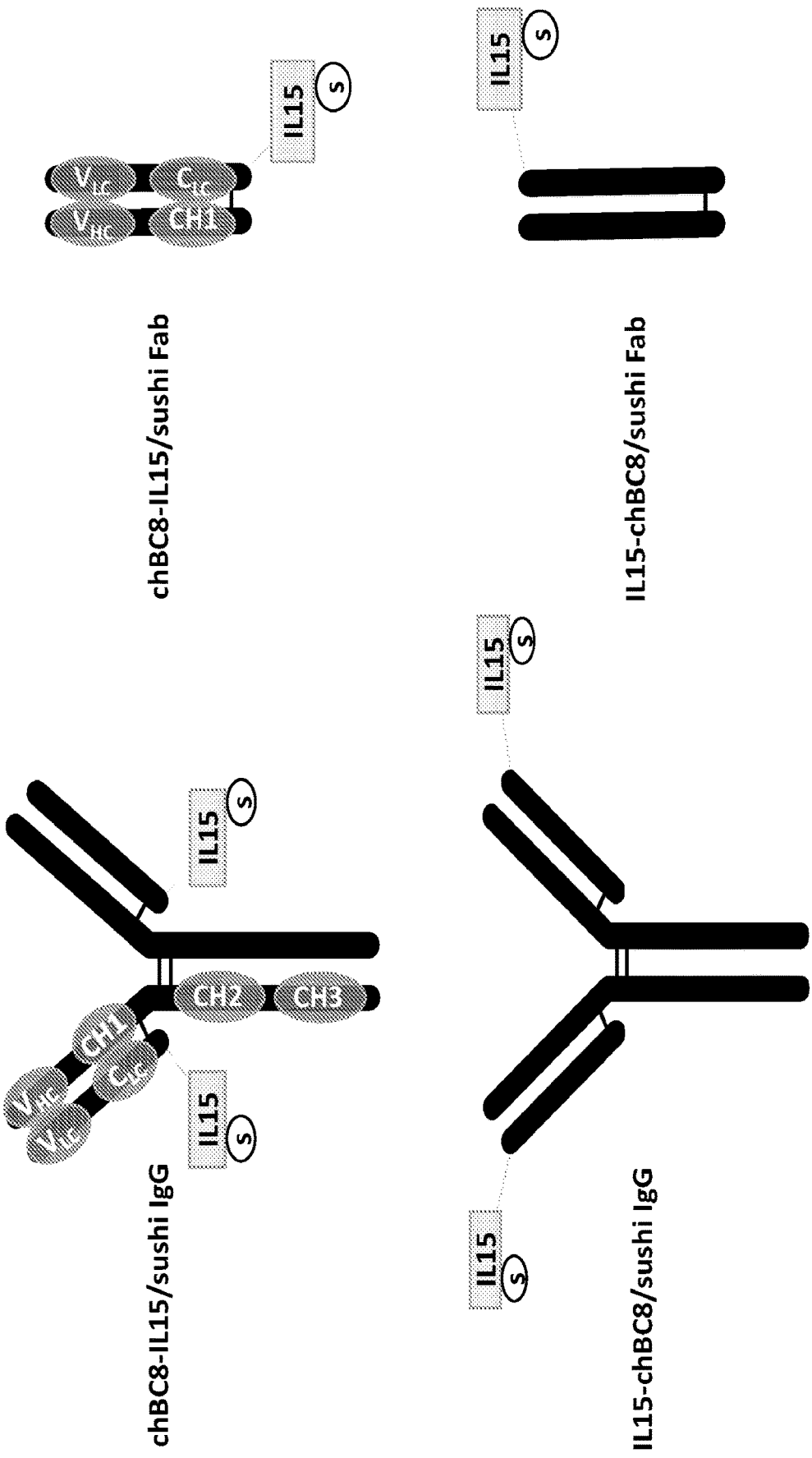


FIG. 3B

FIG. 3A

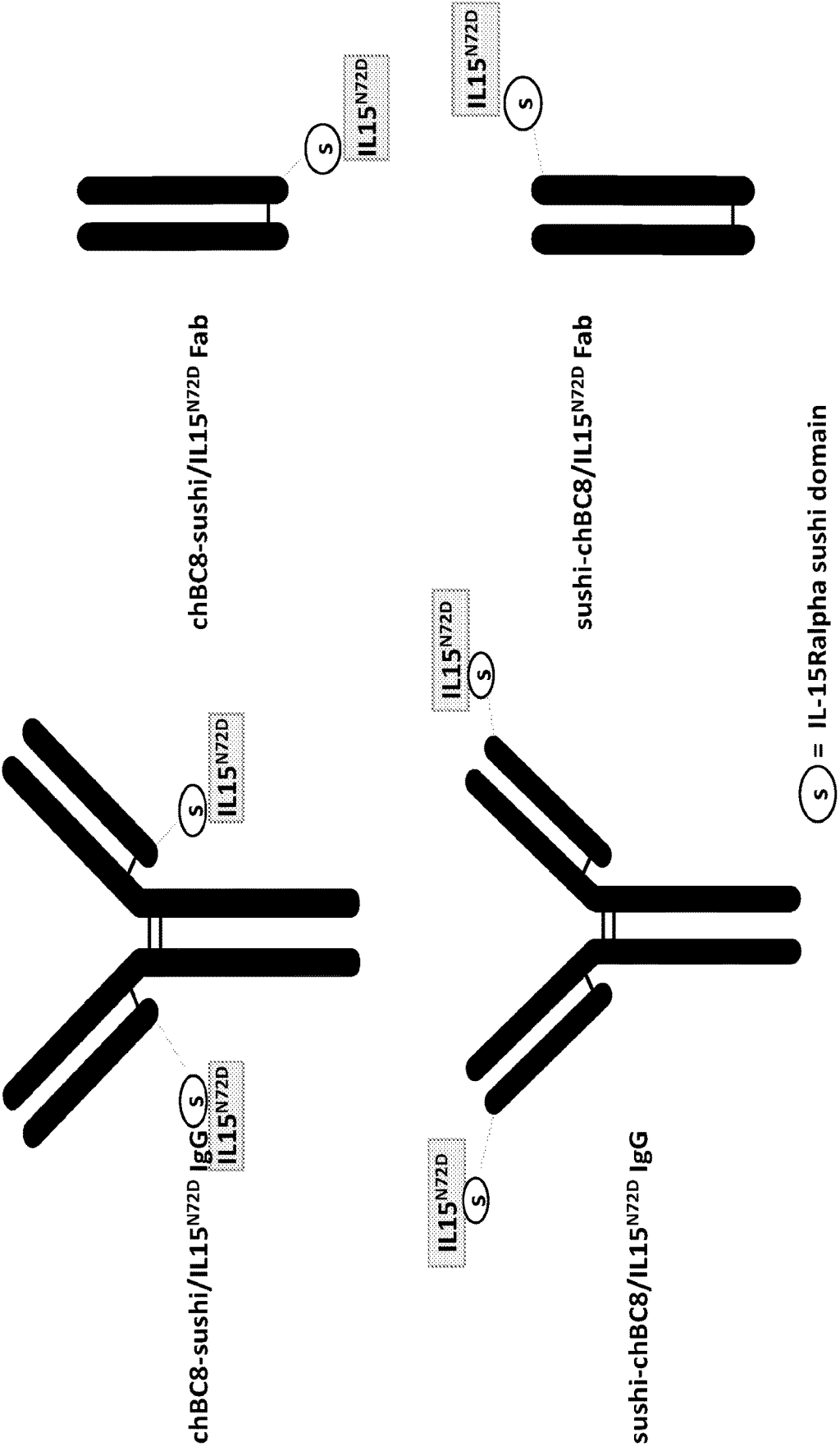


FIG. 3C

FIG. 3D

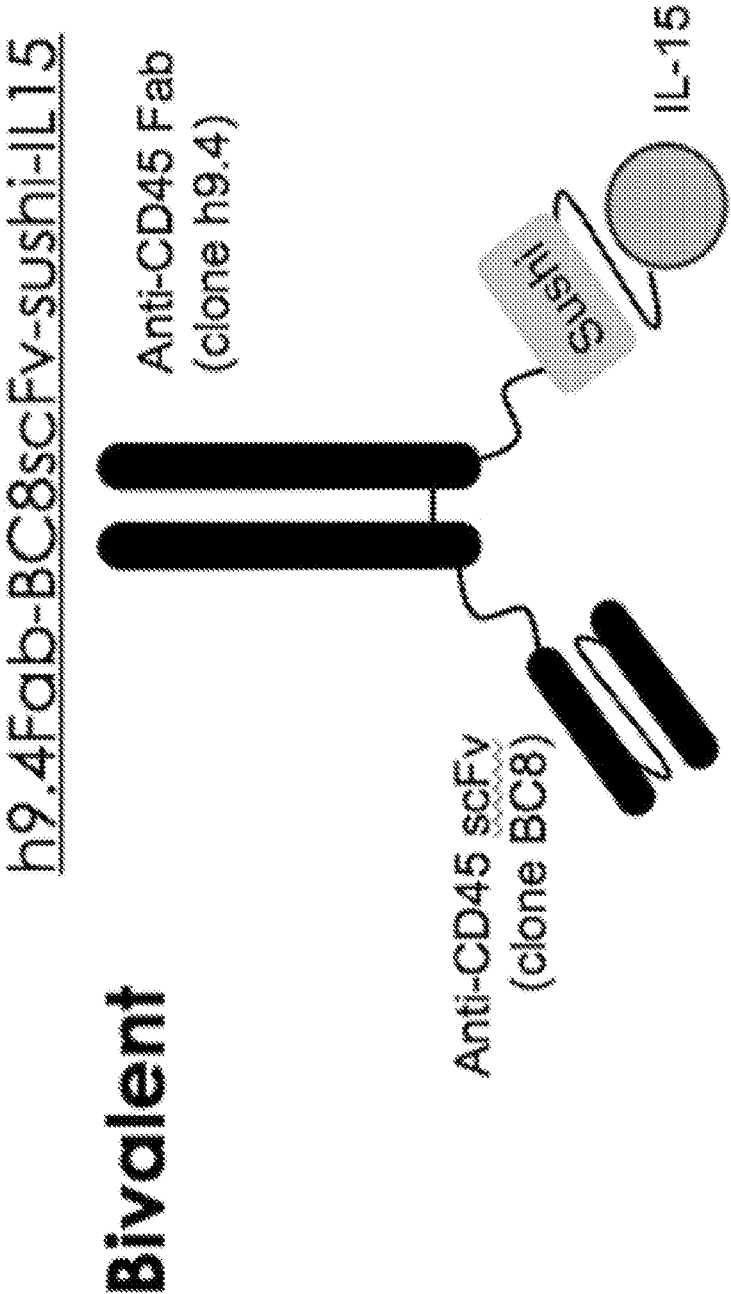


FIG. 3E

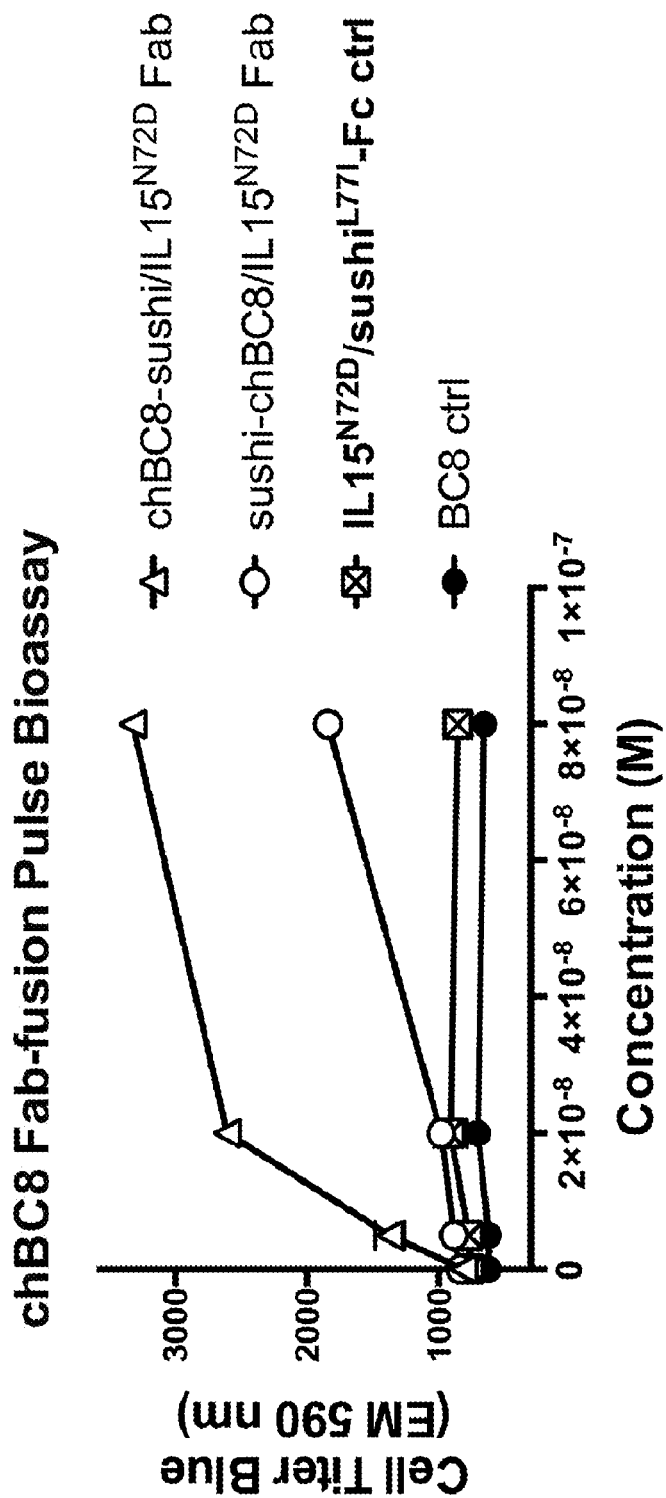


FIG. 4A

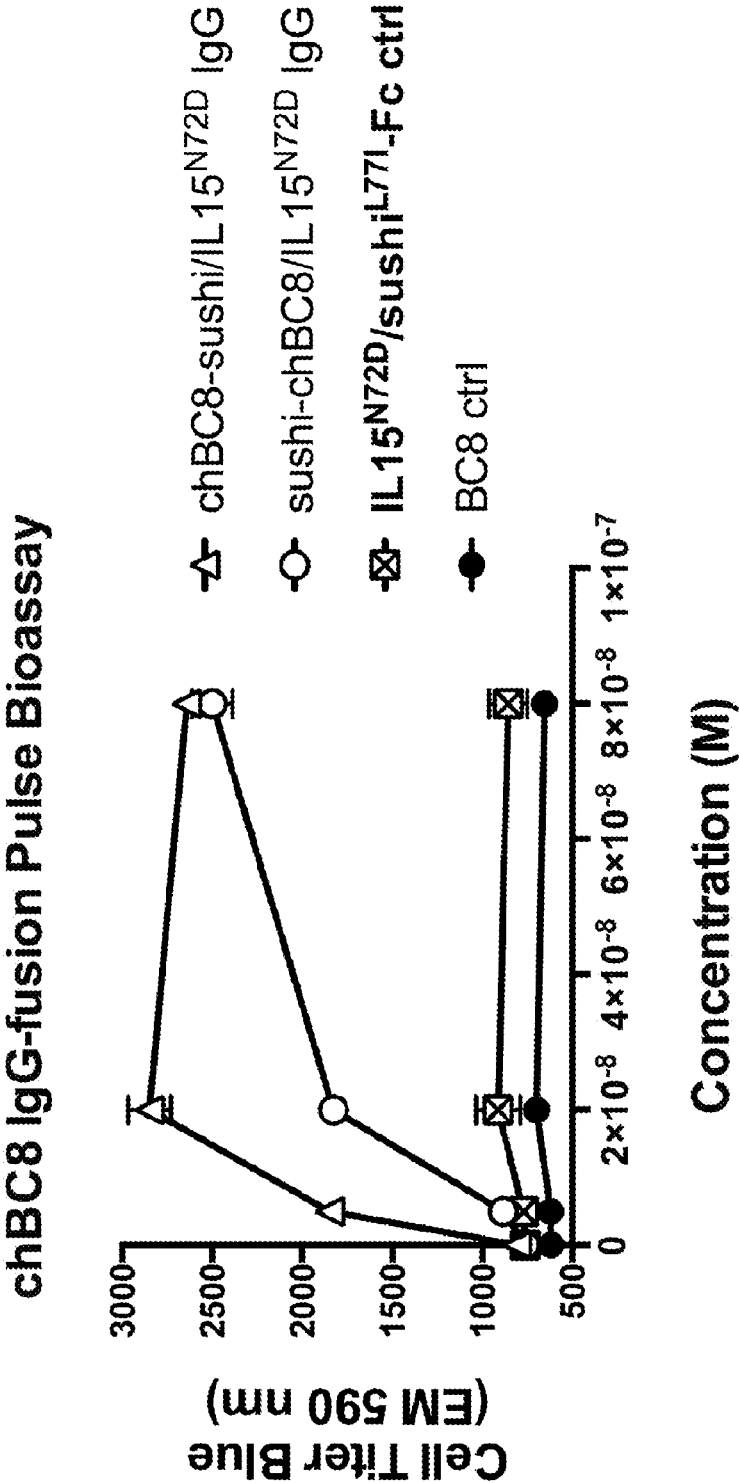


FIG. 4B

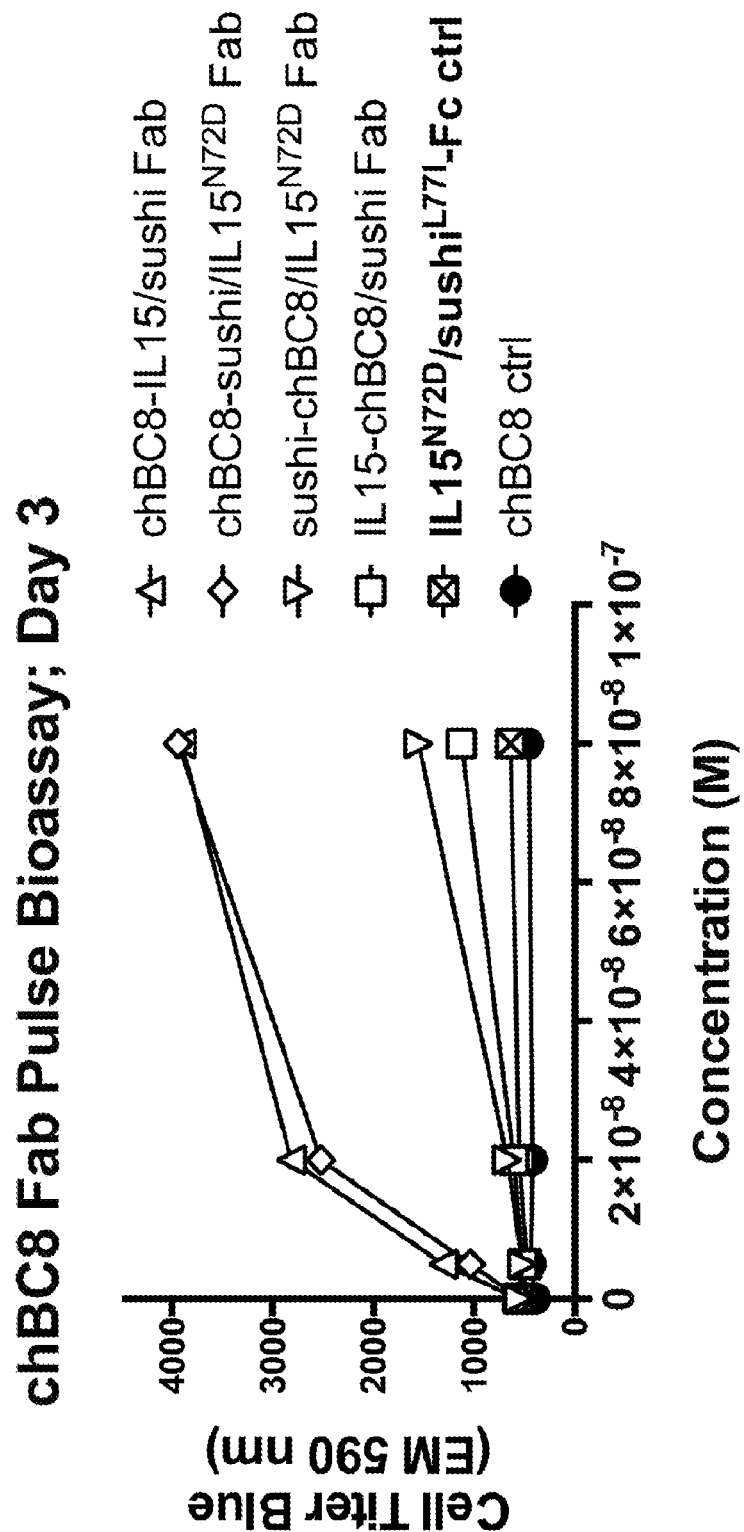


FIG. 5A

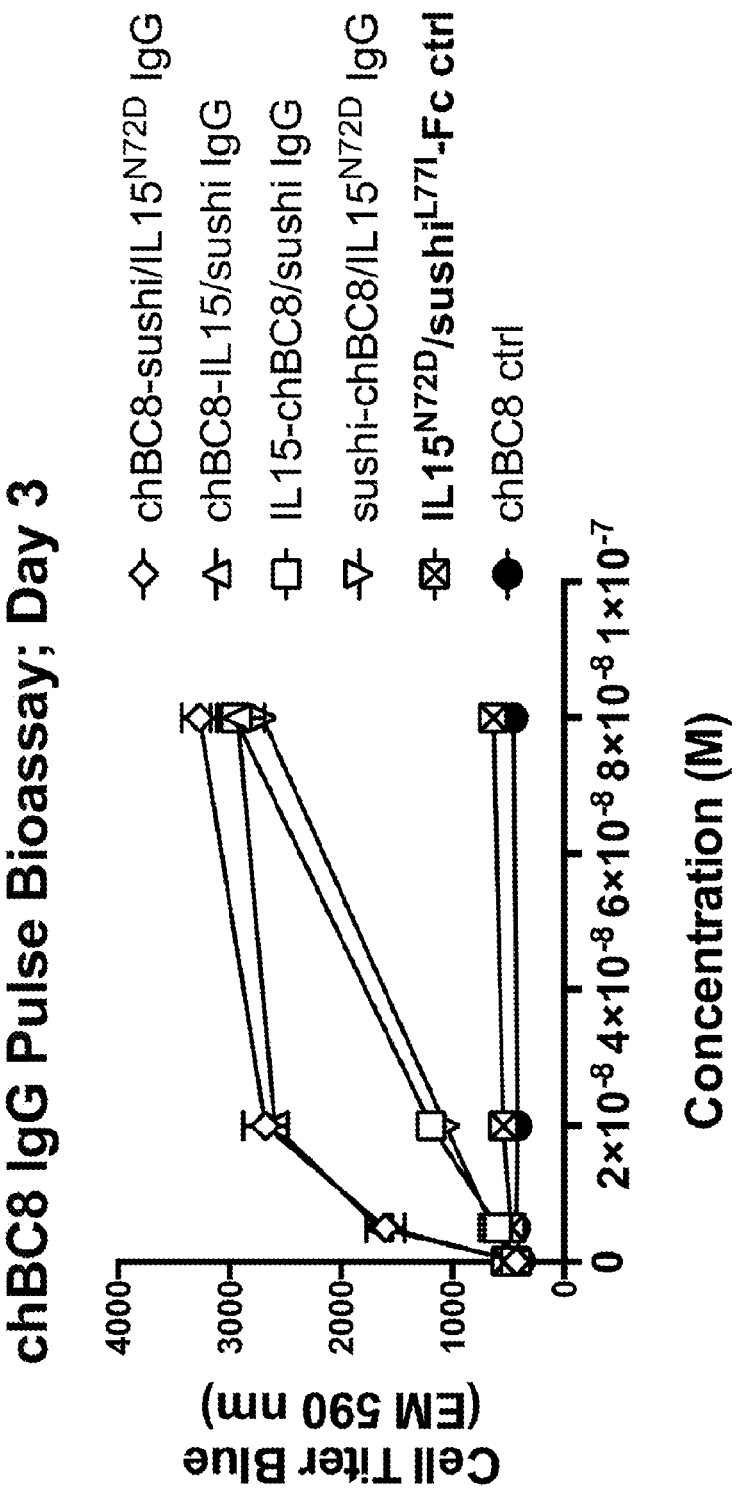


FIG. 5B

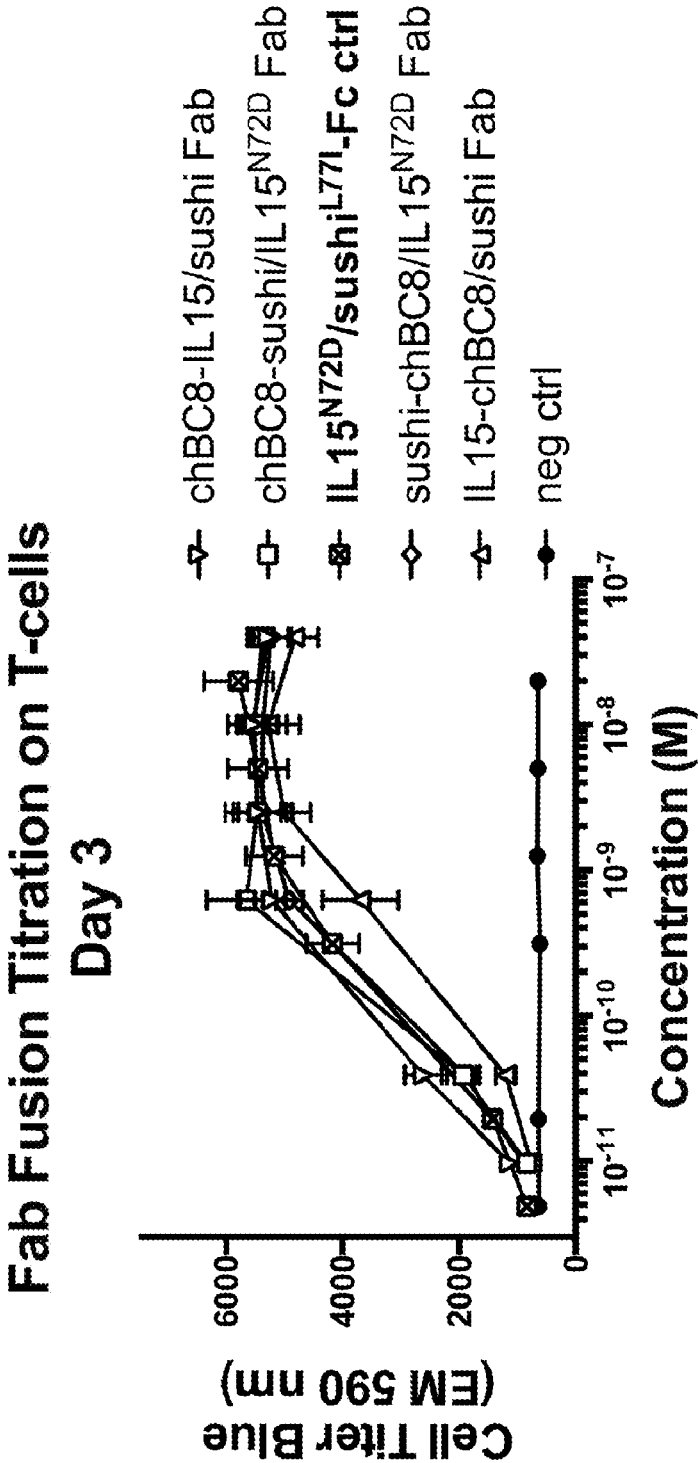


FIG. 5C

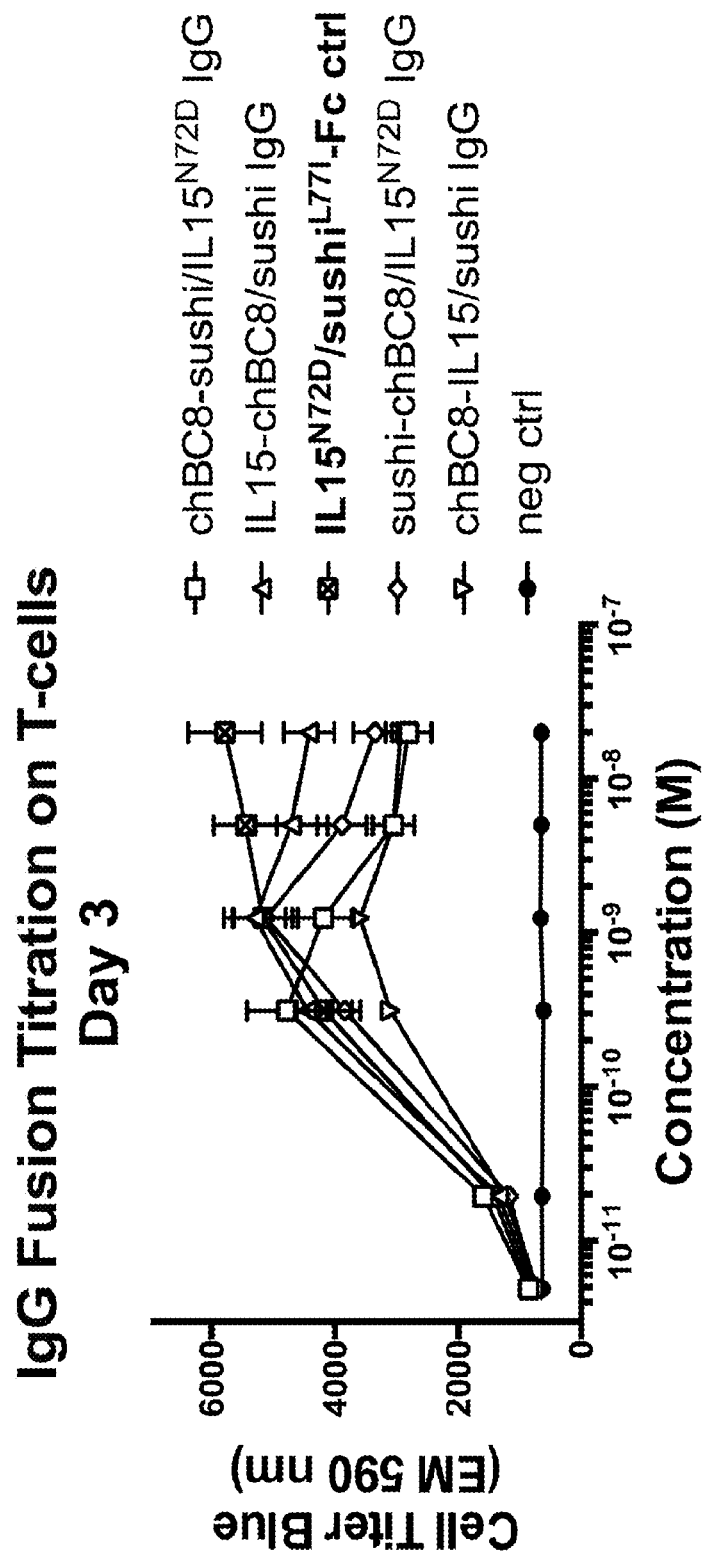


FIG. 5D

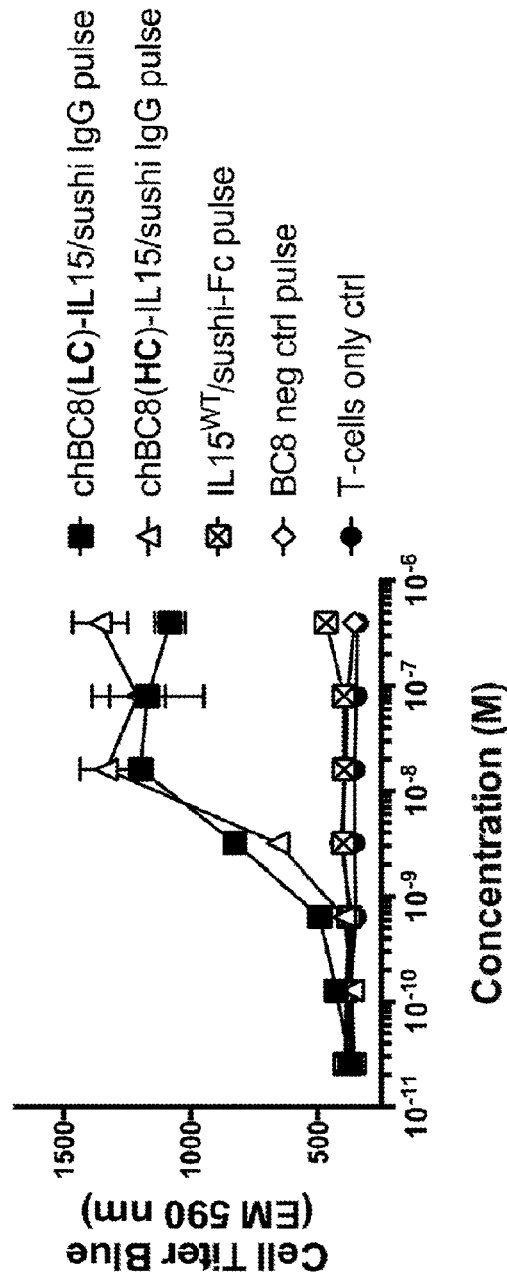
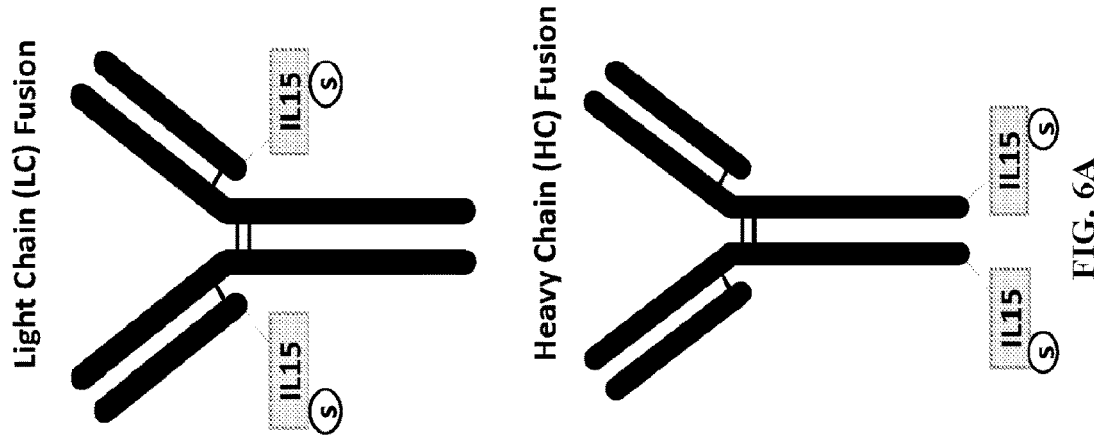


FIG. 6B

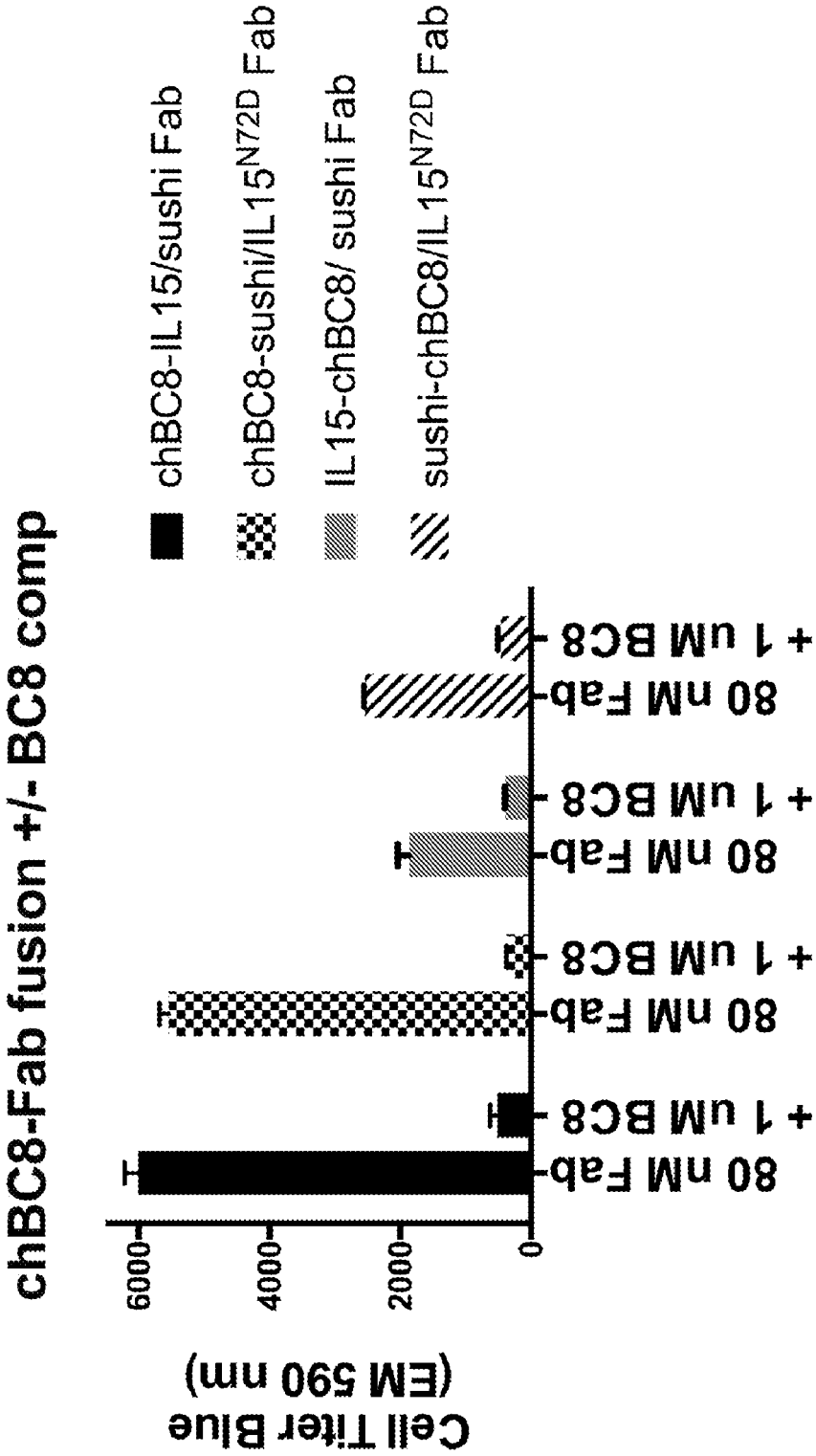


FIG. 7A

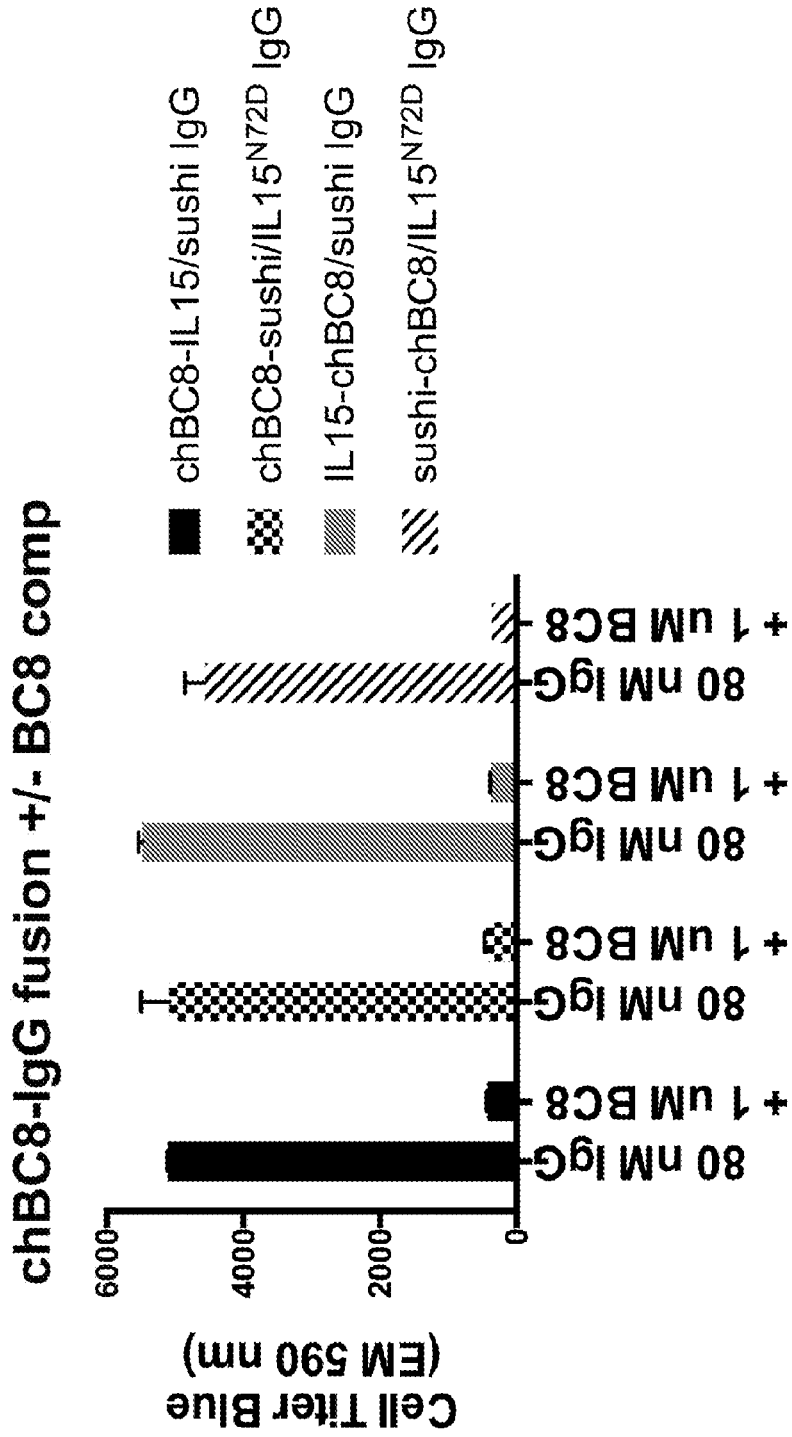


FIG. 7B

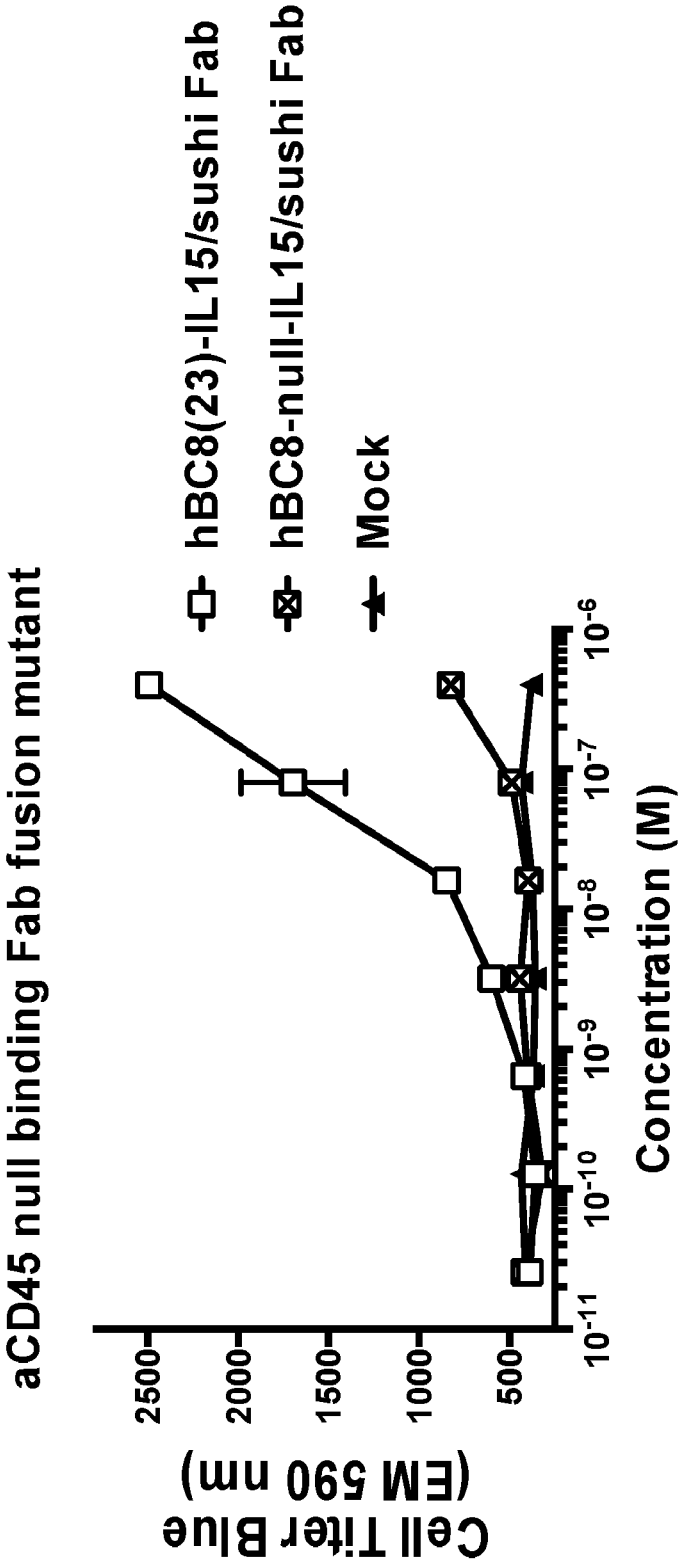


FIG. 7C

Initial cell surface levels

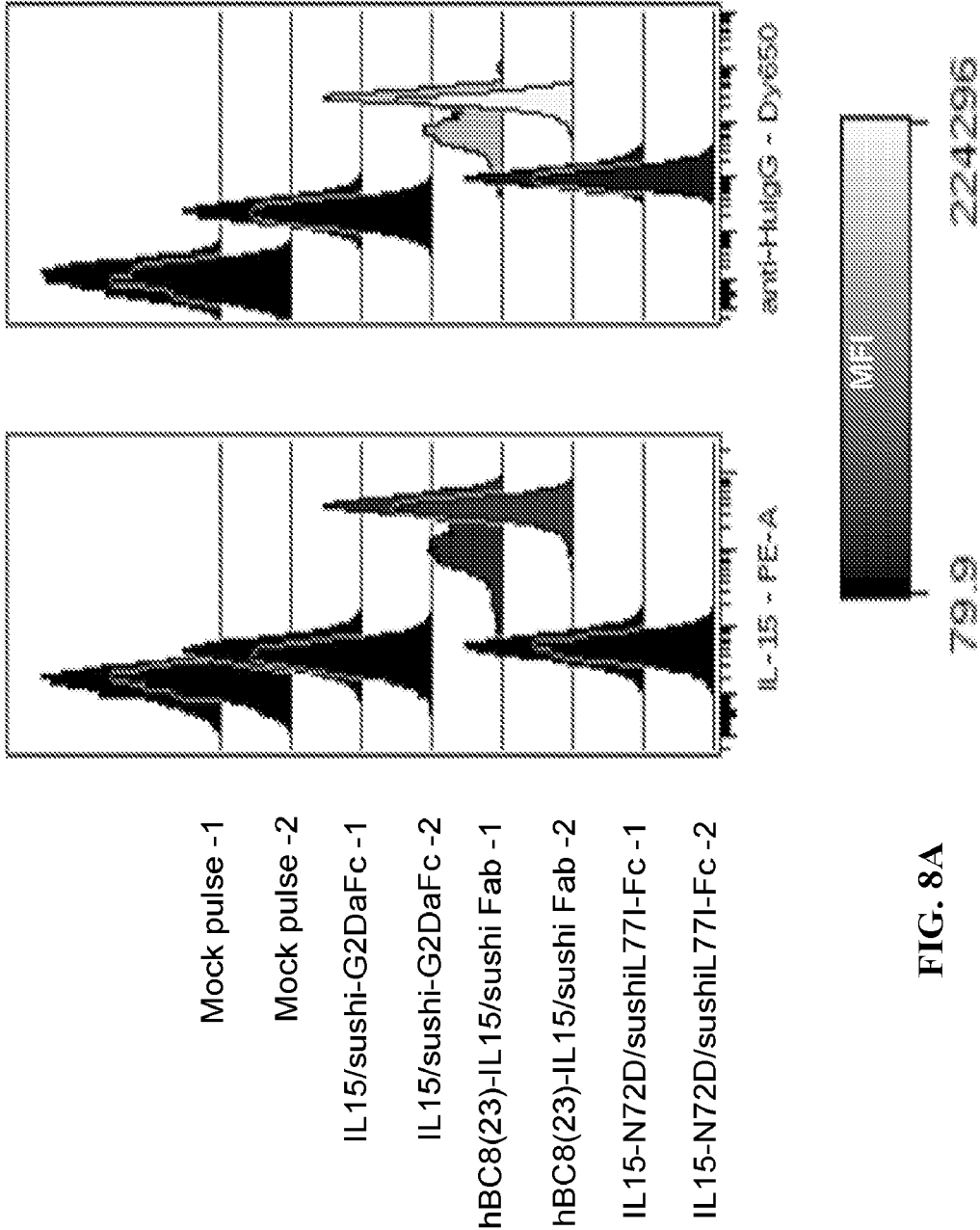


FIG. 8A

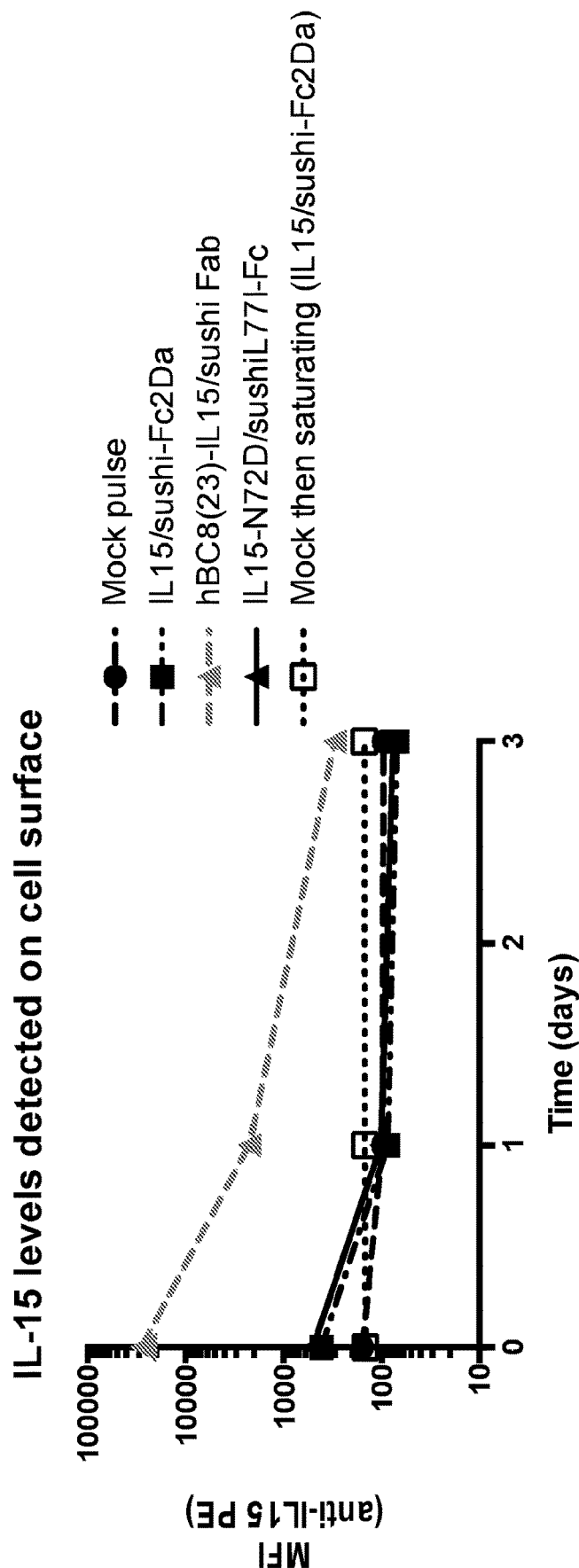


FIG. 8B

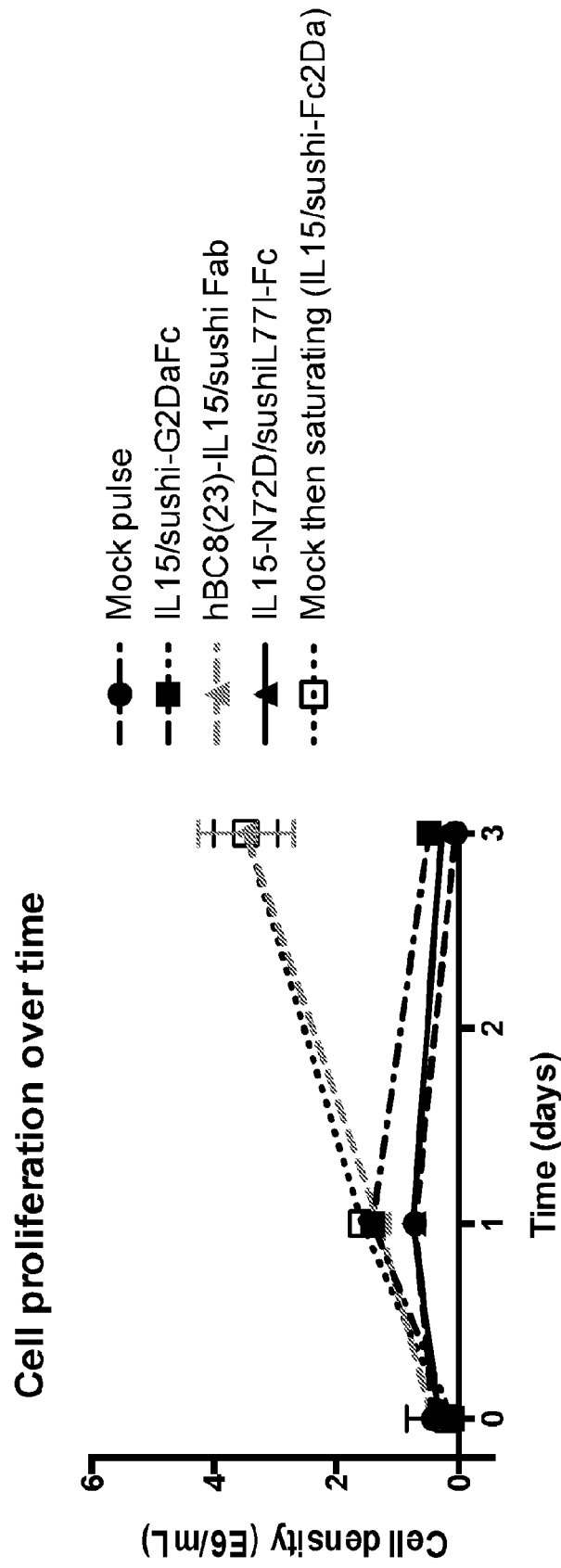


FIG. 8C

Construct	Linker Design amino acid sequence	Linker Length # of amino acids
chBC8-L1-IL15/sushi Fab	(GlyGlyGlySer) x3 GGGGSGGGGGGG GGG	15 GGGS (SEQ ID NO: 125)
chBC8-L2-IL15/sushi Fab	(GlyGlyGlySer) x2 GGGGSGGGG	8 GGGS (SEQ ID NO: 124)
chBC8-L3-IL15/sushi Fab	Amino acids from extended IgG1 hinge: DKTHTSPSPAP	12 (SEQ ID NO: 38)

FIG. 9A

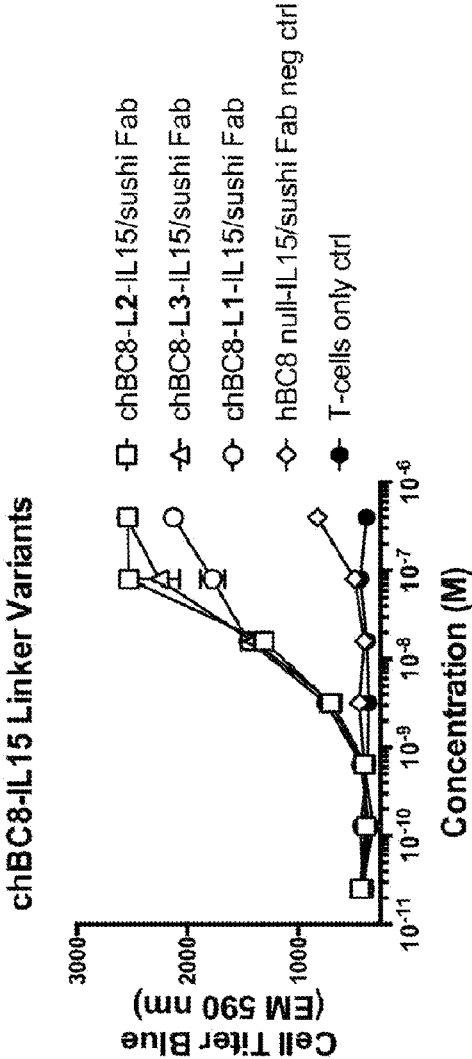


FIG. 9B

Antibody Titration on Human
CD8⁺ T-cells

CD45 mAb	T-Cell binding Apparent K _d (nM)
Humanized BC8(23)	7.8
Chimeric BC8	6.68
Chimeric 4B2	0.44
Chimeric 9.4	0.84
Human BC8 null binding mutant	>500

FIG. 10A

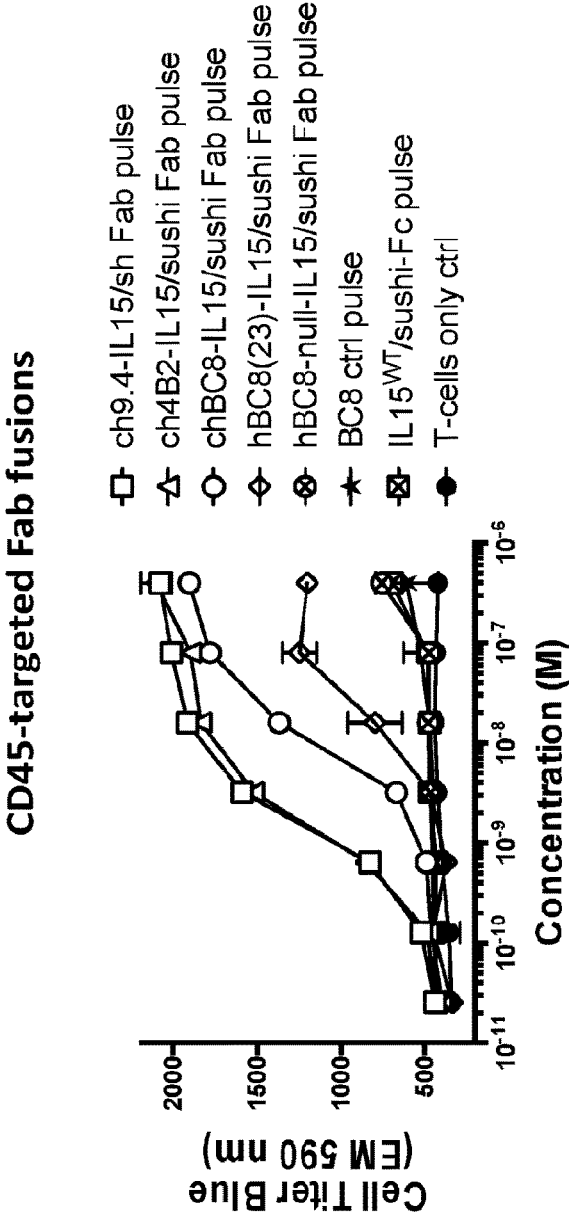
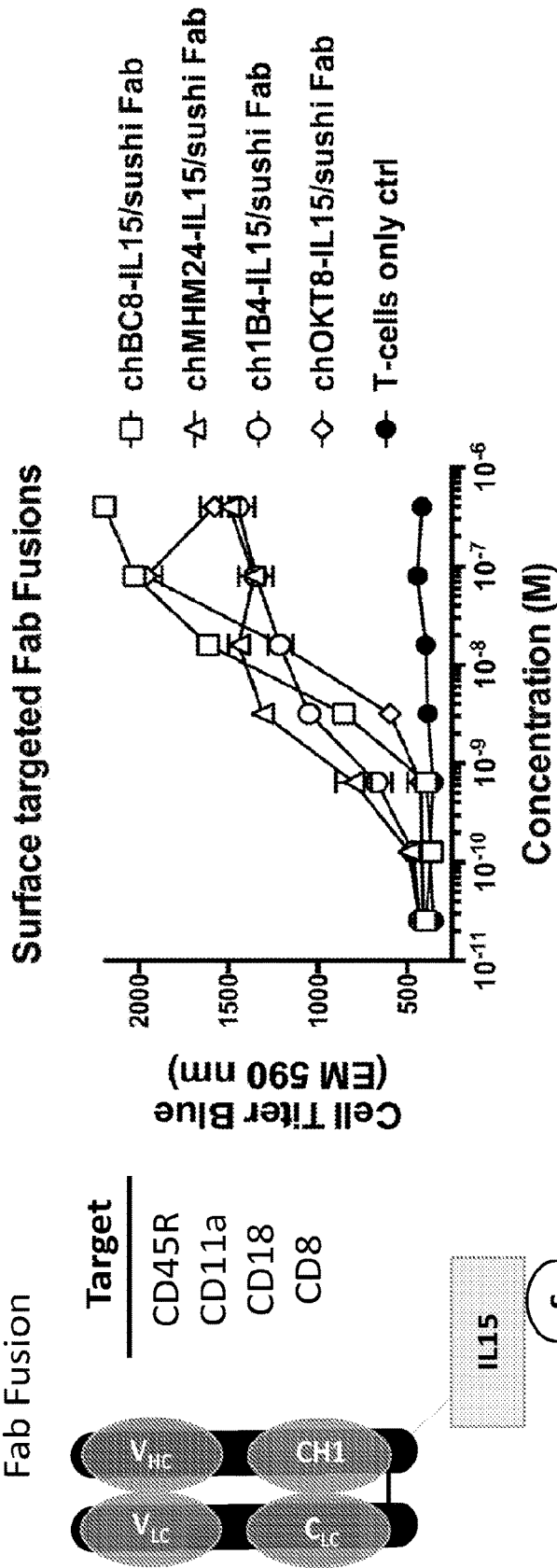


FIG. 10B



(S) = IL15Ralpha sushi domain

FIG. 11A

FIG. 11B

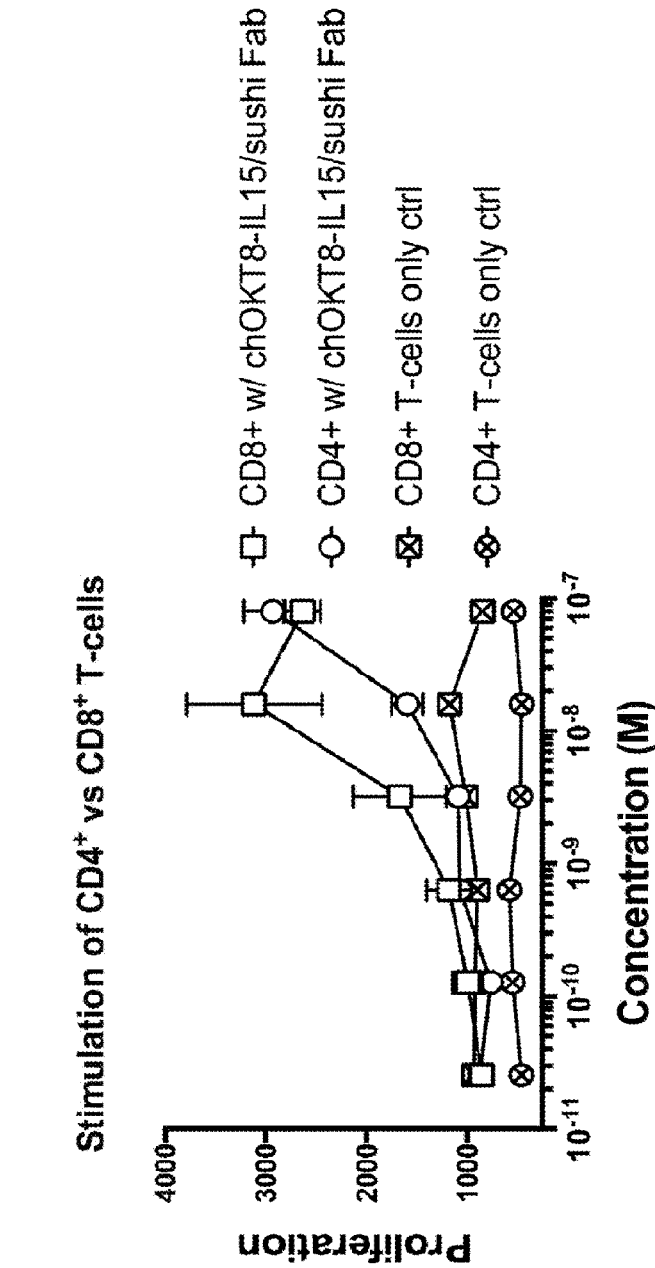


FIG. 12A

(S) = IL15Ralpha sushi domain

FIG. 12B

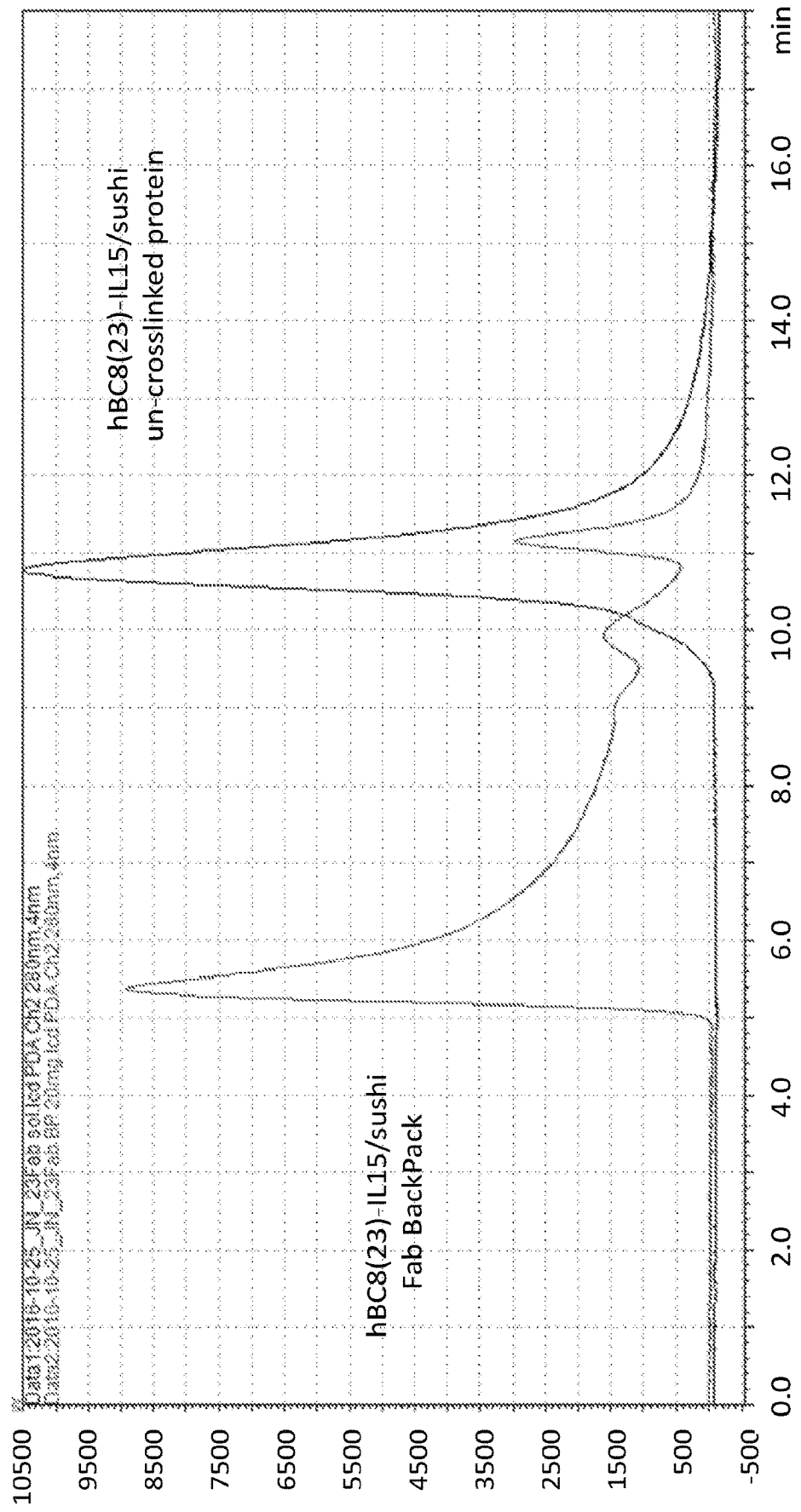


FIG. 13A

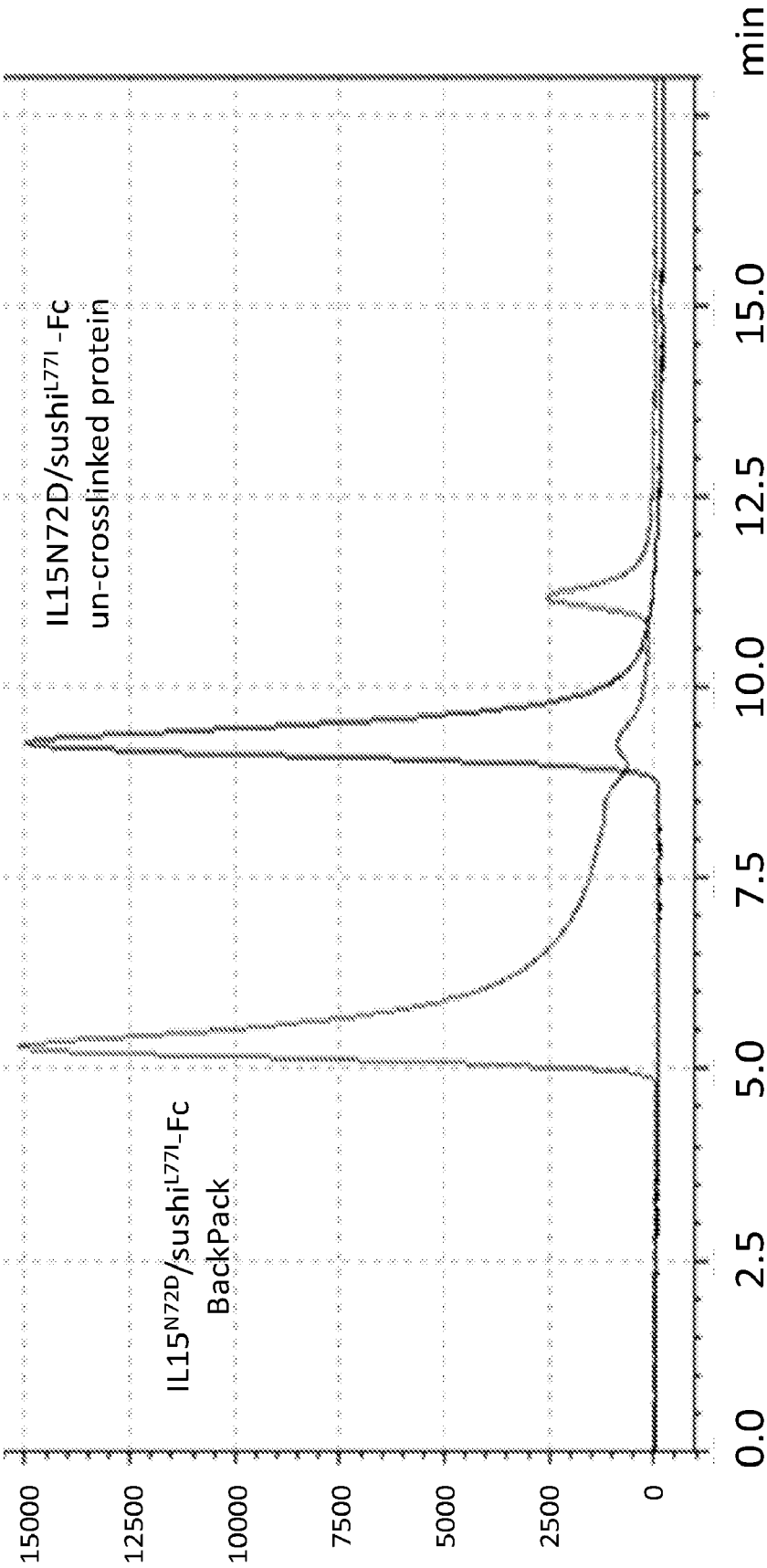


FIG. 13B

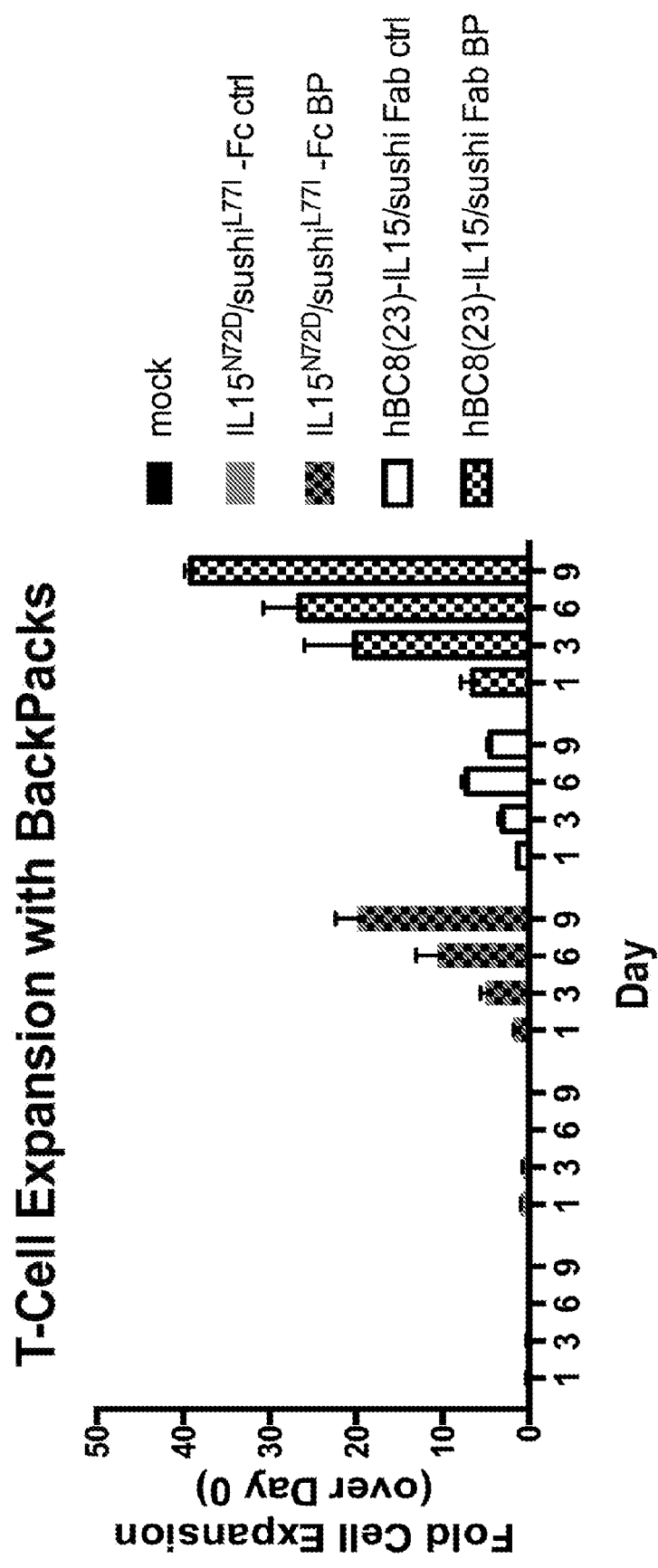


FIG. 13C

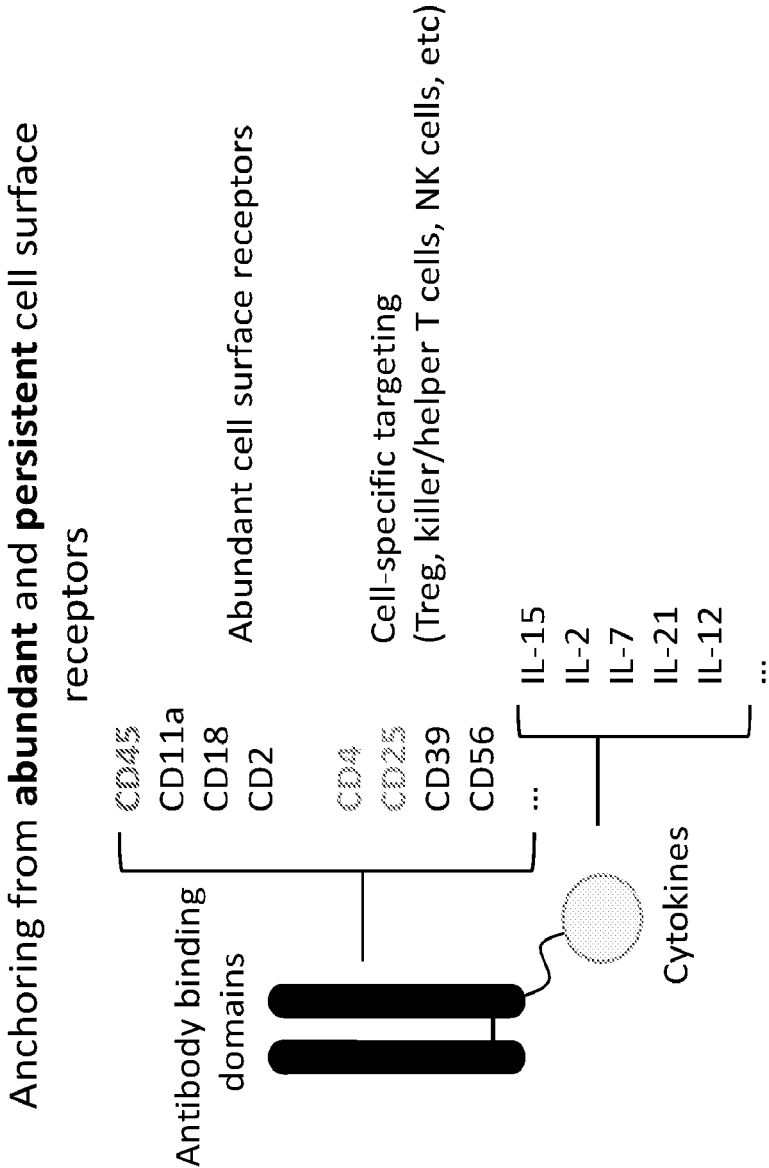


FIG. 14

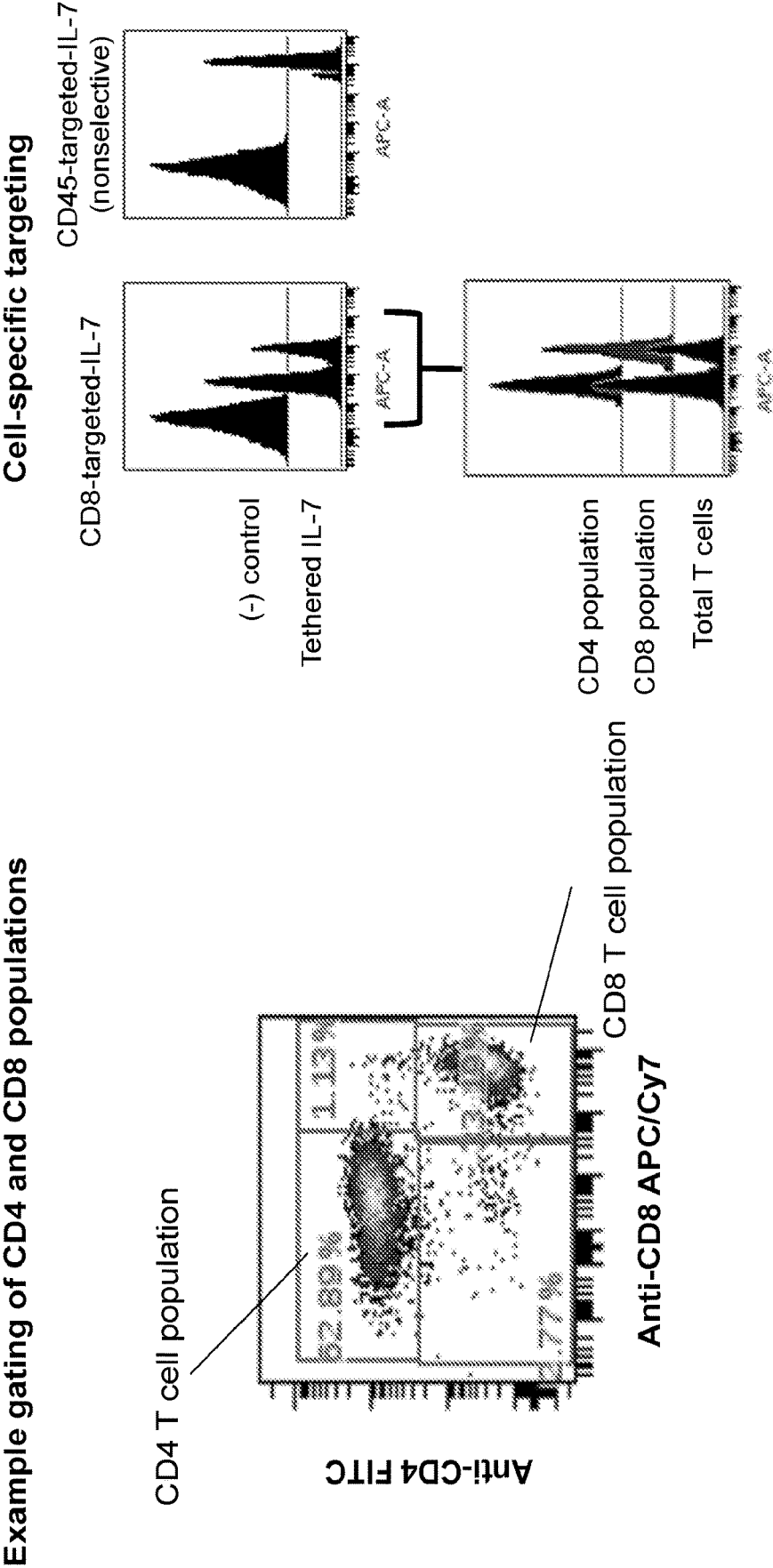
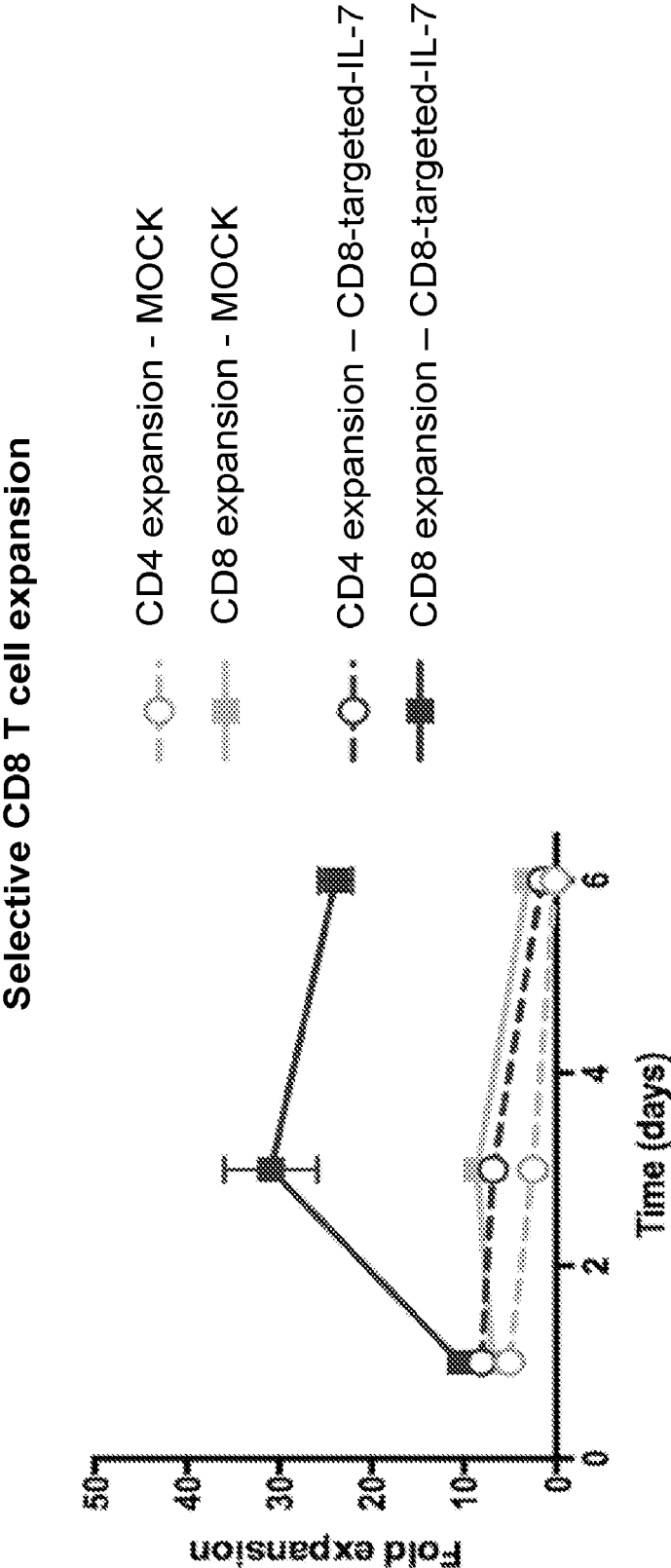


FIG. 15A

FIG. 15B



Expansion of CD4 vs CD8 T cells in cultures of activated total CD3 human T cells by pulse incubation with CD8-targeted IL-7

FIG. 15C

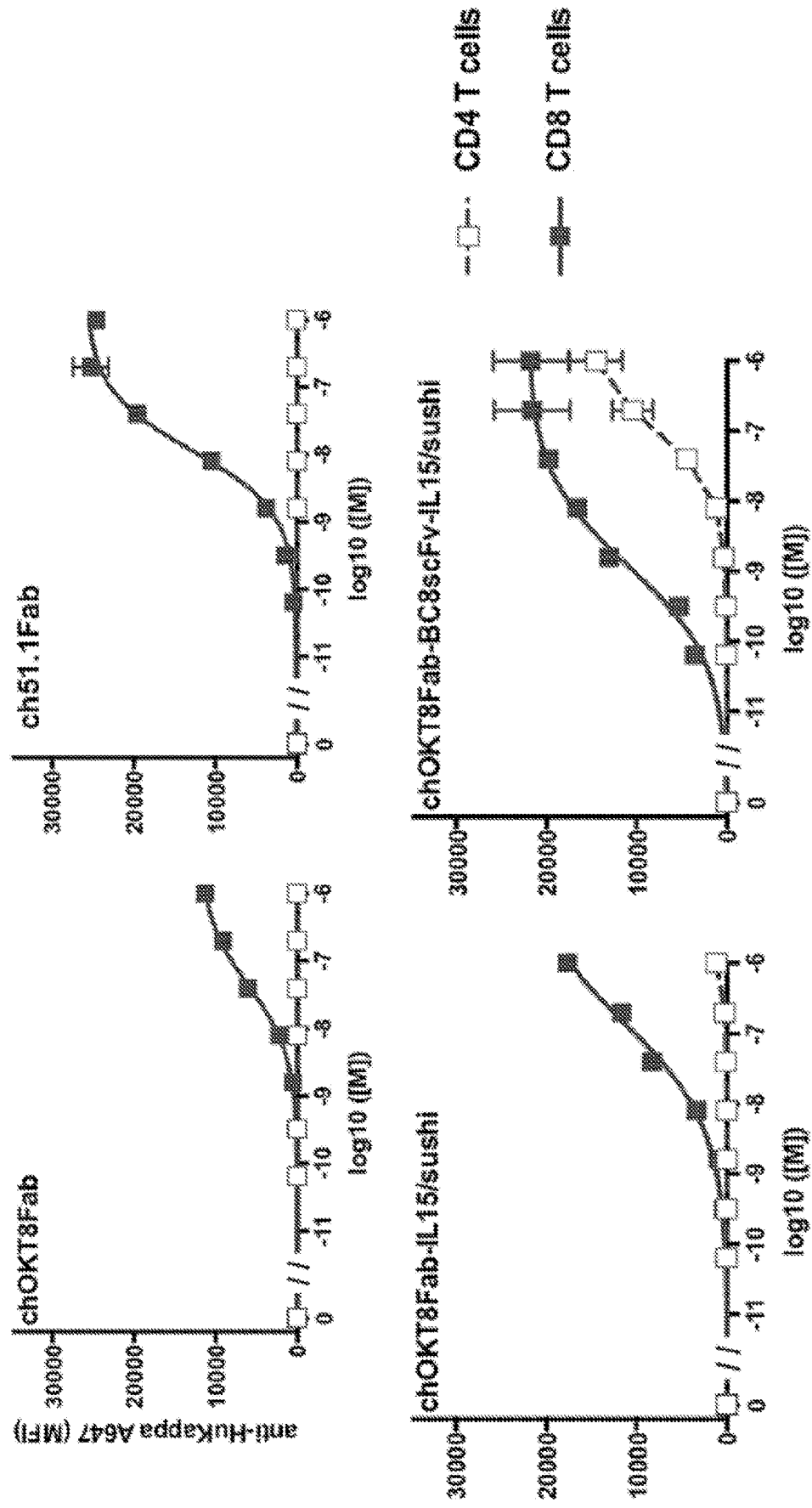


FIG. 15D

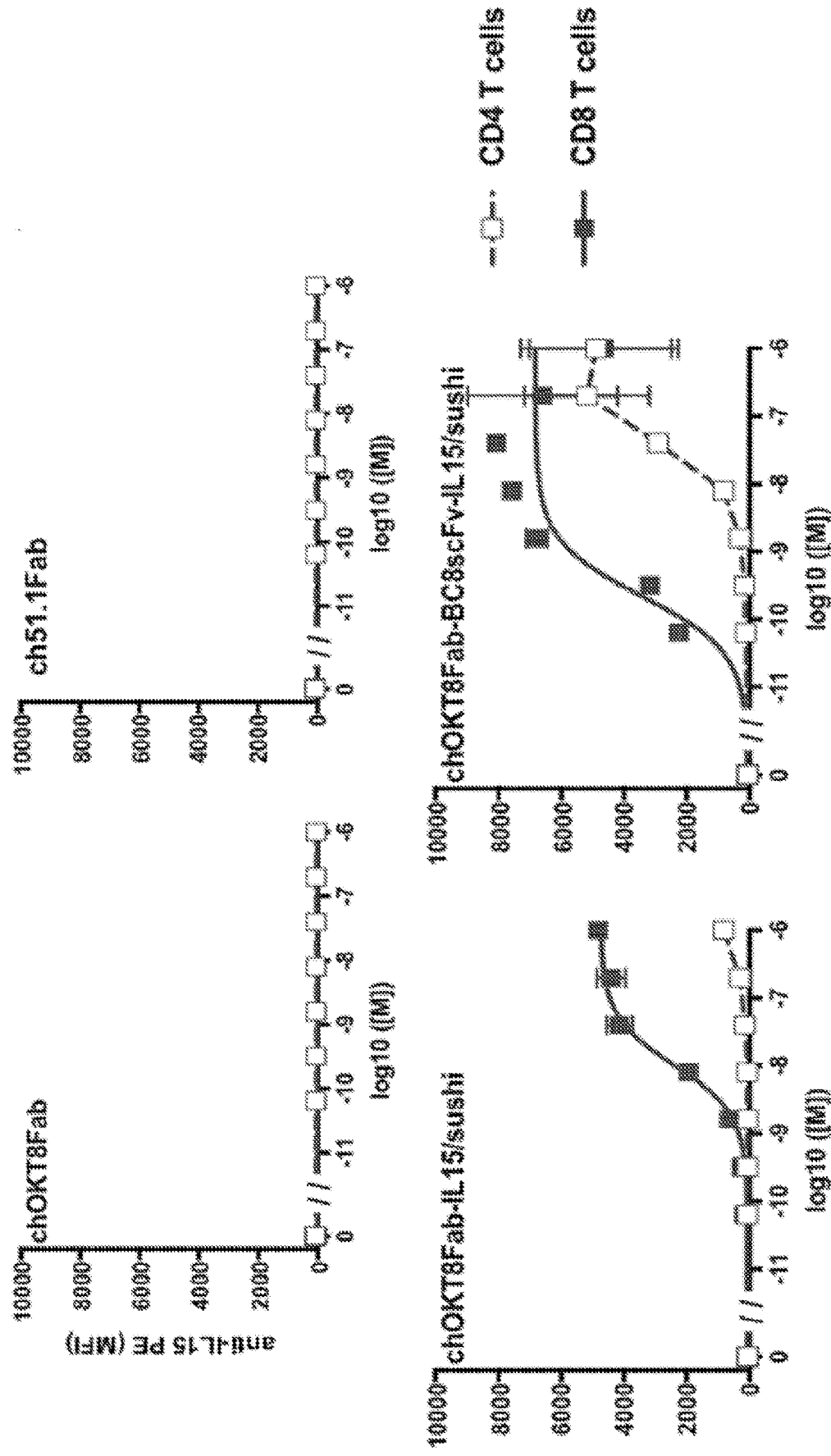


FIG. 15E

Comparison of CD8 T cell binding

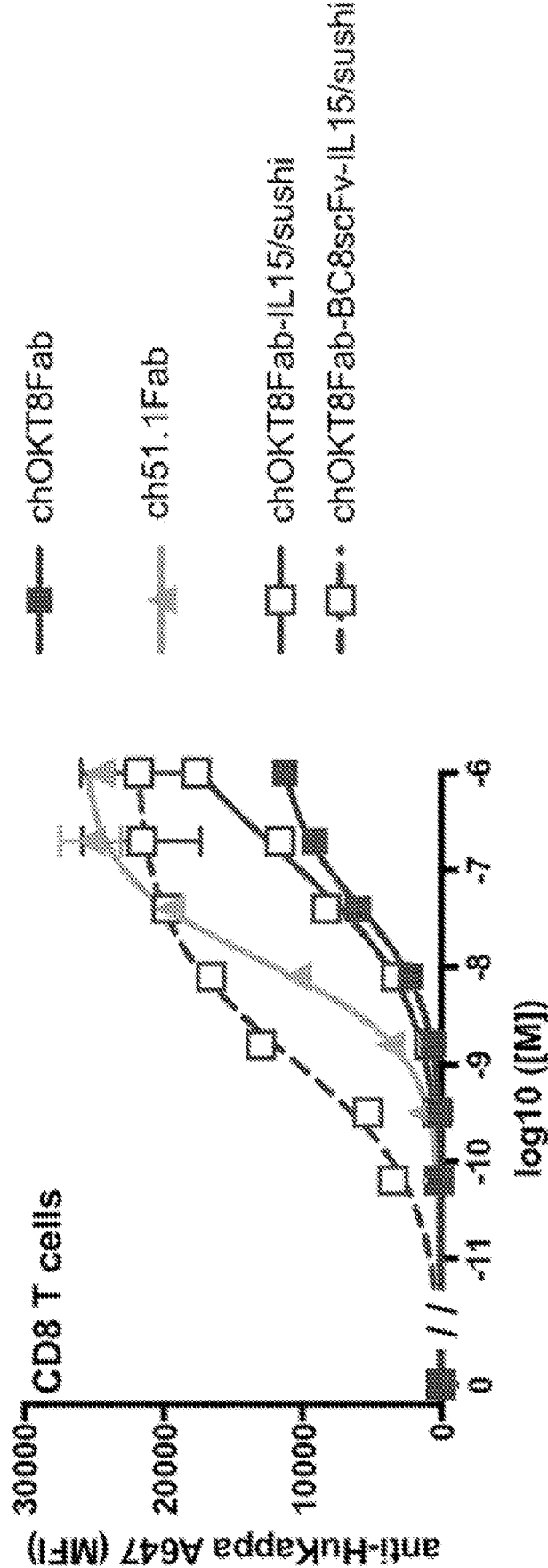


FIG. 15F

T cell binding by BC8 scFv monomer or dimer

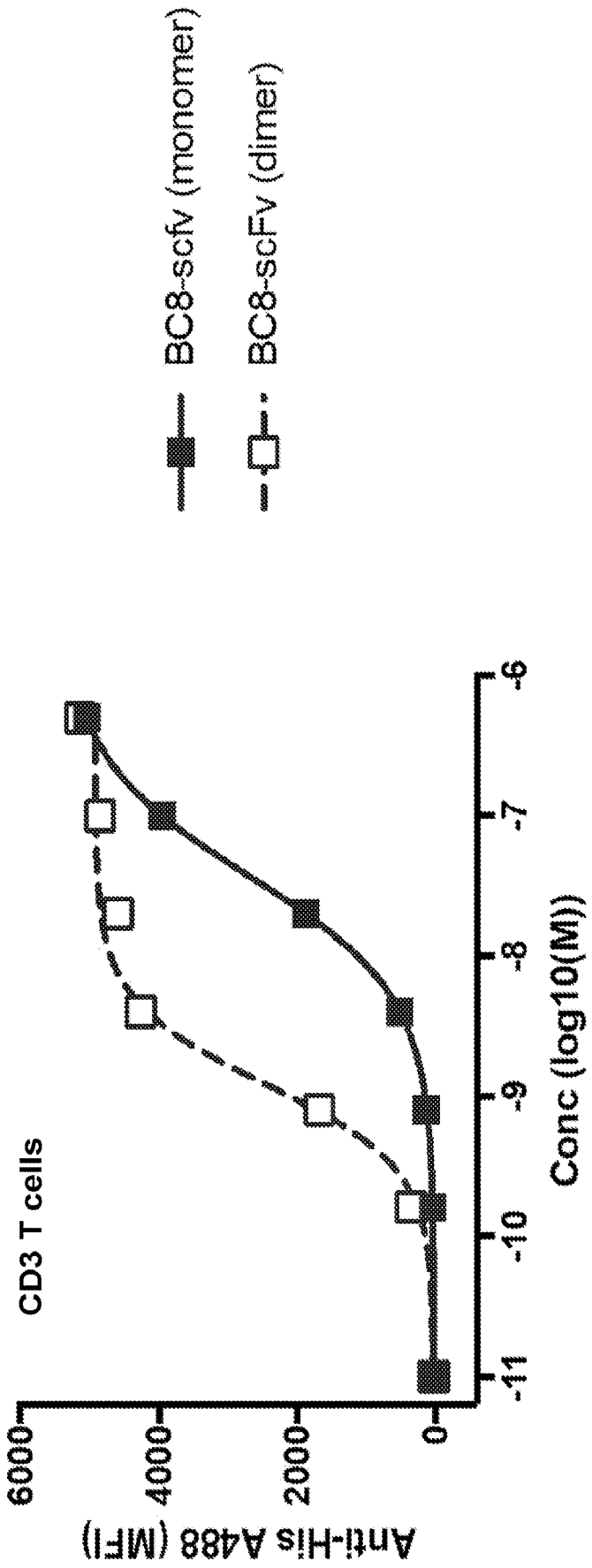


FIG. 15G

Titration of IL-15 variants on human T cells

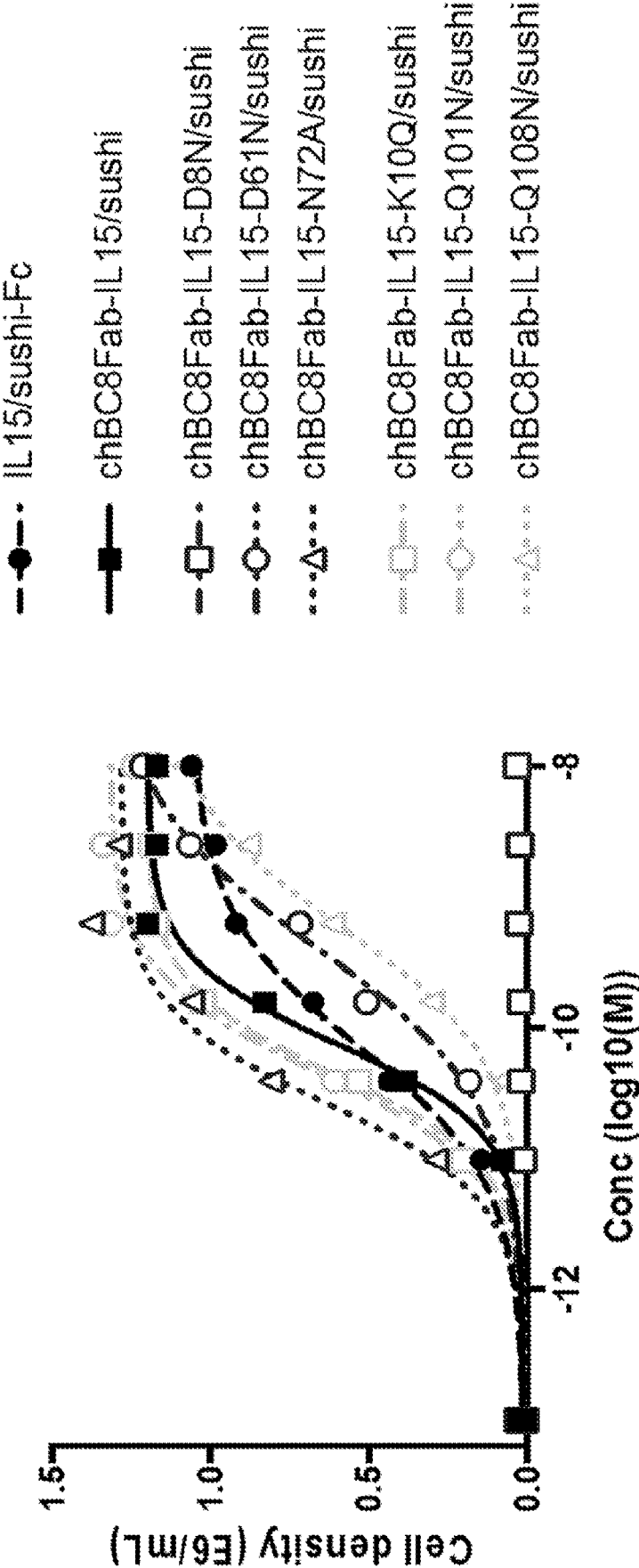


FIG. 16A

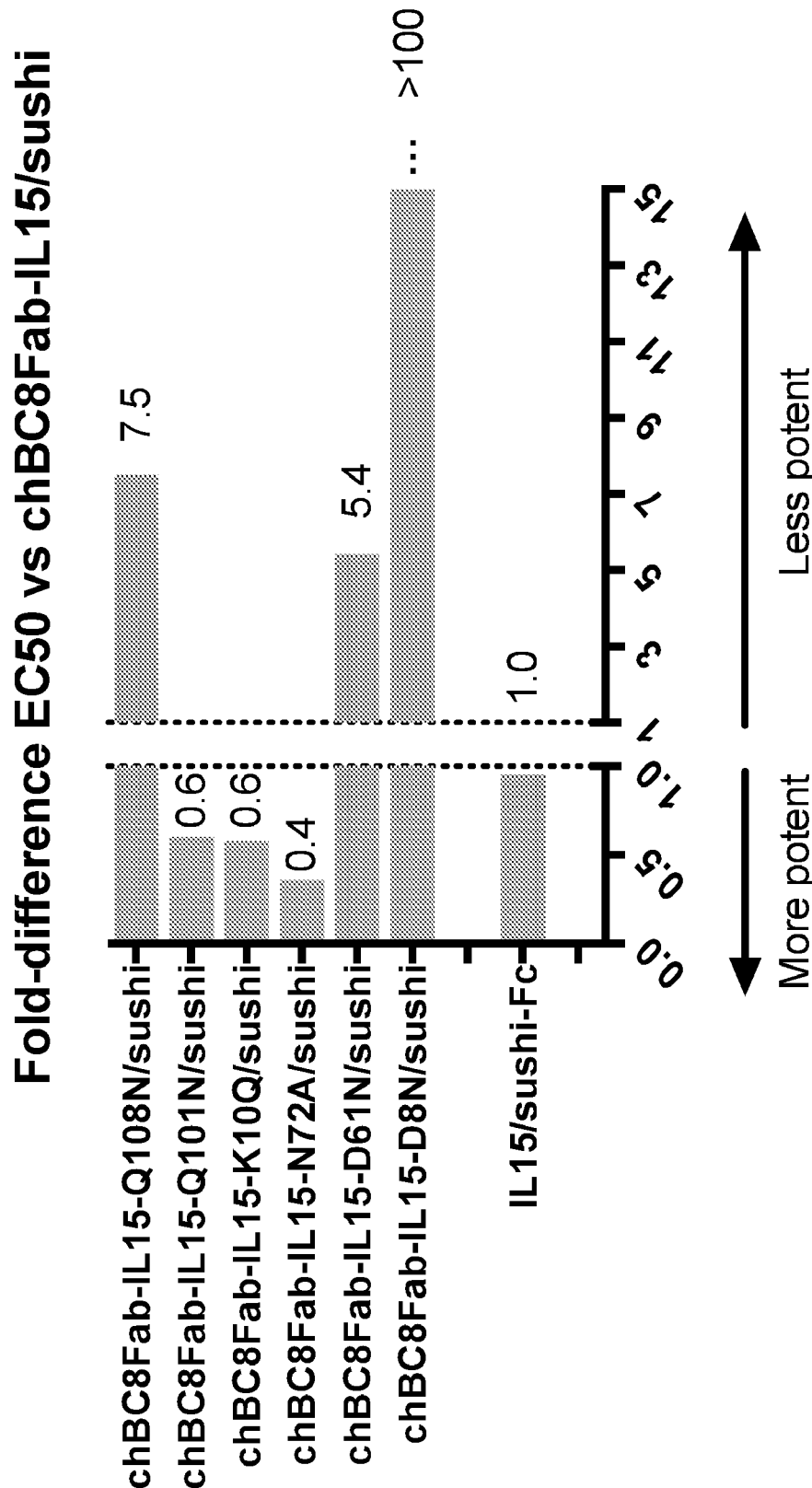


FIG. 16B

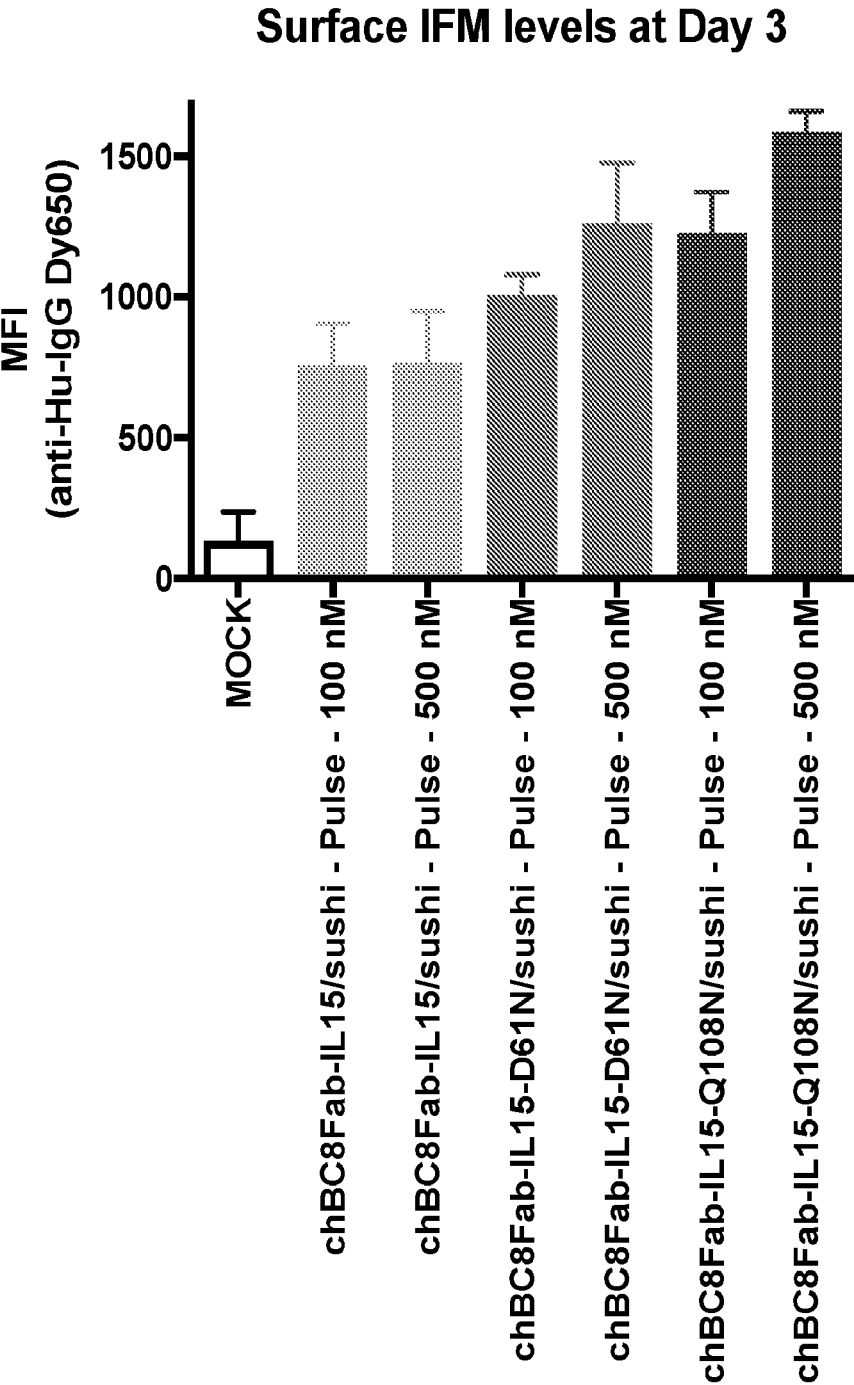


FIG. 16C

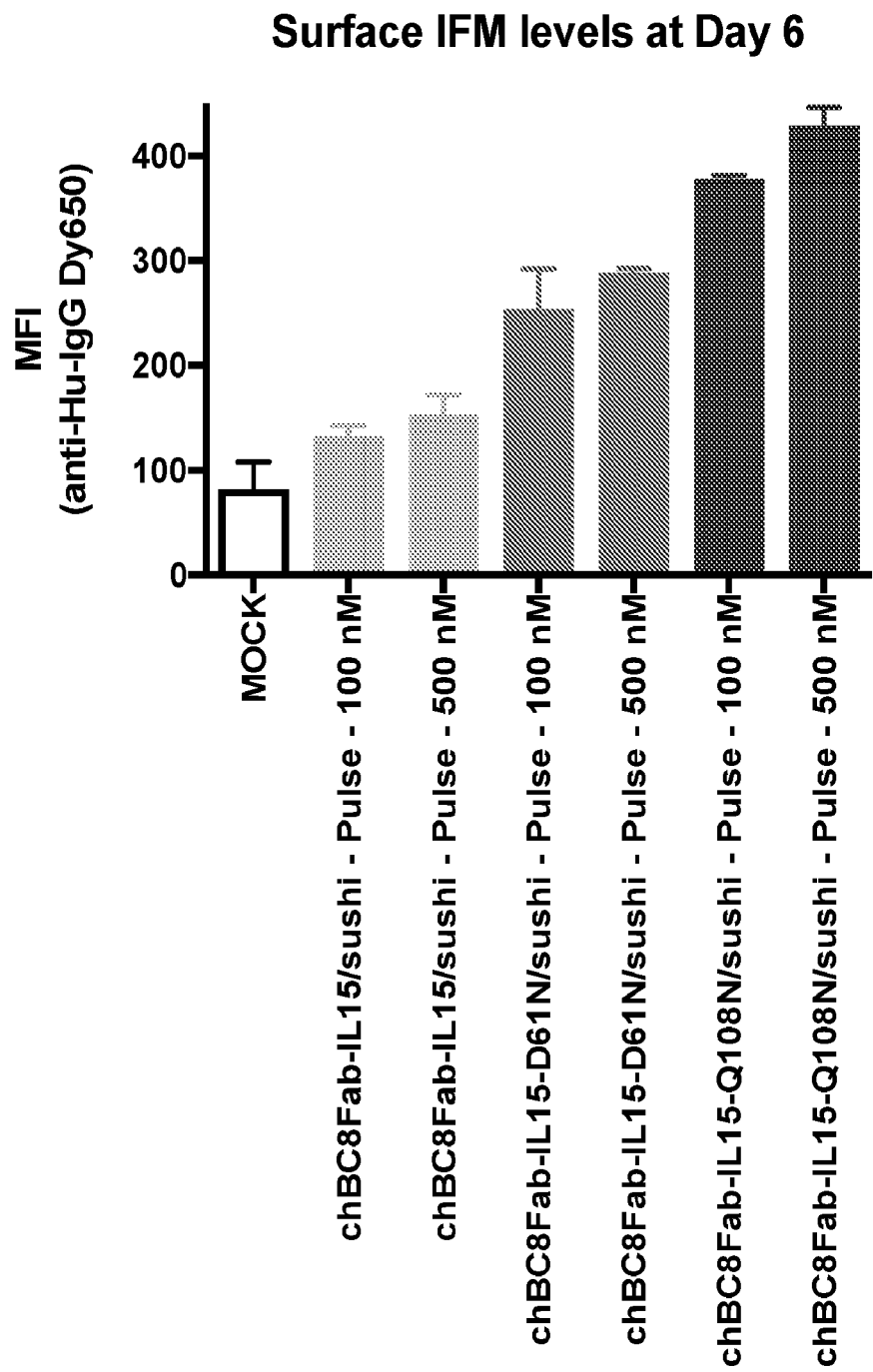


FIG. 16C
(cont.)

CD8-targeted IFM binding to CD4 T cells

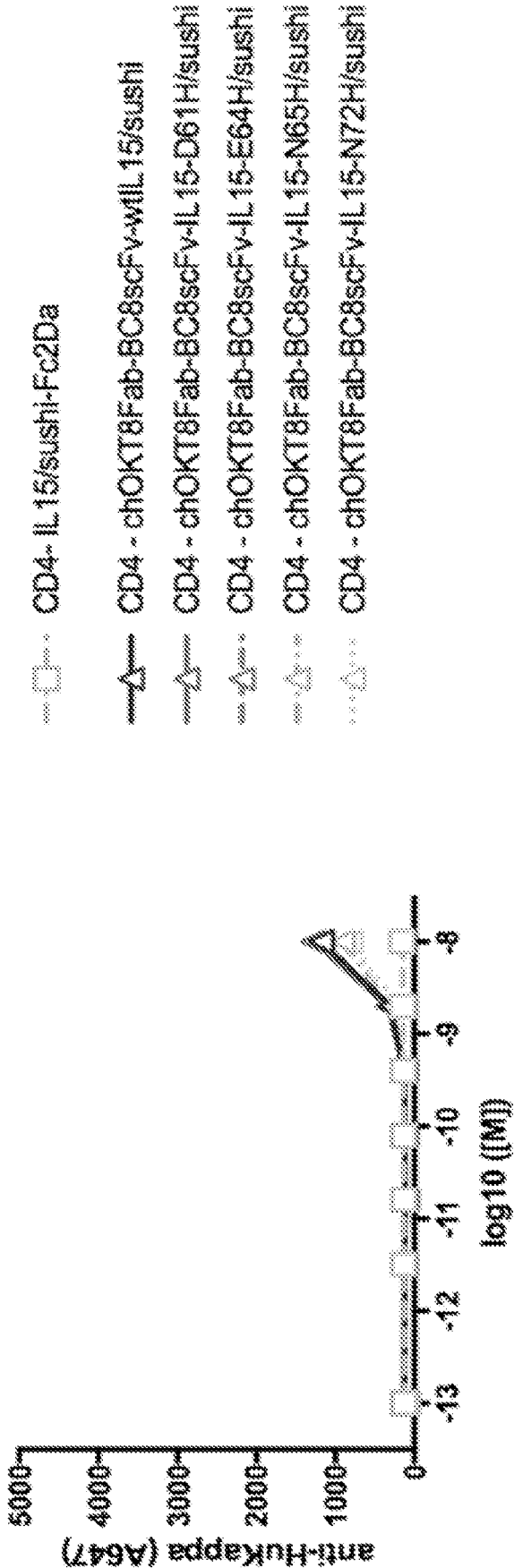


FIG. 16D

CD8-targeted IFM binding to CD8 T cells

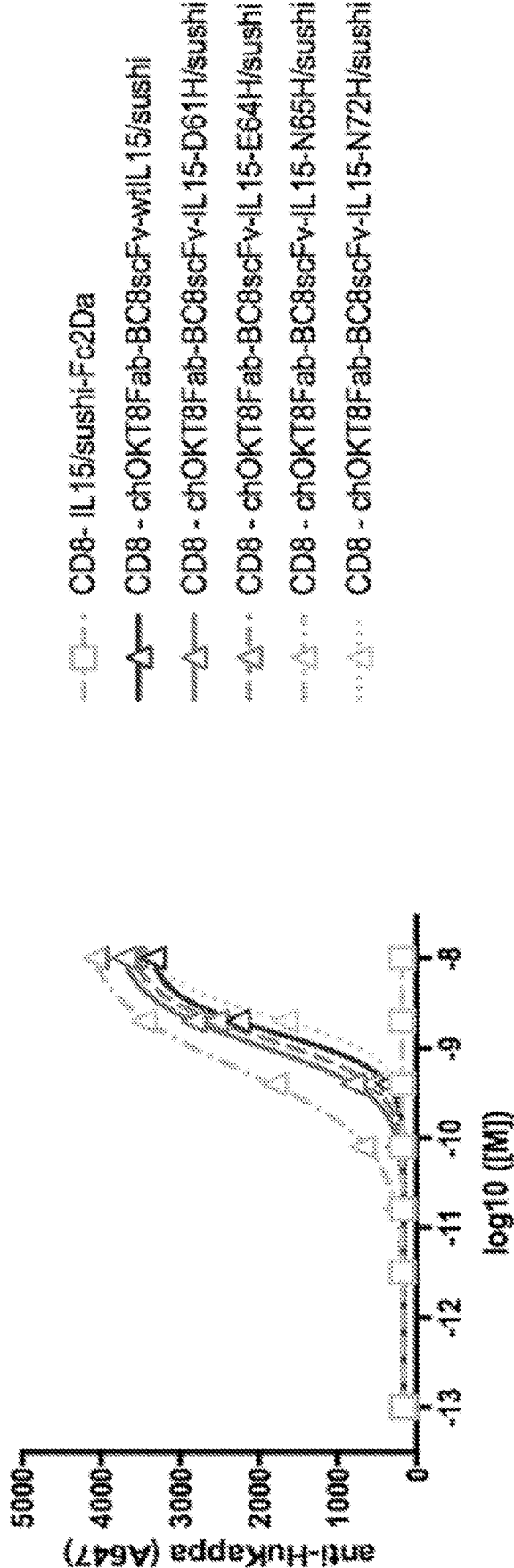


FIG. 16D (cont.)

Cell expansion by IL-15 point mutants

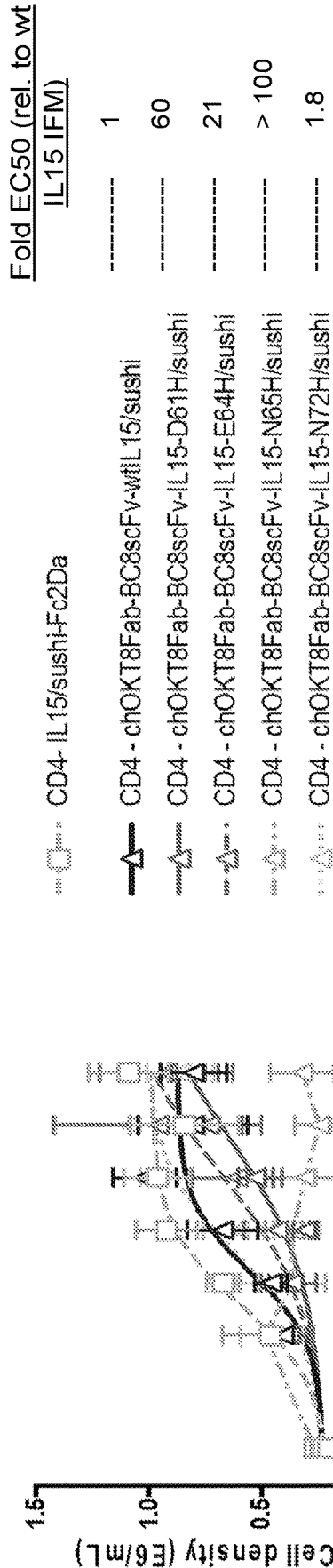


FIG. 16E

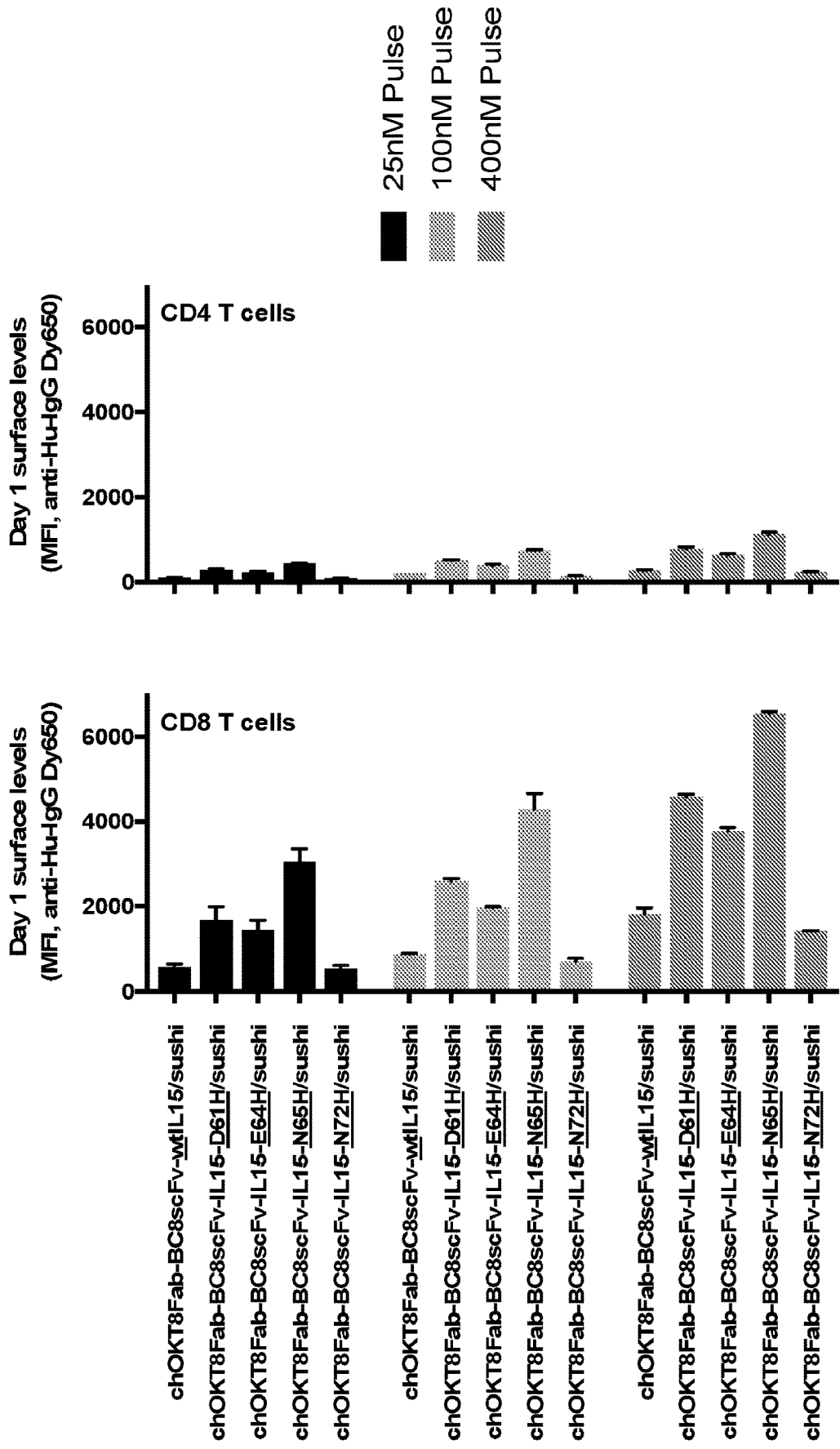


FIG. 17A

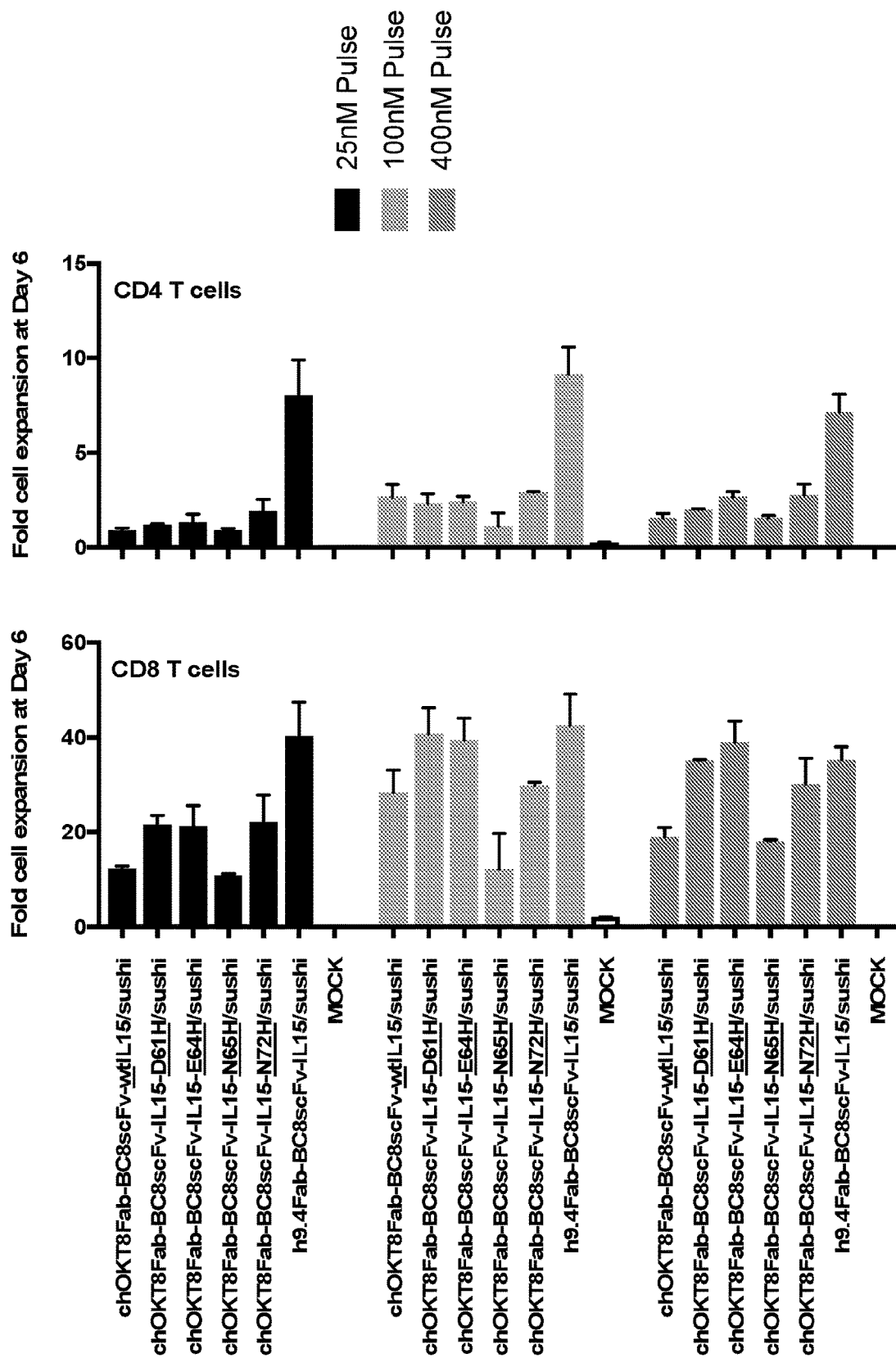
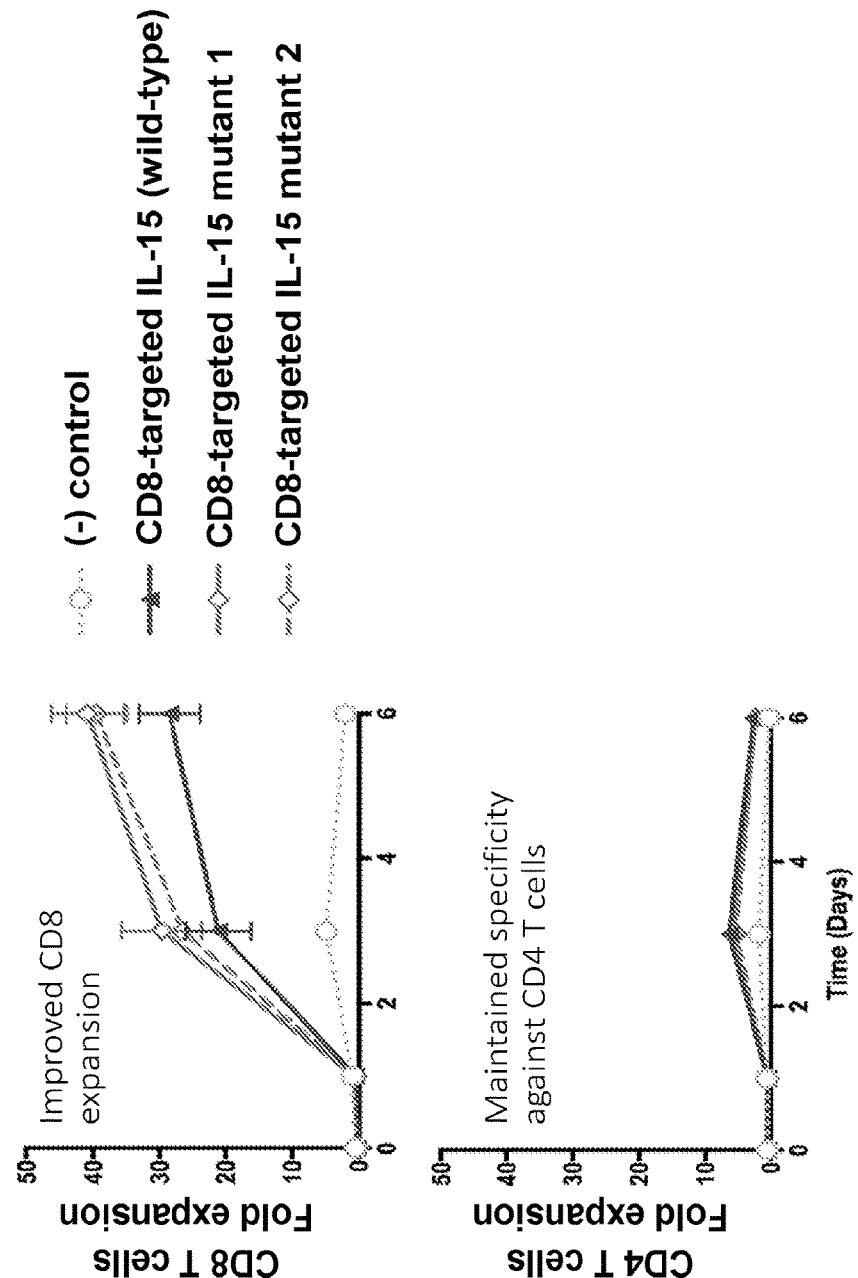


FIG. 17B

Improved CD8 T cell expansion



Expansion of CD4 vs CD8 T cells in cultures of activated total CD3 human T cells by pulse incubation with CD8-targeted IL-15 variants

FIG. 17C

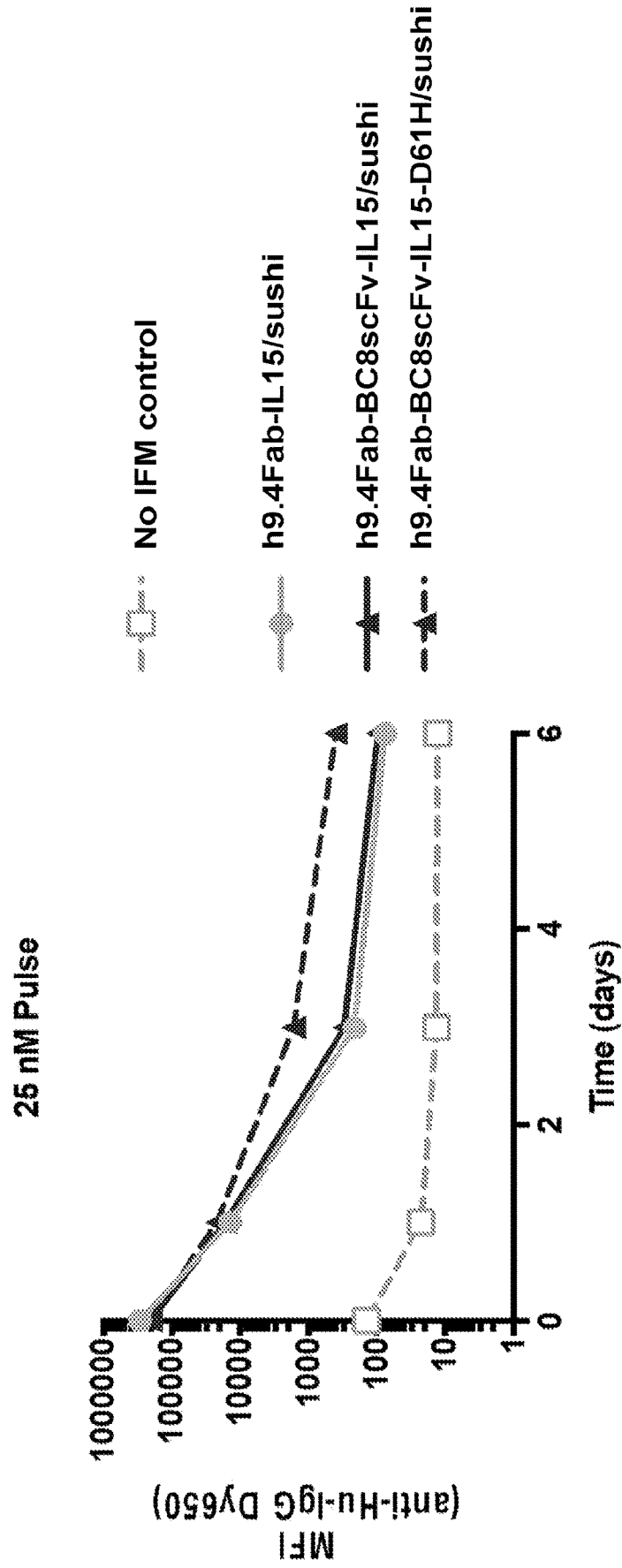


FIG. 17D

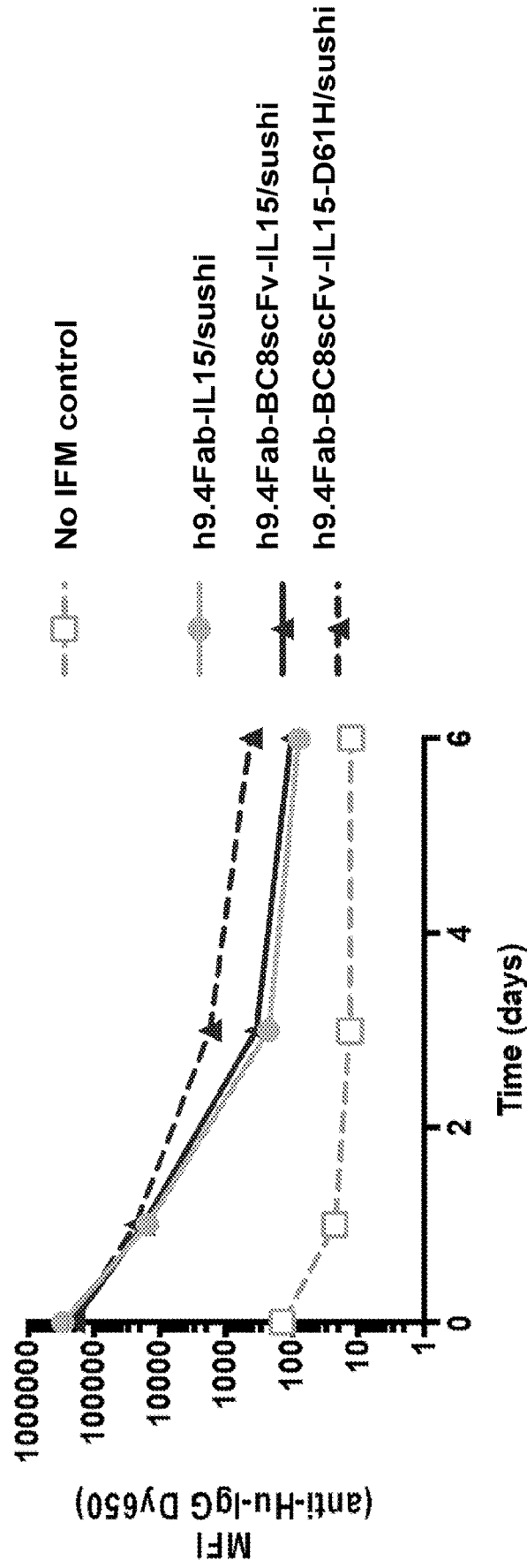


FIG. 17D (cont.)

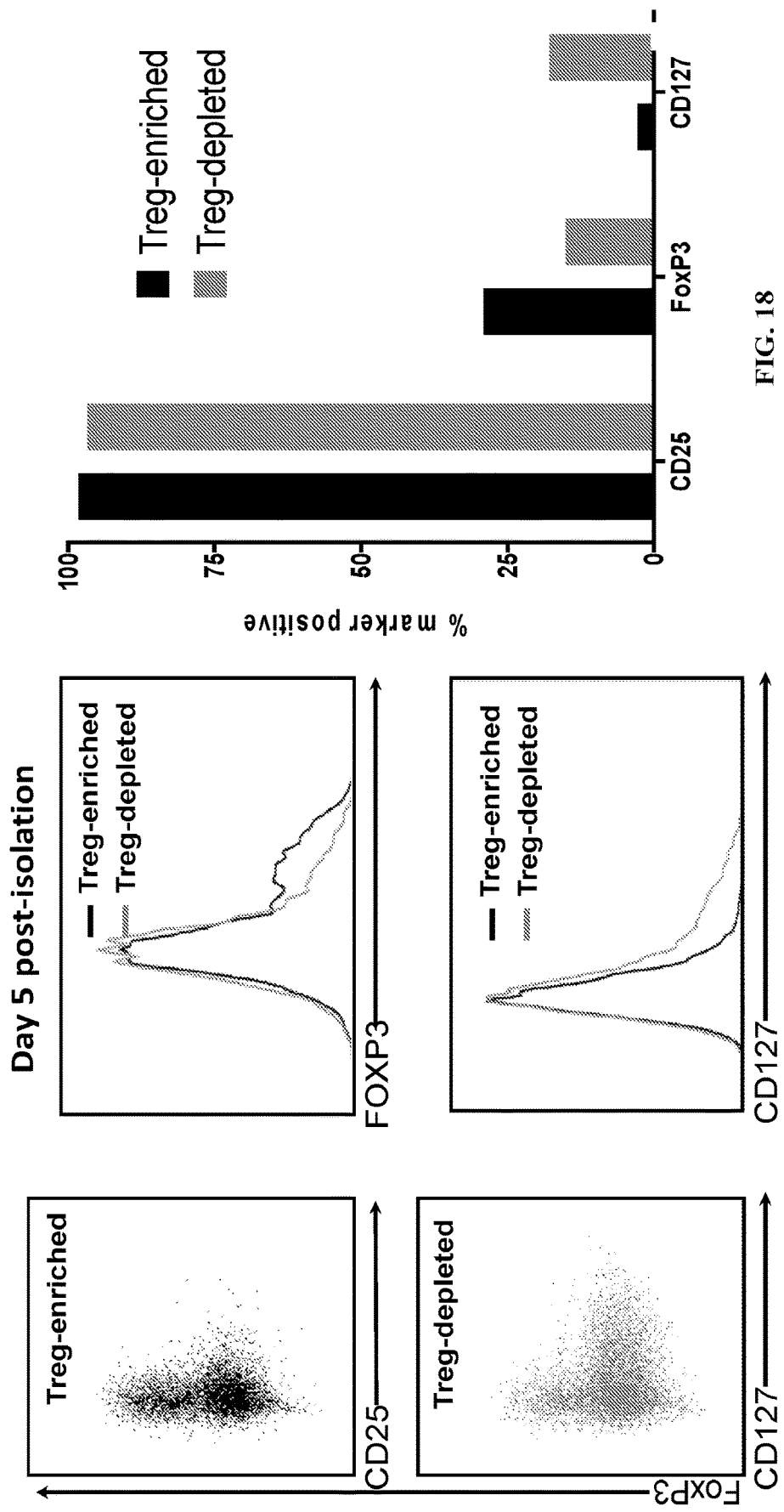


FIG. 18

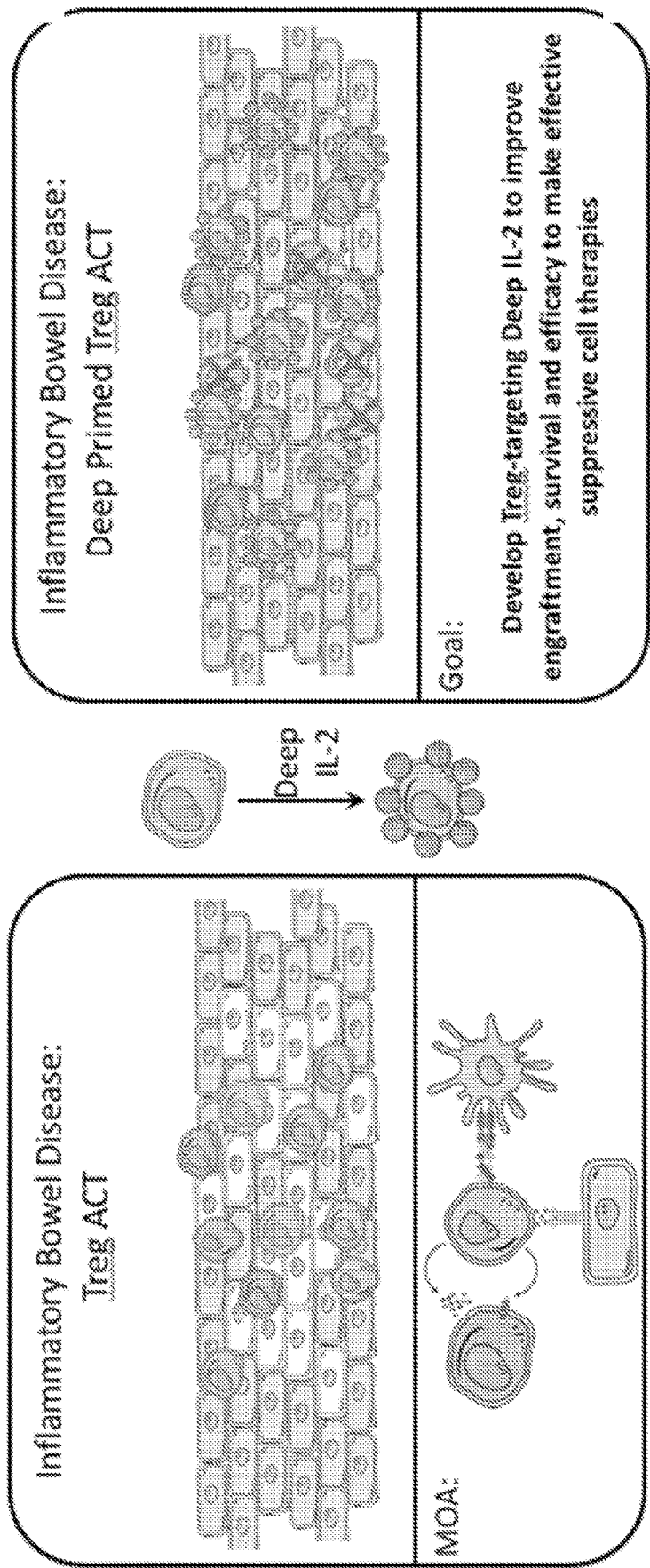


FIG. 19

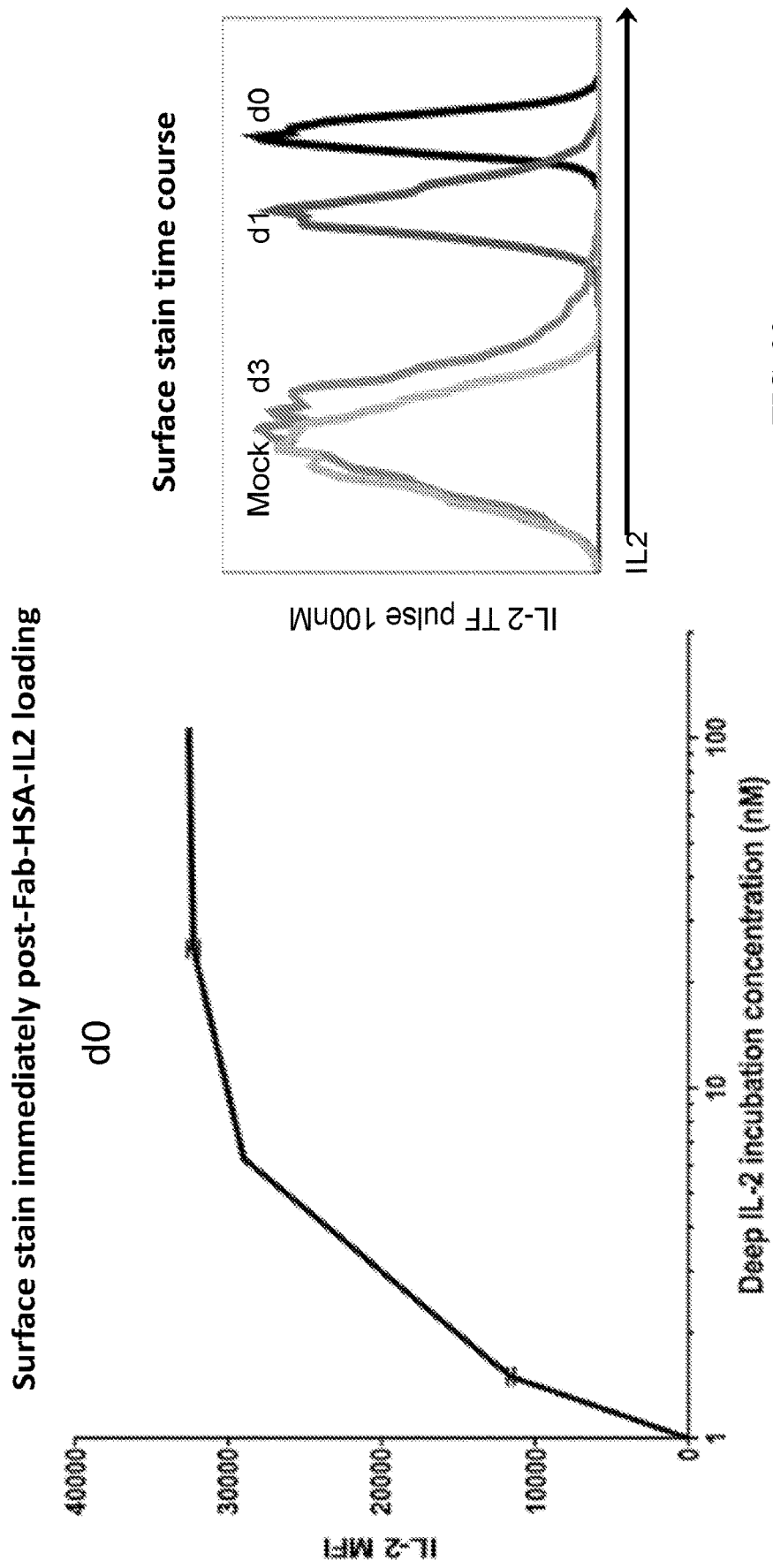


FIG. 20

Expansion and survival 3 Days post-Fab-HSA-IL2 loading

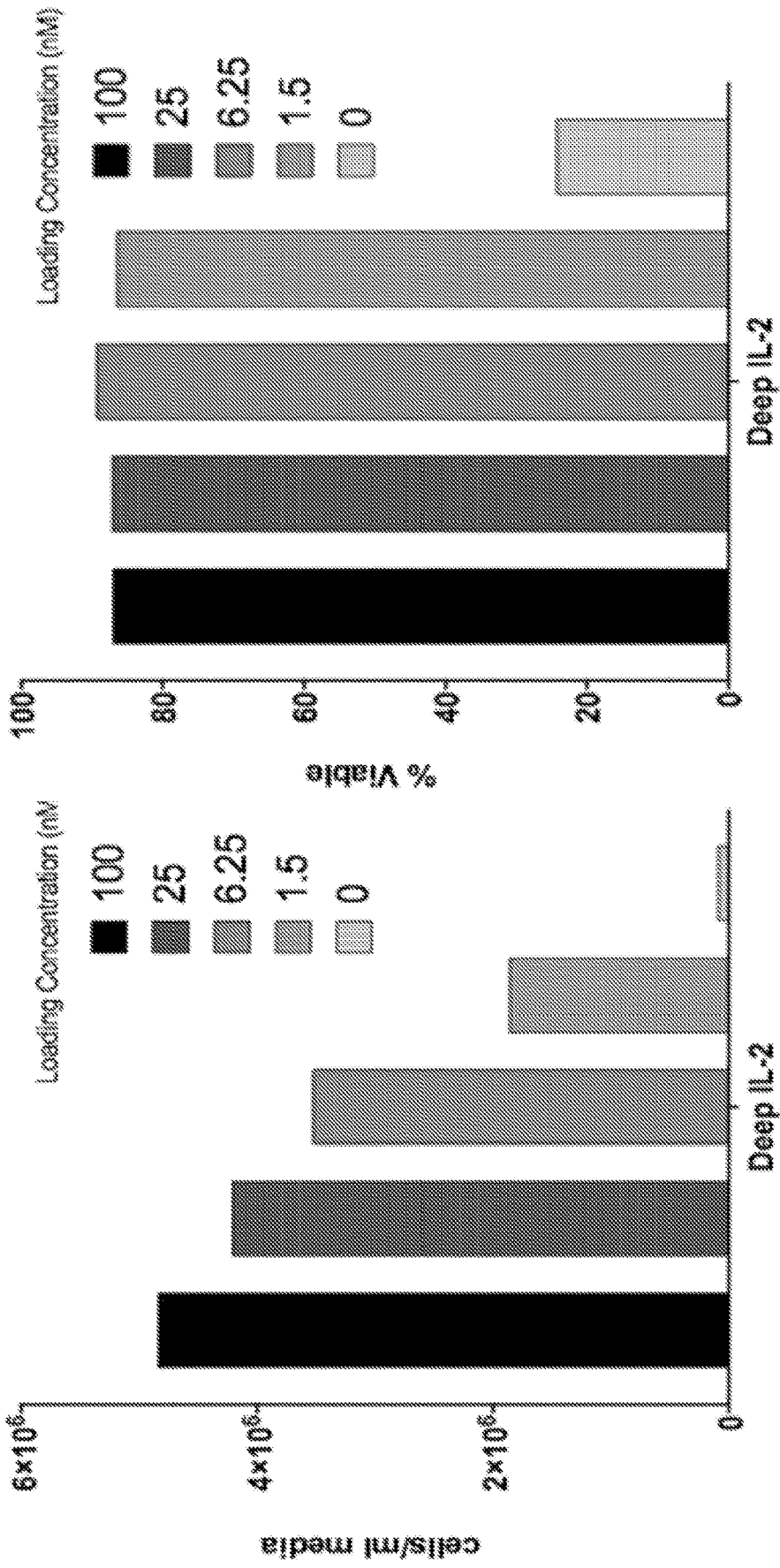


FIG. 20 (cont.)

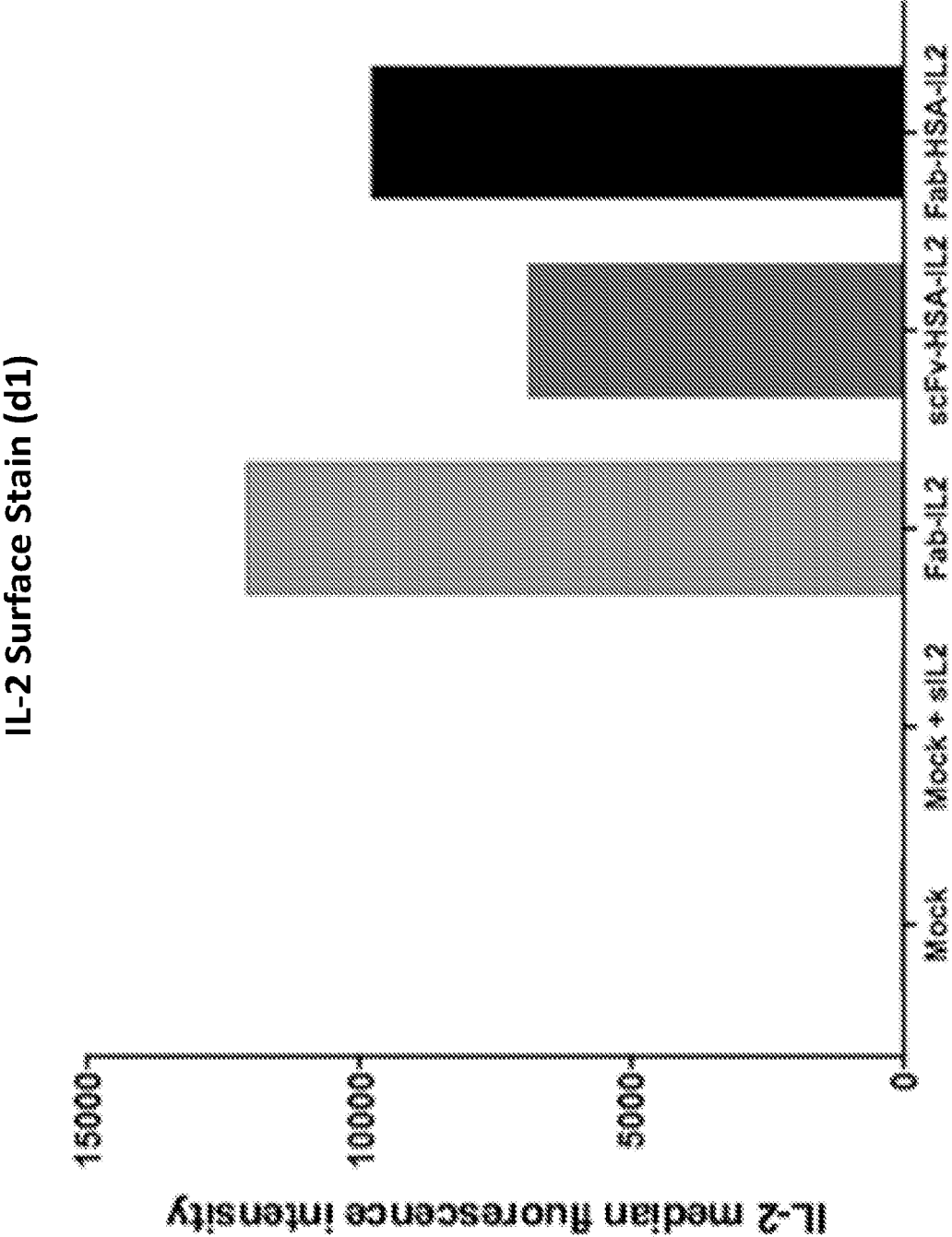


FIG. 21

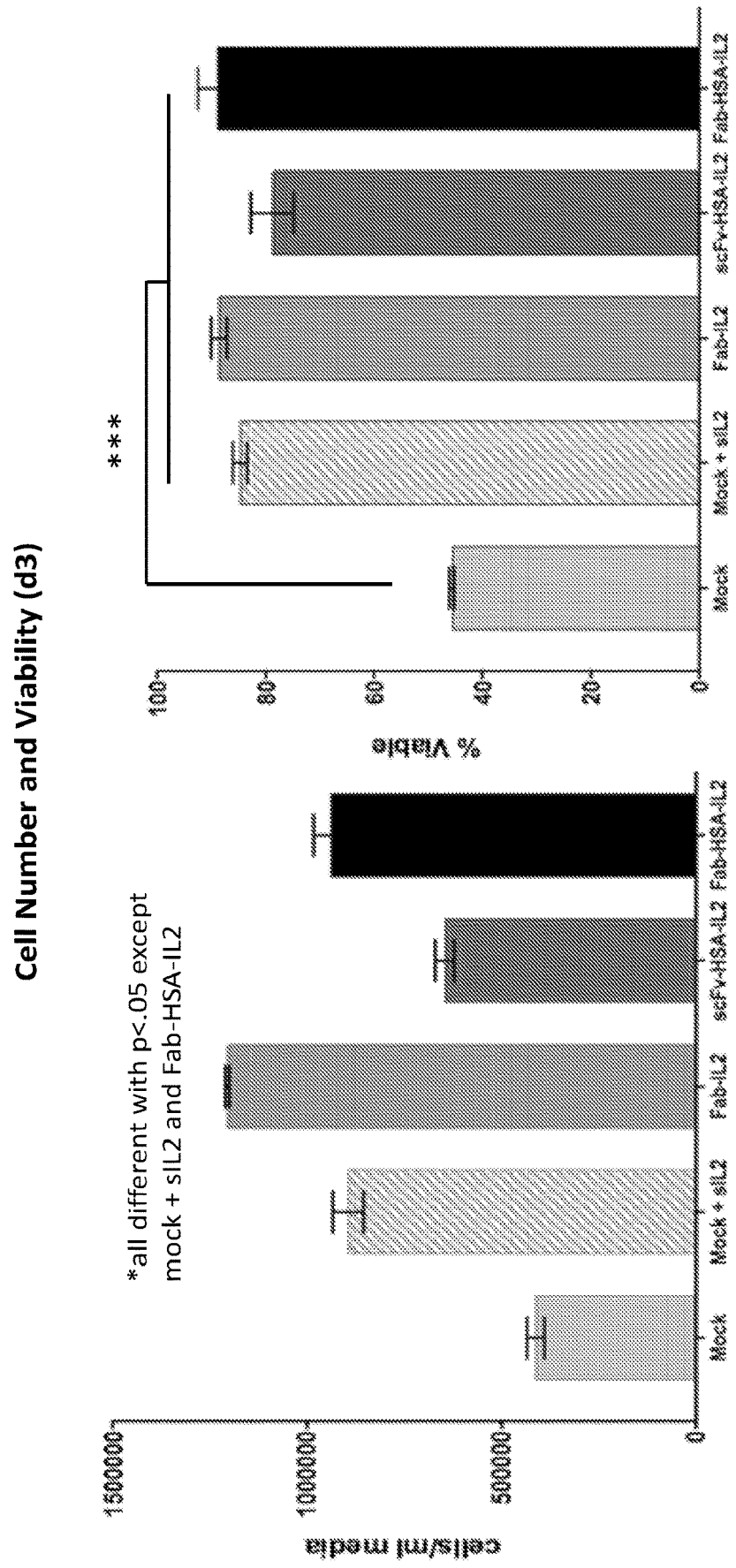


FIG. 21 (cont.)

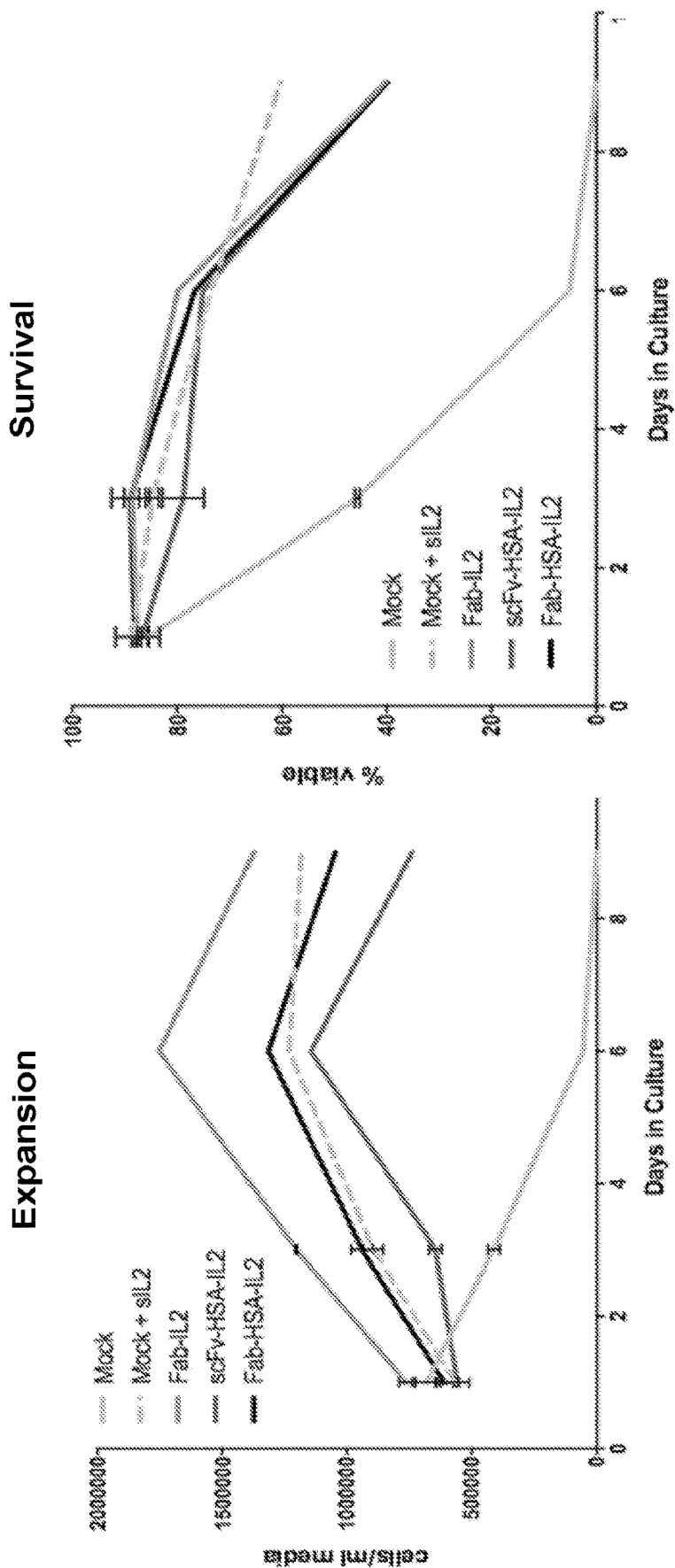


FIG. 22

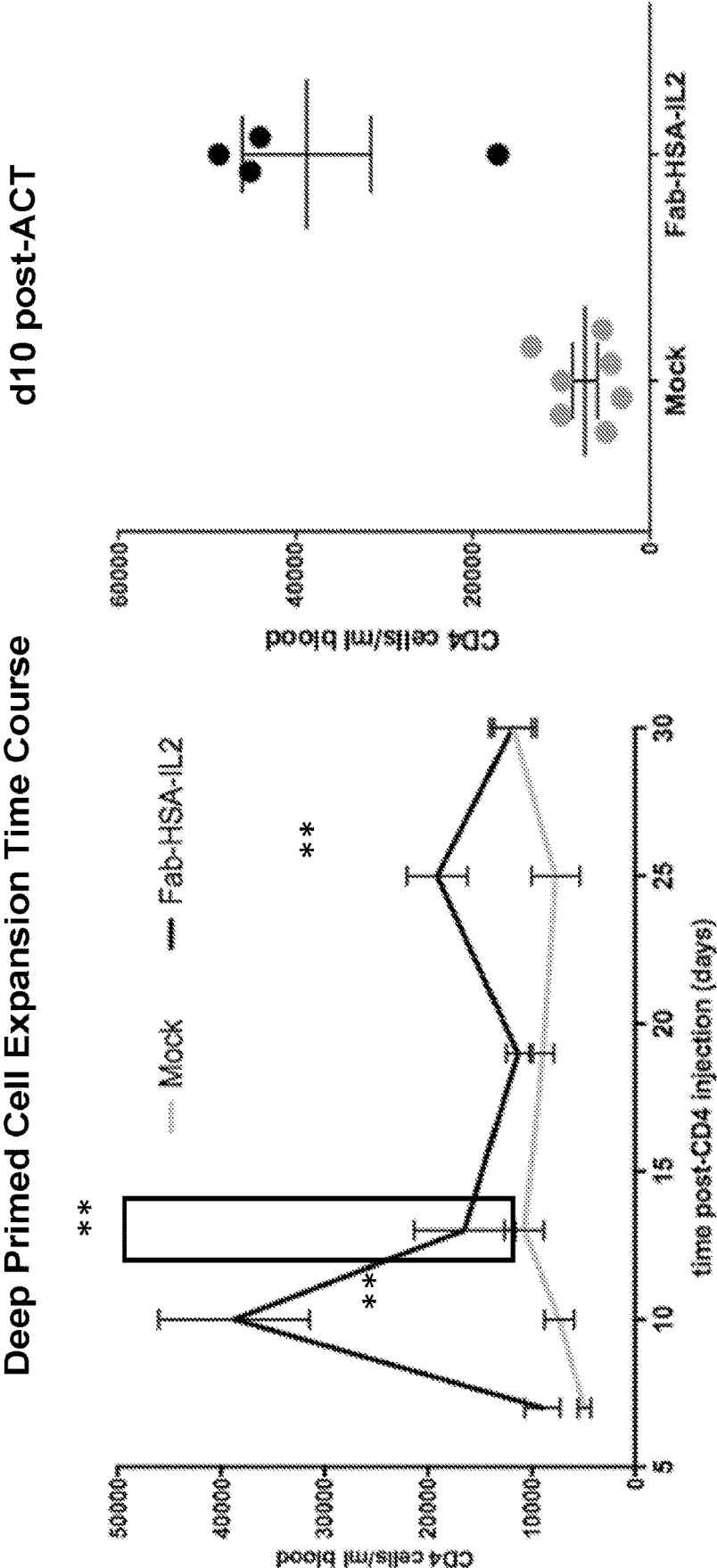


FIG. 23

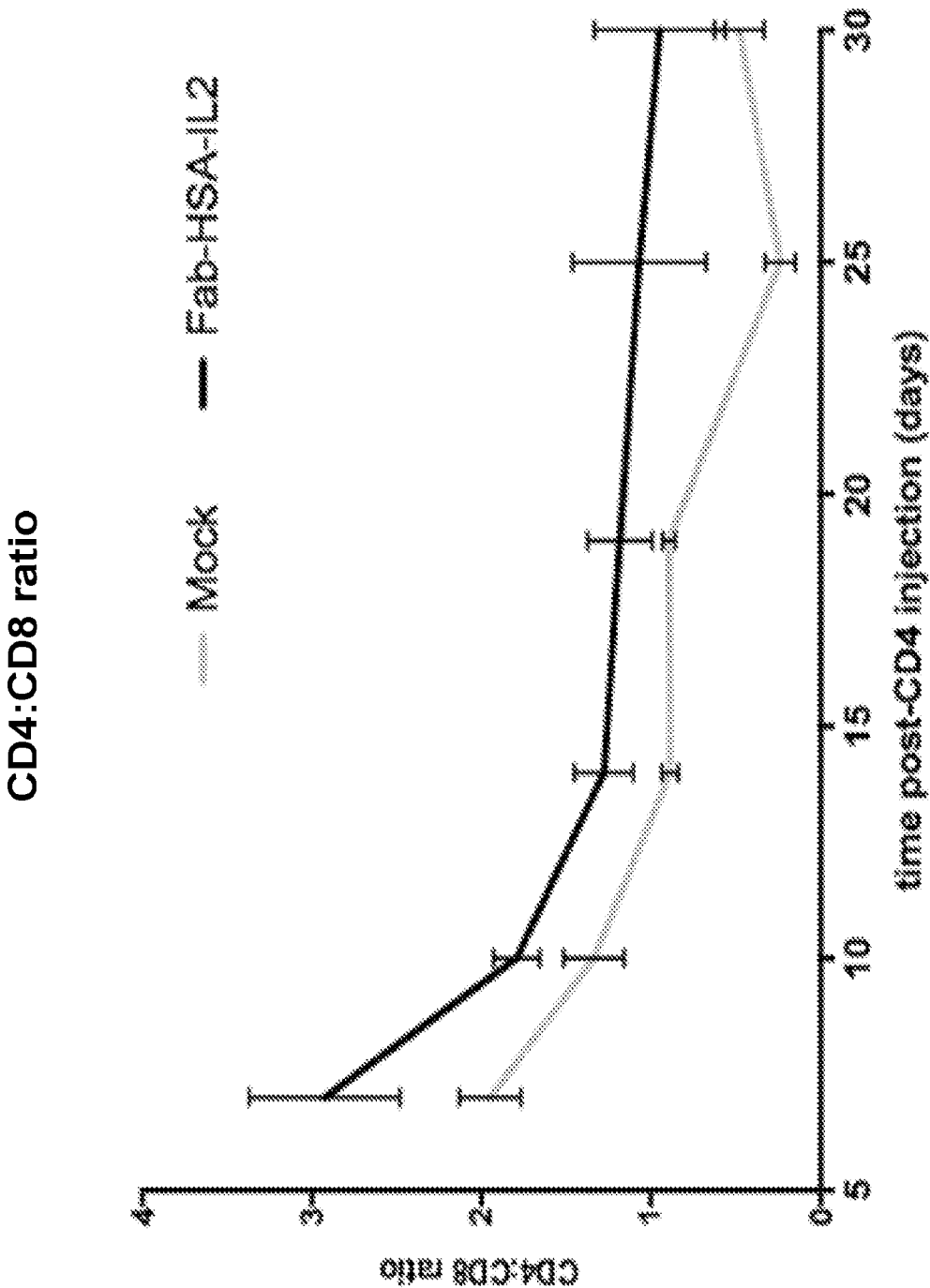


FIG. 24

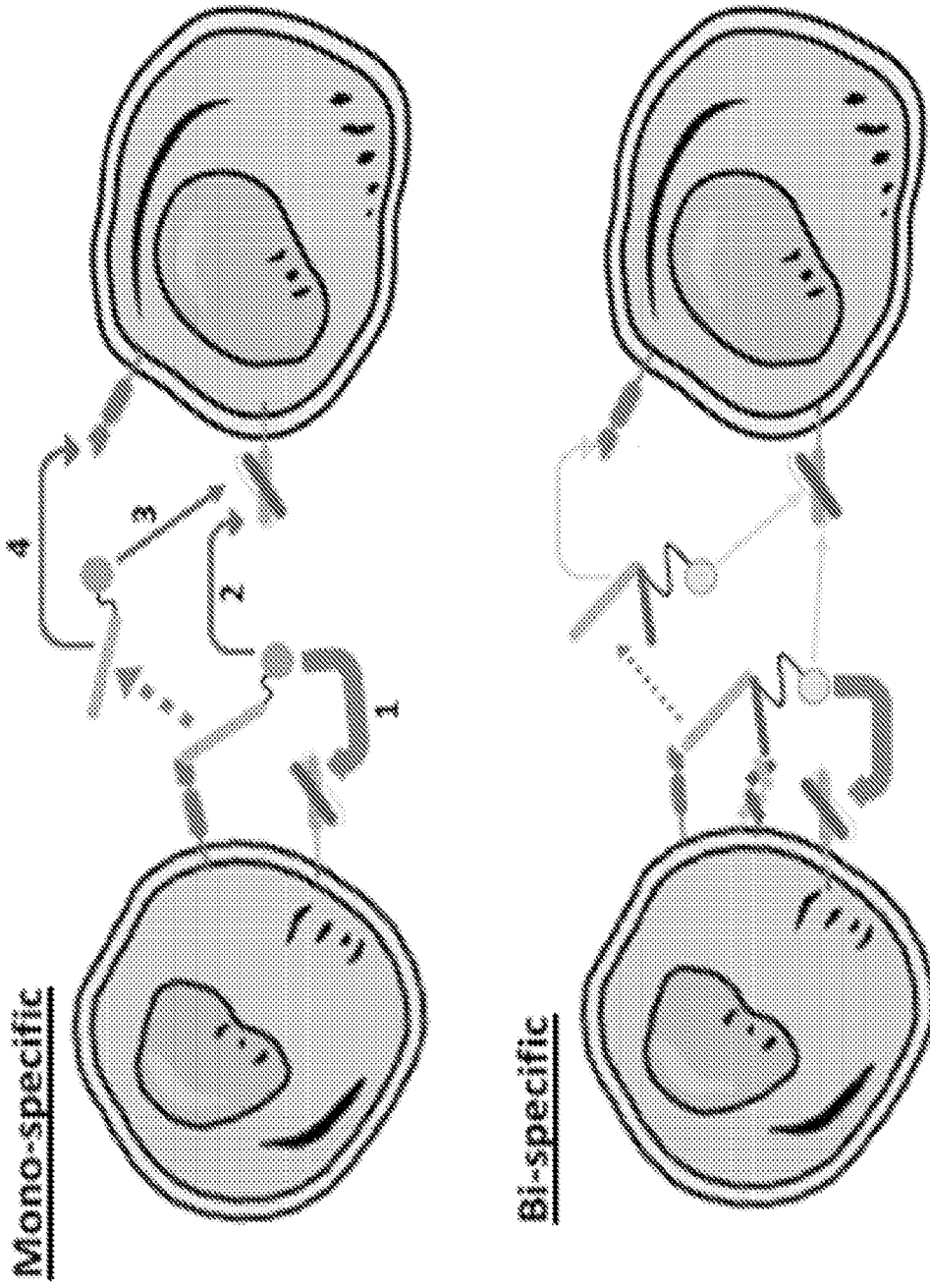


FIG. 25

FUSION MOLECULES TARGETING IMMUNE REGULATORY CELLS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Application Nos. 62/528,411 filed Jul. 3, 2017, 62/598,433 filed Dec. 13, 2017, 62/620,418 filed Jan. 22, 2018, 62/620,107 filed Jan. 22, 2018 and 62/657,455 filed Apr. 13, 2018, the entire disclosure of each of which is incorporated herein by reference.

SEQUENCE LISTING

[0002] The ASCII text file submitted herewith via EFS-Web, entitled “174285_011000_sequence.txt” created on Jul. 3, 2018, having a size of 189,027 bytes, is hereby incorporated by reference in its entirety. c1 FIELD

[0003] The present disclosure relates generally to modified immune regulatory cells and engineered fusion molecules. More specifically, the present disclosure relates to modified regulatory T cells and fusion proteins useful for targeted immune therapy and adoptive cell therapy.

BACKGROUND

[0004] Adoptive cellular therapies (ACT) are demonstrating clinical benefits across a range of disorders, with potentially fewer risks and greater efficacy than traditional pharmacological strategies. Regulatory T cells (Tregs) are currently undergoing clinical trials in various immune-mediated pathologies, including transplant rejection and autoimmune conditions. In general, cell therapy relies upon ex vivo expansion of the cell product. In vitro manipulation of cell therapy products, prior to administration to patients, offers the opportunity to enhance the efficacy of the final cell therapy product in other ways. For example, cells can be exposed to reagents that enhance their longevity or functional potency after transfer into the patient. Genetic modification strategies can even permit the design of cells with bespoke functionality. Crucially, in vitro manipulation of therapeutic cells in isolation can exert these influences upon the biology of the therapeutic cells, without systemic exposure of the patient to the reagents being used.

[0005] Specifically, current Treg manufacturing and ACT requires sorting of Tregs (e.g., CD4+CD25+CD127-by fluorescence-activated cell sorting, FACS) from a patient sample, ex vivo expansion of Tregs, often by expanding the Tregs using interleukin 2 (IL-2) or activating and expanding the T cells by using CD3 and CD28 stimulation and IL-2, and then subsequently administering the Tregs to the patient; see FIG. 1A. However, Treg cell therapy presents several key challenges: it requires GMP (good manufacturing practice) level sorting and long and costly ex vivo expansion; significant deficiencies in Treg viability and activity after freezing and thaw can exist; and cells can exhibit short-term survival following reinfusion into the patient.

[0006] Thus, compositions and methods to improve Treg-based cell therapies hold strong potential for delivering meaningful clinical benefits.

SUMMARY

[0007] Disclosed herein are methods and compositions for Treg-targeting fusion molecules (“FMs”) to improve

engraftment, survival and efficacy of adoptive cell therapies, e.g., immunosuppressive cell therapy. In various embodiments, the Treg-targeting fusions can be combined with the backpack technology as disclosed in, e.g., U.S. Publication No. 2017/0080104, U.S. Pat. No. 9,603,944, U.S. Publication No. 2014/0081012, and PCT Application No. PCT/US2017/037249, each of which is incorporated herein by reference in its entirety.

[0008] In one aspect, provided herein is a fusion molecule, comprising:

[0009] (a) a cytokine or growth factor molecule; and

[0010] (b) a regulatory T cell (Treg) targeting moiety comprising an antigen-binding fragment of an antibody having an affinity to an antigen on the surface of a Treg;

[0011] wherein the cytokine or growth factor molecule is operatively linked to the antigen-binding fragment.

[0012] In some embodiments, the antigen is one or more of CD45, CD4, CD25, CD39, Neuropilin 1 (NRP1), or a variant of any of the foregoing. In some embodiments, the targeting moiety comprises a bispecific molecule having an antigen-binding fragment specific for CD4 and an antigen-binding fragment specific for CD25. In some embodiments, the antigen is one or more of CD4, CD45, CD3, CD2, CD25, CD127, CD197 (CCR7), CXCR3, CXCR4, CXCR5, CD38, CD27, CCR4, CCR5, CD137, CD39, CCR4, CCR5, CCR6 (CD196), CCR8, CCR10, OX40, GITR, CTLA4, LAG3, CD73, CD103, CD62L, CCR2, CCR9, Neuropilin 1 (NRP1), CD8, CD11a, CD18, or a variant of any of the foregoing. In some embodiments, the cytokine or growth factor molecule is one or more of IL-15, IL-2, IL-18, IL-27, IL-10, IL-35, Amphiregulin, IL-33, or TGF- β , or a variant of any of the foregoing. In some embodiments, the cytokine or growth factor molecule is one or more of IL-15, IL-2, IL-18, IL-27, IL-10, IL-35, Amphiregulin, IL-33, TGF- β , IL-7, or IL-21, or a variant of any of the foregoing. In some embodiments, the cytokine or growth factor molecule is one or more of IL-15, IL-2, IL-18, IL-27, IL-10, IL-35, Amphiregulin, IL-33, TGF- β , IL-7, IL-21, IL-6, IL-12, or IL-23, or a variant of any of the foregoing. In some embodiments, the Treg is a healthy and/or non-malignant Treg.

[0013] In some embodiments, the fusion molecule can further include a linker for operably linking the cytokine or growth factor molecule and the targeting moiety. In some embodiments, the linker is selected from one or more of a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, or a non-helical linker. In some embodiments, the peptide linker is a human serum albumin or a variant thereof. In some embodiments, the linker, at either end, is fused to the cytokine or growth factor molecule and the targeting moiety via a peptide comprising Gly and Ser. In some embodiments, the peptide is a (GGGS)_N (SEQ ID NO: 87) or (GGGS)_N (SEQ ID NO: 88) linker, where N indicates the number of repeats of the motif and is an integer selected from 1-10.

[0014] In some embodiments, the antigen-binding fragment is a Fab fragment comprising a light chain and a heavy chain fragment linked by a disulfide bond, and wherein the cytokine or growth factor molecule is operably linked to the Fab fragment at a C-terminus of the light chain, an N-terminus of the light chain, a C-terminus of the heavy chain fragment, or an N-terminus of the heavy chain fragment. In some embodiments, the targeting moiety localizes the cytokine or growth factor molecule to the Treg and enhances its

concentration, distribution, duration of exposure and/or availability on a cell surface of the Treg.

[0015] A further aspect relates to a fusion molecule, comprising:

[0016] (a) a cytokine or growth factor molecule; and

[0017] (b) a Treg targeting moiety comprising an antibody having an antigen-binding site specific for an antigen on the surface of a Treg, wherein the antibody comprises a light chain having a C-terminus and an N-terminus, and a heavy chain having a C-terminus and an N-terminus,

[0018] wherein the light chain is linked to the heavy chain by a disulfide bond, wherein the cytokine or growth factor molecule is operably linked to the antibody at the C-terminus of the light chain, the N-terminus of the light chain, or the N-terminus of the heavy chain portion.

[0019] In some embodiments, the antigen is a CD4 or a CD25 receptor expressed on the cell surface of the Treg. In some embodiments, the cytokine or growth factor molecule comprises an IL-2, or a variant thereof. In some embodiments, the cytokine or growth factor molecule is operably linked to the antibody by a linker. In some embodiments, the linker is selected from a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, and a non-helical linker. In some embodiments, the linker is a peptide linker comprising a Gly and a Ser. In some embodiments, the peptide linker is a (GGGS)_N (SEQ ID NO: 87) or (GGGGS)_N (SEQ ID NO: 88) linker, wherein _N indicates the number of repeats of the motif and is an integer selected from 1-10.

[0020] In another aspect, provided herein is a fusion molecule (FM), comprising: (i) a regulatory T cell (Treg) targeting moiety having an affinity with an immune cell surface receptor on the Treg, wherein the targeting moiety is selected from an antibody or antigen-binding fragment thereof, a non-antibody scaffold, or a ligand that binds to the immune cell surface receptor; and (ii) a cytokine or growth factor molecule; wherein the cytokine or growth factor molecule and the targeting moiety are operably linked together as a fusion molecule.

[0021] In some embodiments, the immune cell surface receptor is selected from one or more of CD45, CD4, CD25, CD39, Neuropilin 1 (NRP1), or variants thereof. In one embodiment, the targeting moiety comprises a bispecific molecule that binds to both CD4 and CD25. In some embodiments, the immune cell surface receptor is selected from one or more of CD4, CD45, CD3, CD2, CD25, CD127, CD197 (CCR7), CXCR3, CXCR4, CXCR5, CD38, CD27, CCR4, CCR5, CD137, CD39, CCR4, CCR5, CCR6 (CD196), CCR8, CCR10, OX40, GITR, CTLA4, LAG3, CD73, CD103, CD62L, CCR2, CCR9, Neuropilin 1 (NRP1), CD8, CD11a, or CD18, or variants thereof.

[0022] In some embodiments, the cytokine or growth factor molecule is selected from one or more of IL-15, IL-2, IL-18, IL-27, IL-10, IL-35, Amphiregulin, IL-33, or TGF- β , or variants thereof. In some embodiments, the cytokine or growth factor molecule is selected from one or more of IL-15, IL-2, IL-18, IL-27, IL-10, IL-35, Amphiregulin, IL-33, TGF- β , IL-7, IL-21, IL-6, IL-12, or IL-23, or variants thereof.

[0023] In various embodiments, the Treg targeted by the FMs disclosed herein is a healthy and/or non-malignant

Treg. In various embodiments, the Treg targeted by the FMs disclosed herein is obtained from patients in need of immunosuppressive therapy that may or may not be marked by Treg dysfunction (e.g., an unhealthy or abnormal Treg). In certain embodiments, the targeting moiety localizes the cytokine or growth factor molecule to the Treg and enhances its concentration, distribution, duration of exposure and/or availability on a cell surface of the Treg.

[0024] The FM, in some embodiments, can further include a linker for operably linking the cytokine or growth factor molecule and the targeting moiety. The linker can be selected from one or more of a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, or a non-helical linker. The peptide linker can be human serum albumin or an Fc domain. The linker, at either end, can be fused to the cytokine or growth factor molecule and the targeting moiety via a peptide comprising Gly and Ser. In some embodiments, the peptide is a (GGGS)_N (SEQ ID NO: 87) or (GGGGS)_N (SEQ ID NO: 88) linker, where N indicates the number of repeats of the motif and is an integer selected from 1-10.

[0025] In some embodiments, any of the fusion molecules disclosed herein can further include a nanoparticle, a liposome and/or a biodegradable polymer. In some embodiments, the nanoparticle comprises a protein nanogel, a nucleotide nanogel, a polymer nanoparticle, or a solid nanoparticle. In some embodiments, the nanoparticle comprises a protein nanogel. In some embodiments, the nanoparticle optionally comprises at least one polymer, cationic polymer, or cationic block co-polymer on the nanoparticle surface. In some embodiments, the nanoparticle comprises a nanogel that is cross linked by a reversible linker that is sensitive to redox (disulfide) or pH (hydrolysable groups) or enzymes (proteases).

[0026] Another aspect relates to a modified Treg comprising:

[0027] (a) a fusion molecule comprising

[0028] (i) a cytokine or growth factor molecule; and

[0029] (ii) a Treg targeting moiety having an affinity to a Treg cell surface antigen; and

[0030] (b) a Treg, wherein the fusion molecule is bound to the surface of Treg through interaction with the cell surface antigen.

[0031] A further aspect relates to a modified Treg comprising a Treg and any of the fusion molecule disclosed herein bound thereto.

[0032] Also provided herein is a method of preparing modified Tregs, comprising:

[0033] (a) providing a population of Tregs; and

[0034] (b) incubating the fusion molecule disclosed herein with the population of Tregs so as to permit targeted binding of the fusion molecule thereto, thereby producing a population of Tregs having fusion molecules bound on the cell surface.

[0035] Another aspect relates to a composition for use in cell therapy, the composition comprising:

[0036] (a) a plurality of fusion molecules, each fusion molecule comprising

[0037] (i) a cytokine or growth factor molecule; and

[0038] (ii) a Treg targeting moiety having an affinity to a cell surface antigen of a Treg;

[0039] (b) a population of Tregs, wherein the plurality of fusion molecules are bound to the surface of the Treg through interaction with the cell surface antigen; and

[0040] (c) a pharmaceutically acceptable carrier, excipient, or stabilizer.

[0041] A further aspect relates to a method for the suppressing or preventing an immune response in a human subject, the method comprising administering to the human subject a cell therapeutic composition, the composition comprising:

[0042] (a) a plurality of fusion molecules, each fusion molecule comprising

[0043] (i) a cytokine or growth factor molecule; and

[0044] (ii) a Treg targeting moiety having an affinity to a cell surface antigen of a Treg; and

[0045] (b) a population of Tregs,

[0046] wherein the plurality of fusion molecules are bound to the surface of the Tregs, and wherein the cytokine or growth factor molecule acts in vivo upon the population of Tregs in the human subject to suppress or prevent an immune response in the human subject.

[0047] Also provided herein is a composition comprising:

[0048] (a) a fusion molecule comprising

[0049] (i) a cytokine or growth factor molecule; and

[0050] (ii) an immune cell targeting moiety having an affinity to a Treg cell surface antigen;

[0051] (b) a Treg expressing or otherwise displaying the cell surface antigen, wherein the fusion molecule is bound to the surface of the Treg through interaction with the cell surface antigen; and

[0052] (c) a nanoparticle, nanogel, or liposome.

[0053] Another aspect relates to an isolated nucleic acid molecule engineered to encode the FM disclosed herein.

[0054] A further aspect relates to a vector comprising the nucleic acid molecule disclosed herein.

[0055] Still another aspect relates to a host cell comprising the nucleic acid molecule disclosed herein or the vector disclosed herein.

[0056] Also provided herein is method of making the FM disclosed herein, comprising culturing the host cell disclosed herein under suitable conditions.

[0057] In another aspect, a pharmaceutical composition comprising the FM disclosed herein and a pharmaceutically acceptable carrier, excipient, or stabilizer is provided.

[0058] In a further aspect, a modified Treg, comprising a healthy and/or non-malignant Treg and the FM disclosed herein bound or targeted thereto is provided.

[0059] In still another aspect, provided herein is a method for in vitro or ex vivo expansion of Tregs, comprising providing a population of PBMCs from a subject, and selectively expanding Tregs therein in the presence of a plurality of FMs disclosed herein, thereby producing a plurality of expanded Tregs.

[0060] In another aspect, provided herein is a method for providing immunosuppressive therapy, comprising administering to a subject in need thereof a plurality of the fusion molecules disclosed herein, a plurality of the modified Tregs disclosed herein, or the plurality of expanded Tregs disclosed herein.

[0061] In still another aspect, provided herein is a method for in vivo expansion of Tregs, comprising loading a population of sorted Tregs or PBMCs obtained from a subject with CD3/CD28/IL-2 backpacks to provide loaded cells, and administering the loaded cells to the subject without in vitro culturing.

[0062] In a further aspect, provided herein is a method for providing immunosuppressive therapy, comprising admin-

istering to a subject in need thereof a plurality of FMs disclosed herein, a plurality of the modified Tregs disclosed herein, the plurality of expanded Tregs disclosed herein, or the loaded cells disclosed herein. The immunosuppressive therapy can be used to treat diseases such as allo-immune diseases, auto-immune diseases, allergy, and inflammatory diseases. The allo-immune diseases can include, for example, organ transplant rejection, graft versus host disease or rejection (GVHD) (e.g., post-allogeneic hematopoietic stem cell transplant (HSCT) and other post-allogeneic stem cell transplantation (SCT), and rejection of autologous stem cells and/or genetically modified autologous stem cells (e.g., where CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) or the like is used to modify cells ex vivo to fix some deficiency which are then transplanted back into the patient, resulting in rejection due to any new or modified proteins being recognized as antigenic). The auto-immune diseases can include, for example, Type 1 diabetes, Multiple Sclerosis and Alopecia. The inflammatory diseases can include, for example, Inflammatory Bowel Disease, Rheumatoid Arthritis and Lupus.

[0063] In another aspect, the disclosure provides a particle, e.g., a nanoparticle, that comprises an FM as described herein, e.g., nanoparticle that comprises a protein (e.g., a protein nanogel as described herein). In one embodiment, the particle comprises the same FM. In other embodiments, the particle comprises one or more different types of FM. Nanoparticles and methods of making are disclosed in PCT International Application No. PCT/US2017/037249 filed Jun. 13, 2017, e.g., on pages 57-79, which is incorporated herein by reference in its entirety.

[0064] Compositions, e.g., pharmaceutical compositions, comprising the FMs and/or the particles disclosed herein, are also disclosed. In embodiments, the pharmaceutical compositions further include a pharmaceutically acceptable carrier, excipient, or stabilizer.

[0065] In some embodiments, the FM is a bifunctional or bispecific molecule, e.g., it has at least two different kinds of members, e.g., with different functions and/or binding specificities. For example, the FM can comprise, or consist of, a modulatory moiety, e.g., a cytokine molecule, and a targeting moiety, wherein the modulatory moiety and the targeting moiety bind to two different cell surface targets or receptors in the same or different immune regulatory cells. In some embodiments, the modulatory moiety and the targeting moiety bind to two different targets on the same regulatory T cell. A bifunctional or bispecific molecule can further comprise additional moieties, e.g., further binding and/or functional moieties. For example, the FM can be a multifunctional or multispecific molecule, e.g., it is a trifunctional or trispecific, or a tetrafunctional or tetraspecific, fusion molecule.

[0066] In certain embodiments, the FM can be represented with the following formula in an N to C terminal orientation: R1-(optionally L1)-R2 or R2-(optionally L1)-R1; wherein R1 comprises a targeting moiety, L1 comprises a linker (e.g., a peptide linker described herein), and R2 comprises a modulatory moiety, e.g., a cytokine or growth factor molecule.

[0067] In some embodiments, the modulatory moiety, e.g., the cytokine molecule, is functionally linked, e.g., covalently linked (e.g., by chemical coupling, genetic or protein fusion, noncovalent association or otherwise) to the targeting moiety. For example, the modulatory moiety can be

covalently coupled indirectly, e.g., via a linker to the targeting moiety. In some embodiments, the linker is chosen from: a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, or a non-helical linker. In some embodiments, the linker is a peptide linker. The peptide linker can be 1-30, 1-5, 5-20, 8-18, 10-15, or about 8, 9, 10, 11, 12, 13, 14, 15-20, 20-25, or 25-30 amino acids long. In some embodiments, the peptide linker can be 30 amino acids or longer; e.g., 30-35, 35-40, 40-50 50-60 amino acids long. In some embodiments, the peptide linker comprises Gly and Ser, e.g., a linker comprising the amino acid sequence $(\text{Gly}_3\text{-Ser})_n$ or $(\text{Gly}_4\text{-Ser})_2$, wherein n indicates the number of repeats of the motif, e.g., $n=1, 2, 3, 4$ or 5 (e.g., a $(\text{Gly}_3\text{-Ser})_2$ or $(\text{Gly}_4\text{Ser})_2$, or a $(\text{Gly}_3\text{-Ser})_3$ or a $(\text{Gly}_4\text{Ser})_3$ linker). In some embodiments, the linker comprises the amino acid sequence of SEQ ID NO: 36, 37, 38, or 39, or an amino acid sequence substantially identical thereto (e.g., having 1, 2, 3, 4, or 5 amino acid substitutions). In one embodiment, the linker comprises an amino acid sequence GGGSGGGS (SEQ ID NO: 37). In another embodiment, the linker comprises amino acids derived from an antibody hinge region. In certain embodiments, the linker comprises amino acids derived from the hinge regions of IgG1, IgG2, IgG3, IgG4, IgGM, or IgGA antibodies. In embodiments, the linker comprises amino acids derived from an IgG hinge region, e.g., an IgG1, IgG2 or IgG4 hinge region. For example, the linker comprises a variant amino acid sequence from an IgG hinge, e.g., a variant having one or more cysteines replaced, e.g., with serines. In some embodiments, the linker comprises DKTHTCPPSPCAPE (SEQ ID NO: 91), having one or both cysteines replaced with another amino acid, e.g., a serine. In some embodiments, the linker comprises amino acids DKTHTSPPSPAP (SEQ ID NO: 38), EPKSSDKTH-TSPSPCAPE (SEQ ID NO: 92), or a derivative thereof. In embodiments, the linker comprises amino acids derived from an IgG2 hinge region, e.g., amino acids SVESPPSP (SEQ ID NO: 93), ERKSSVESPPSP (SEQ ID NO: 94), or a derivative thereof. In embodiments, the linker comprises amino acids derived from an IgG4 hinge region, e.g., amino acids PPSPSP (SEQ ID NO: 95), ESKYGPPSPSP (SEQ ID NO: 96), or a derivative thereof.

[0068] In other embodiments, the linker is a non-peptide, chemical linker. For example, the modulatory moiety is covalently coupled to the targeting moiety by crosslinking. Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (e.g., *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). In yet other embodiments, the modulatory moiety is directly covalently coupled to the targeting moiety, without a linker. In yet other embodiments, the modulatory moiety and the targeting moiety of the FM are not covalently linked, e.g., are non-covalently associated.

[0069] In other embodiments, the linker can be a protein or a fragment or derivative thereof, e.g., human albumin or an Fc domain, or a fragment or derivative thereof. In some embodiments, the targeting moiety is linked to the N-terminus and the modulatory moiety is linked to the C-terminus.

[0070] In other embodiments, the linker non-covalently associates the targeting moiety to the modulatory moiety. For example, the linker comprises a dimerization domain, e.g., a coiled coil or a leucine zipper.

[0071] In other embodiments, the linker is a reversible linker, in which the association between targeting moiety and the modulatory moiety can be reversed. In some embodiments the linker can be degraded by hydrolysis, reducing environments, or enzymes. In other embodiments, the linker can be degraded by hydrolysis in a pH-dependent manner. In other embodiments the linker can be reversed by redox state (e.g. linker containing disulfide bonds). In other embodiments the linker can be reversed by enzymatic cleavage. In some embodiments the linker can be cleaved by a matrix metalloproteinase (MMP; e.g. MMP-1, MMP-2, MMP-3, or MMP-9), a disintegrin and metalloprotease (ADAM; e.g. ADAM-17), a plasminogen activator (e.g. urokinase-type plasminogen activator, uPA, or tissue plasminogen activator, tPA), or a granzyme (e.g. granzyme A or granzyme B).

[0072] Other features, objects, and advantages of the present disclosure will be apparent from the description and drawings, and from the claims.

[0073] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this present disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

[0074] FIG. 1A depicts the current Treg manufacturing and ACT, and obstacles associated therewith.

[0075] FIG. 1B depicts exemplary fusion proteins of the present disclosure combining a cytokine and an immunoglobulin moiety for cell-surface targeting and stimulation.

[0076] FIG. 1C depicts an exemplary process for ex vivo expansion of Tregs.

[0077] FIG. 1D depicts an exemplary process for in vivo expansion of Tregs, without culturing ex vivo.

[0078] FIG. 1E depicts exemplary process for IL-2 loading-driven survival, expansion and engraftment of Tregs.

[0079] FIG. 1F depicts exemplary applications of Treg cell therapy in connection with various cytokines or growth factors.

[0080] FIG. 1G depicts generation of Treg specific scFvs via phase display and Treg-targeting of exemplary tethered fusions.

[0081] FIG. 1H depicts exemplary diseases that can benefit from Treg cell therapies disclosed herein.

[0082] FIGS. 2A-2C depict FMs comprising various antibody formats. FIG. 2A depicts a schematic of IL-15 FMs comprising various antibody formats. FIGS. 2B and 2C depict evaluation of FMs on CD8 T cell expansion in pulse assay using CellTiter Blue (FIG. 2B) or flow cytometry counting beads (FIG. 2C).

[0083] FIGS. 3A-3D depict a schematic for various fusion strategies between the IL-15/sushi complex and an anti-CD45 antibody. FIGS. 3A and 3B depict schematics for fusion of IL-15 to the N- or C-terminus of chBC8 IgG (FIG. 3A) or chBC8 Fab (FIG. 3B) light-chain. Antibody fusions comprise a complex with IL-15R α -sushi through a high-affinity interaction with IL-15.

[0084] FIGS. 3C and 3D depict schematics for fusion of IL-15R α -sushi to the N- or C-terminus of chBC8 IgG (FIG. 3C) or chBC8Fab (FIG. 3D) light-chain. Antibody fusions comprise a complex with IL-15^{N72D} through a high-affinity interaction with IL-15R α -sushi.

[0085] FIG. 3E depicts a schematic design for a bivalent (bispecific) fusion, comprising 2 anti-CD45 antibodies (h9.4 Fab and BC8 scFv) fused to the IL-15/sushi complex.

[0086] FIGS. 4A-4B depict the effect of FMs comprising various fusion strategies on CD8 T cell expansion. (FIGS. 4A and 4B) FMs comprising IL-15^{N72D} and chBC8 Fab (FIG. 4A) or chBC8 IgG (FIG. 4B) were evaluated for T cell expansion in pulse bioassay. CD8 T cell proliferation was analyzed using CellTiter Blue; IL15^{N72D}/sushi^{L77L}-Fc and unstimulated T-cells (neg ctrl) were included for comparison.

[0087] FIGS. 5A-5D depict biological activity of FMs comprising wild-type or mutated IL-15 and various fusion strategies of IL-15/sushi complex to anti-CD45 antibody. FIGS. 5A and 5B depict FMs comprising wild-type IL-15 or IL-15^{N72D} and chBC8 Fab (FIG. 5A) or chBC8 IgG (FIG. 5B) that were evaluated for CD8 T cell expansion in pulse assay using CellTiter Blue three days after pulse incubation with FMs. FIGS. 5C and 5D depict the evaluation of FMs comprising chBC8 Fab (FIG. 5C) or chBC8 IgG (FIG. 5D) on CD8 T cell expansion in a constant exposure, e.g., static, assay format using CellTiter Blue following incubation with FMs for three days.

[0088] FIGS. 6A-6B depict FMs comprising C-terminal fusion to chBC8 IgG light- or heavy-chain. FIG. 6A depicts schematics of FMs comprising IL-15 fused to the C-terminus of chBC8 IgG light- or heavy-chain and a noncovalent complex between IL-15 and IL-15R α -sushi. FIG. 6B depicts effects of FMs on CD8 T cell expansion in a pulse bioassay.

[0089] FIGS. 7A-7C depict FMs require functional binding of the antibody fragment for improved activity. FIGS. 7A and 7B depict IL-15 FMs comprising chBC8 Fab (FIG. 7A) or chBC8 IgG (FIG. 7B) in the presence or absence of soluble BC8 IgG competitor that were evaluated for expansion of CD8 T cells in pulse assay using CellTiter Blue. FIG. 7C depicts evaluation of IL-15 FM comprising a humanized BC8 Fab fragment or a humanized BC8 Fab fragment containing a mutated CDR-H3 that ablates binding to CD45 for CD8 T cell expansion in pulse bioassay.

[0090] FIGS. 8A-8C depict cell surface loading and persistence of FMs. FIG. 8A depicts flow cytometry histograms showing MFI after pulse of FMs as detected by staining with fluorescently labeled anti-IL15 or anti-IgG antibodies. FIG. 8B depicts persistence of cell surface staining of IL-15 over time. FIG. 8C depicts cell density over time, measured using flow cytometry counting beads.

[0091] FIG. 9A-9B depict evaluation of FMs comprising varied linker composition between IL-15 and chBC8. FIG. 9A depicts amino acid sequences of the three different linkers for fusion of IL-15 to the C-terminus of chBC8 Fab light-chain. FIG. 9B depicts evaluation of FMs comprising different linker compositions for CD8 T cell expansion in pulse bioassay using CellTiter Blue.

[0092] FIGS. 10A-10B depict evaluation of FMs comprising alternative CD45 antibody clones. FIG. 10A depicts binding affinity of various CD45 antibody clones to CD45 expressed on T cells. FIG. 10B depicts evaluation of IL-15 FMs comprising different anti-CD45 antibody clones in pulse bioassay using CellTiter Blue.

[0093] FIGS. 11A-11B depict FMs comprising antibodies targeting CD8, CD11a, or CD18. FIG. 11A depicts a schematic of IL-15 FMs comprising fusion of IL-15 to the light-chain C-terminus of a Fab antibody fragment targeting alternative cell surface receptors; FMs comprise a noncovalent association between IL-15 and IL-15R α -sushi. FIG. 11B depicts evaluation of IL-15 FMs for CD8 T cell expansion in pulse bioassay using CellTiter Blue.

[0094] FIGS. 12A-12B depict evaluation of FM potency comprising anti-CD8 on CD4 or CD8 T cells. FIG. 12A depicts a schematic of IL-15 FM comprising fusion of IL-15 to the C-terminus of an anti-CD8 Fab light-chain, and a noncovalent association with IL-15R α -sushi. FIG. 12B depicts evaluation of potency of anti-CD8 FM on purified CD4 or CD8 T cells in pulse bioassay using CellTiter Blue. CD4 or CD8 T cells were purified from total T cells (which naturally comprise mixtures of CD4 and CD8 T cells) using magnetic bead sorting.

[0095] FIGS. 13A-13C depict graphs showing that nanoparticles comprising IL-15 FMs support T cell expansion. FIGS. 13A and 13B depict crosslinking of FM into protein nanogels analyzed by size-exclusion chromatography (FIG. 13A); protein nanogels comprising IL15^{N72D}/sushiL77L-Fc are shown for comparison (FIG. 13B). Backpacks comprising IL-15 FM or IL15^{N72D}/sushiL77L-Fc were evaluated for CD8 T cell expansion in pulse bioassay (FIG. 13C). Cell density was analyzed using flow cytometry counting beads; data are presented as fold expansion.

[0096] FIG. 14 shows example immunotargeted surface receptors and cytokines, illustrating versatility of the platform.

[0097] FIGS. 15A-15G show tethered fusion platform enables selective cell targeting and improved expansion of CD8 T cells in cultures of activated total CD3 human T cells by pulse incubation with CD8-targeted FMs.

[0098] FIGS. 16A-16E shows cellular activities of various mutated forms of IL-15 and surface persistence of FMs comprising IL-15 mutants.

[0099] FIGS. 17A-17C shows selective cell targeting and CD8 T cell expansion by CD8-targeted FMs comprising wild-type or mutated IL-15.

[0100] FIG. 17D shows surface persistence of CD45-targeted FM comprising wild-type or mutated IL-15.

[0101] FIG. 18 shows enrichment of Tregs following bead-based magnetic sorting for CD4⁺/CD25⁺/CD127^{dim} cells. These Treg enriched CD4s were used to test in vitro and in vivo expansion driven by IL-2 tethered fusions.

[0102] FIG. 19 depicts a strategy for limiting inflammatory and auto-immune diseases via specific targeting of IL-2 to Treg cells.

[0103] FIG. 20 shows titration of Fab-HSA-IL2 on Treg-enriched CD4s. Fab-HSA-IL2 shows titratable surface binding immediately post-loading and for more than 3 days. In addition, Fab-HSA-IL2 -loaded cells show dose-dependent proliferation, and even very low doses maintains viability.

[0104] FIG. 21 relates surface staining of pulsed IL-2 tethered fusions (left panel) with proliferation and survival of Treg-enriched cells (right panels). All three tested tethered fusions exhibited strong staining and drove proliferation and survival of the cells. Proliferation and survival were comparable to constant stimulation with recombinant IL-2.

[0105] FIG. 22 illustrates the effects of pulsed IL-2 tethered fusions over time. Cells pulsed with any of the TF constructs showed equal or better expansion compared to

constant stimulation with rhIL2 through 6 days post-pulse, and comparable expansion and survival out to 9 days. IL-2 tethered fusions promote proliferation and long-term survival of Treg-enriched cell populations in vitro.

[0106] FIG. 23 shows increased expansion of IL-2 tethered fusion pulsed Treg-enriched CD4 cells in the blood of NSG mice. There were increased numbers of CD4 cells in the IL-2 tethered fusion-pulsed group throughout the course of the experiment. This indicates that IL-2 tethered fusions can increase expansion and engraftment in vivo.

[0107] FIG. 24 shows that the Fab-HSA-IL2 preferentially supports proliferation and survival of loaded cells. Despite having some pro-proliferative effect on non-pulsed cells, there is still stronger effects on the pulsed cells, and the CD4:CD8 ratio is higher in the Fab-HSA-IL2 group than the control group.

[0108] FIG. 25 provides a schematic describing how dual-targeting, and cytokine affinity modulation will increase on-target effects while reducing off-target effects of IL-2 antibody tethered fusion molecules.

DETAILED DESCRIPTION

[0109] The present disclosure provides, inter alia, compositions and methods for preparation and use of fusion molecules or FMs. An “FM” as described herein includes a modulatory moiety, e.g., a cytokine or growth factor molecule (e.g., a biologically active cytokine), and a targeting moiety, e.g., an antibody molecule (e.g., an antibody or antibody fragment) capable of binding to Tregs. In embodiments, the modulatory moiety and the targeting moiety are operably linked or tethered together (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise). As such, the fusion molecules are also referred to as “tethered fusions” herein. In some embodiments, the targeting moiety is capable of binding to Treg cell surface target, thereby targeting the modulatory moiety, e.g., cytokine molecule, to the Treg.

[0110] In embodiments, the Treg-targeting tethered fusions disclosed herein can be used to improve engraftment, survival and efficacy of adoptive cell therapies, e.g., immunosuppressive cell therapy. In various embodiments, the Treg-targeting tethered fusions, or the cytokine molecules alone (e.g., without Treg-targeting moiety) can be combined with nanoparticle, nanogel and liposome technology as disclosed in, e.g., U.S. Publication No. 2017/0080104, U.S. Pat. No. 9,603,944, U.S. Publication No. 2014/0081012, and PCT Application No. PCT/US2017/037249, each of which is incorporated herein by reference in its entirety.

[0111] The Treg-targeting tethered fusions and related immunosuppressive cell therapy disclosed herein are advantageous compared to conventional Treg manufacturing and ACT. Conventional Treg manufacturing requires GMP sorting of pure Tregs. In contrast, as shown in FIG. 1C, using Treg-targeting tethered fusions such as Treg-targeted IL-2, selective expansion (ex vivo) of Tregs from a mixed population of peripheral blood mononuclear cells (PBMC) can be achieved, thereby avoiding expensive and time-consuming GMP sorting of Tregs. Furthermore, by further subjecting the cells to loading (e.g., with IL-2 backpacks), survival and activity of the cells post-thaw can be improved.

[0112] Conventional Treg manufacturing additionally requires long and costly ex vivo expansion. In contrast, as shown in FIG. 1D, sorted Tregs or PBMC without sorting

can be subject to loading (e.g., CD3/CD28/IL-2) followed by direct infusion to patient, whereby the Tregs are expanded in vivo, without in vitro culturing. Furthermore, loaded Tregs (e.g., with IL-2 backpacks), prepared either from ex vivo expansion or without culturing, can receive pro-survival signal in vivo, increasing expansion and engraftment (FIG. 1E).

[0113] Without wishing to be bound by theory, binding of the tethered fusions to Tregs, e.g., via Treg cell surface target is believed to increase the concentration, e.g., the concentration over time, of the cytokine or growth factor molecule, on the surface of the Treg. This can result in an effect (e.g., stimulatory) on the Treg itself bound by the tethered fusions (autocrine signaling), or on another (e.g., neighboring) immune cell (paracrine signaling). In embodiments, the targeting moiety results in an increase in one or more of: binding, availability, activation and/or signaling of the cytokine or growth factor on the Treg, e.g., over a specified amount of time. For example, CD4/CD25 targeting moieties can result in binding specifically to Tregs. CD45 targeting moieties can be used to target Tregs, although not specifically. In embodiments, the FM does not substantially interfere with the signaling function of the cytokine or growth factor. Such targeting effect results in localized and prolonged stimulation of proliferation and activation of the Tregs, thus inducing controlled Treg expansion and/or activation, and Treg-mediated suppression of an immune response.

[0114] In some embodiments, the targeting moiety can be an antibody molecule or a ligand molecule that binds to a Treg cell surface receptor, e.g., CD45, CD4, CD25, CD39, or Neuropilin 1 (NRP1). In some embodiments, the Treg cell surface receptor is selected from one or more of CD4, CD45, CD3, CD2, CD25, CD127, CD197 (CCR7), CXCR3, CXCR4, CXCR5, CD38, CD27, CCR4, CCR5, CD137, CD39, CCR4, CCR5, CCR6 (CD196), CCR8, CCR10, OX40, GITR, CTLA4, LAG3, CD73, CD103, CD62L, CCR2, CCR9, NRP1, CD8, CD11a, CD18, or variants thereof.

[0115] In some embodiments, the cytokine or growth factor molecule is selected from one or more IL-15, IL-2, IL-18, IL-27, IL-10, IL-35, Amphiregulin, IL-33, or TGF- β , or variants thereof. In some embodiments, the cytokine or growth factor molecule is selected from one or more of IL-15, IL-2, IL-18, IL-27, IL-10, IL-35, Amphiregulin (Areg), IL-33, TGF- β , IL-7, or IL-21, or variants thereof. In some embodiments, the cytokine or growth factor molecule is selected from one or more of IL-15, IL-2, IL-18, IL-27, IL-10, IL-35, Amphiregulin, IL-33, TGF- β , IL-7, IL-21, IL-6, IL-12, or IL-23, or variants thereof. For example, as shown in FIG. 1F, TGF- β , IL10 and IL-33 can promote Treg differentiation and prevent conversion to inflammatory cells. IL-35 and IL-10 can promote iTreg in the periphery. Areg drives tissue regeneration during inflammation.

[0116] In one exemplary embodiment, the targeting moiety is derived from an anti-CD45 antibody molecule and the cytokine molecule is IL-2 or variants thereof.

[0117] Exemplary applications of immunosuppressive cell therapy include allo-immune diseases, auto-immune diseases, allergy, and inflammatory diseases (FIG. 1H). Allo-immune diseases can include, for example, organ transplant rejection, graft versus host disease or rejection (GVHD) (e.g., post-allogeneic hematopoietic stem cell transplant (HSCT) and other post-allogeneic stem cell transplantation

(SCT), and rejection of autologous stem cells and/or genetically modified autologous stem cells (e.g., where CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) or the like is used to modify cells ex vivo to fix some deficiency which are then transplanted back into the patient, resulting in rejection due to any new or modified proteins being recognized as antigenic). Treg number has been shown to correlate with GVHD control and organ transplant tolerance. For allergy, expansion of antigen-specific Tregs has been shown to associate with loss of milk allergy, and Treg depletion enhances allergy. Auto-immune diseases include Type 1 diabetes, Multiple Sclerosis and alopecia. These diseases have functional defects in Tregs, phenotypic instability and decreased number of Tregs. Inflammatory diseases include Crohn's Disease, Inflammatory Bowel Disease, Rheumatoid Arthritis and Lupus. These diseases can have increased Treg number, but are marked by decreased Treg function. Tregs have been shown to control/prevent these diseases in mouse models.

Definitions

[0118] Certain terms are defined herein below. Additional definitions are provided throughout the application.

[0119] As used herein, the articles “a” and “an” refer to one or more than one, e.g., to at least one, of the grammatical object of the article. The use of the words “a” or “an” when used in conjunction with the term “comprising” herein may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.”

[0120] As used herein, “about” and “approximately” generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are within 20 percent (%), typically, within 10%, and more typically, within 5% of a given range of values. The term “substantially” means more than 50%, preferably more than 80%, and most preferably more than 90% or 95%.

[0121] As used herein the term “comprising” or “comprises” is used in reference to compositions, methods, and respective component(s) thereof, that are present in a given embodiment, yet open to the inclusion of unspecified elements.

[0122] As used herein the term “consisting essentially of” refers to those elements required for a given embodiment. The term permits the presence of additional elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the disclosure.

[0123] The term “consisting of” refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[0124] The term “autologous” refers to any material derived from the same individual to whom it is later to be re-introduced into the individual.

[0125] The term “allogeneic” refers to any material derived from a different animal of the same species as the individual to whom the material is introduced.

[0126] “Antibody” or “antibody molecule” as used herein refers to a protein, e.g., an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable domain sequence. An antibody molecule encompasses antibodies (e.g., full-length antibodies) and antibody fragments. In an embodiment, an antibody molecule comprises an antigen binding or functional fragment of a full length

antibody, or a full length immunoglobulin chain. For example, a full-length antibody is an immunoglobulin (Ig) molecule (e.g., IgG) that is naturally occurring or formed by normal immunoglobulin gene fragment recombinatorial processes). In embodiments, an antibody molecule refers to an immunologically active, antigen-binding portion of an immunoglobulin molecule, such as an antibody fragment. An antibody fragment, e.g., functional fragment, is a portion of an antibody, e.g., Fab, Fab', F(ab')₂, F(ab)₂, variable fragment (Fv), domain antibody (dAb), or single chain variable fragment (scFv). A functional antibody fragment binds to the same antigen as that recognized by the intact (e.g., full-length) antibody. The terms “antibody fragment” or “functional fragment” also include isolated fragments consisting of the variable regions, such as the “Fv” fragments consisting of the variable regions of the heavy and light chains or recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker (“scFv proteins”). In some embodiments, an antibody fragment does not include portions of antibodies without antigen binding activity, such as Fc fragments or single amino acid residues. Exemplary antibody molecules include full length antibodies and antibody fragments, e.g., dAb (domain antibody), single chain, Fab, Fab', and F(ab')₂ fragments, and single chain variable fragments (scFvs). The terms “Fab” and “Fab fragment” are used interchangeably and refer to a region that includes one constant and one variable domain from each heavy and light chain of the antibody, i.e., V_L, C_L, V_H, and C_H1.

[0127] As used herein, an “immunoglobulin variable domain sequence” refers to an amino acid sequence which can form the structure of an immunoglobulin variable domain. For example, the sequence may include all or part of the amino acid sequence of a naturally-occurring variable domain. For example, the sequence may or may not include one, two, or more N- or C-terminal amino acids, or may include other alterations that are compatible with formation of the protein structure.

[0128] In embodiments, an antibody molecule is monospecific, e.g., it comprises binding specificity for a single epitope. In some embodiments, an antibody molecule is multispecific, e.g., it comprises more than one immunoglobulin variable domain sequence, where a first immunoglobulin variable domain sequence has binding specificity for a first epitope and a second immunoglobulin variable domain sequence has binding specificity for a second epitope. In some embodiments, an antibody molecule is a bispecific antibody molecule. “Bispecific antibody molecule” as used herein refers to an antibody molecule that has specificity for at least two (e.g., two, three, four, or more) epitopes and/or antigens.

[0129] “Antigen” (Ag) as used herein refers to a macromolecule, including all proteins or peptides. In some embodiments, an antigen is a molecule that can provoke activation of certain immune cells (including immune regulatory cells) and/or antibody generation. Any macromolecule, including almost all proteins or peptides, can be an antigen. Antigens can also be derived from genomic recombinant or DNA. For example, any DNA comprising a nucleotide sequence or a partial nucleotide sequence that encodes a protein capable of eliciting an immune response encodes an “antigen.” In embodiments, an antigen does not need to be encoded solely by a full-length nucleotide sequence of a gene, nor does an antigen need to be encoded

by a gene at all. In embodiments, an antigen can be synthesized or can be derived from a biological sample, e.g., a tissue sample, a tumor sample, a cell, or a fluid with other biological components.

[0130] The “antigen-binding site” or “antigen-binding fragment” or “antigen-binding portion” (used interchangeably herein) of an antibody molecule refers to the part of an antibody molecule, e.g., an immunoglobulin (Ig) molecule such as IgG, that participates in antigen binding. In some embodiments, the antigen-binding site is formed by amino acid residues of the variable (V) regions of the heavy (H) and light (L) chains. Three highly divergent stretches within the variable regions of the heavy and light chains, referred to as hypervariable regions, are disposed between more conserved flanking stretches called “framework regions” (FRs). FRs are amino acid sequences that are naturally found between, and adjacent to, hypervariable regions in immunoglobulins. In embodiments, in an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface, which is complementary to the three-dimensional surface of a bound antigen. The three hypervariable regions of each of the heavy and light chains are referred to as “complementarity-determining regions,” or “CDRs.” The framework region and CDRs have been defined and described, e.g., in Kabat, E.A., et al. (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) *J. Mol. Biol.* 196:901-917. Each variable chain (e.g., variable heavy chain and variable light chain) is typically made up of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the amino acid order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. Variable light chain (VL) CDRs are generally defined to include residues at positions 27-32 (CDR1), 50-56 (CDR2), and 91-97 (CDR3). Variable heavy chain (VH) CDRs are generally defined to include residues at positions 27-33 (CDR1), 52-56 (CDR2), and 95-102 (CDR3). One of ordinary skill in the art would understand that the loops can be of different length across antibodies and the numbering systems such as the Kabat or Chotia control so that the frameworks have consistent numbering across antibodies.

[0131] In some embodiments, the antigen-binding fragment of an antibody (e.g., when included as part of the fusion molecule of the present disclosure) can lack or be free of a full Fc domain. In certain embodiments, an antibody-binding fragment does not include a full IgG or a full Fc but may include one or more constant regions (or fragments thereof) from the light and/or heavy chains. In some embodiments, the antigen-binding fragment can be completely free of any Fc domain. In some embodiments, the antigen-binding fragment can be substantially free of a full Fc domain. In some embodiments, the antigen-binding fragment can include a portion of a full Fc domain (e.g., CH2 or CH3 domain or a portion thereof). In some embodiments, the antigen-binding fragment can include a full Fc domain. In some embodiments, the Fc domain is an IgG domain, e.g., an IgG1, IgG2, IgG3, or IgG4 Fc domain. In some embodiments, the Fc domain comprises a CH2 domain and a CH3 domain.

[0132] As used herein, “backpacks” refer to protein clusters that can be prepared by reacting, e.g., cross-linking

various therapeutic protein monomers. Optionally, a surface modification such as polycation can be introduced on the protein cluster, which can be loaded onto cell surface as cell membranes are negatively charged. Exemplary backpacking technology is disclosed in, e.g., U.S. Publication No. 2017/0080104, U.S. Pat. No. 9,603,944, U.S. Publication No. 2014/0081012, U.S. Provisional App. No. 62/554,058 filed Sep. 5, 2017 and PCT Application No. PCT/US2017/037249, each of which is incorporated herein by reference in its entirety.

[0133] As used herein, a “cytokine molecule” refers to a full length, a fragment, or a variant of a naturally-occurring, wild type cytokine (including fragments and functional variants thereof having at least 10% of the activity of the naturally-occurring cytokine molecule). In embodiments, the cytokine molecule has at least 30, 50, or 80% of the activity, e.g., the immunomodulatory activity, of the naturally-occurring molecule. In embodiments, the cytokine molecule further comprises a receptor domain, e.g., a cytokine receptor domain, optionally, coupled to an immunoglobulin Fc region. In other embodiments, the cytokine molecule is coupled to an immunoglobulin Fc region. In other embodiments, the cytokine molecule is coupled to an antibody molecule (e.g., an immunoglobulin Fab or scFv fragment, a Fab fragment, a FAB₂ fragment, or an affibody fragment or derivative, e.g. a sdAb (nanobody) fragment, a heavy chain antibody fragment, single-domain antibody, a bispecific or multispecific antibody), or non-antibody scaffolds and antibody mimetics (e.g., lipocalins (e.g. anticalins), affibodies, fibronectin (e.g., monobodies or Adnectins), knottins, ankyrin repeats (e.g., DARPins), and A domains (e.g. avimers)).

[0134] A “cytokine agonist,” as used herein can include an agonist of a cytokine receptor, e.g., an antibody molecule (e.g., an agonistic antibody) to a cytokine receptor that elicits at least one activity of a naturally-occurring cytokine.

[0135] As used herein, an “immune cell” refers to any of various cells that function in the immune system, e.g., to protect against agents of infection and foreign matter. In embodiments, this term includes leukocytes, e.g., neutrophils, eosinophils, basophils, lymphocytes, and monocytes. The term “immune cell” includes immune regulatory cells (e.g., Tregs) and immune effector cells described herein. “Immune cell” also refers to modified versions of cells involved in an immune response, e.g. modified NK cells, including NK cell line NK-92 (ATCC cat. No. CRL-2407), haNK (an NK-92 variant that expresses the high-affinity Fc receptor FcγRIIIa (158V)) and taNK (targeted NK-92 cells transfected with a gene that expresses a CAR for a given tumor antigen), e.g., as described in Klingemann et al. *supra*.

[0136] “Immune effector cell,” as that term is used herein, refers to a cell that is involved in an immune response, e.g., in the promotion of an immune effector response. Examples of immune effector cells include, but are not limited to, T cells, e.g., CD4⁺ T cells, CD8⁺ T cells, alpha T cells, beta T cells, gamma T cells, and delta T cells; B cells; natural killer (NK) cells; natural killer T (NKT) cells; dendritic cells; and mast cells. In some embodiments, the immune cell is an immune cell (e.g., T cell or NK cell) that comprises, e.g., expresses, a Chimeric Antigen Receptor (CAR), e.g., a CAR that binds to a cancer antigen. In other embodiments, the immune cell expresses an exogenous high affinity Fc receptor. In some embodiments, the immune cell comprises, e.g., expresses, an engineered T-cell receptor. In some

embodiments, the immune cell is a tumor infiltrating lymphocyte. In some embodiments, the immune cells comprise a population of immune cells and comprise T cells that have been enriched for specificity for a tumor-associated antigen (TAA), e.g. enriched by sorting for T cells with specificity towards MHCs displaying a TAA of interest, e.g. MART-1. In some embodiments, immune cells comprise a population of immune cells and comprise T cells that have been “trained” to possess specificity against a TAA by an antigen presenting cell (APC), e.g. a dendritic cell, displaying TAA peptides of interest. In some embodiments, the T cells are trained against a TAA chosen from one or more of MART-1, MAGE-A4, NY-ESO-1, SSX2, Survivin, or others. In some embodiments the immune cells comprise a population of T cells that have been “trained” to possess specificity against a multiple TAAs by an APC, e.g. a dendritic cell, displaying multiple TAA peptides of interest. In some embodiments, the immune cell is a cytotoxic T cell (e.g., a CD8+ T cell). In some embodiments, the immune cell is a helper T cell, e.g., a CD4+ T cell.

[0137] The term “effector function” or “effector response” refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines.

[0138] “Immune regulatory cell”, as used herein, refers to an immune cell that participates in immunomodulation or suppression of immune responses, e.g., a regulatory T cell or regulatory B cell.

[0139] “Tregs” or “Treg cells” refer to Regulatory T cells. Regulatory T cells are a class of T cells that suppress the activity of other immune cells, and are defined using flow cytometry by the cell marker phenotype CD4+CD25+FOXP3+. Because FOXP3 is an intracellular protein and requires cell fixation and permeabilization for staining, the cell surface phenotype CD4+CD25+CD127—can be used for defining live Tregs. Tregs also include various Treg subclasses, such as tTregs (thymus-derived) and pTregs (peripherally-derived, differentiated from naïve T cells in the periphery). All Tregs express the IL2R $\alpha\beta\gamma$ receptor, do not produce their own IL-2 and are dependent on IL-2 for growth, and someone skilled in the art will recognize that both classes will be selectively activated by an IL2R $\alpha\beta\gamma$ selective agonist.

[0140] “Cytotoxic T lymphocytes” (CTLs) as used herein refer to T cells that have the ability to kill a target cell. CTL activation can occur when two steps occur: 1) an interaction between an antigen-bound MHC molecule on the target cell and a T cell receptor on the CTL is made; and 2) a costimulatory signal is made by engagement of costimulatory molecules on the T cell and the target cell. CTLs then recognize specific antigens on target cells and induce the destruction of these target cells, e.g., by cell lysis. In some embodiments, the CTL expresses a CAR. In some embodiments, the CTL expresses an engineered T-cell receptor.

[0141] “Modulation” “modulatory” as used herein refers to any alteration of an existing or potential immune responses against an autoimmune or allergy provoking epitope, including, e.g., nucleic acids, lipids, phospholipids, carbohydrates, self-polypeptides, protein complexes, or ribonucleoprotein complexes, that occurs as a result of administration of the compositions disclosed herein. Such modulation includes any alteration in presence, capacity, or function of an immune regulatory cell, such as Treg, involved in, or capable of being involved in, an immune

response. “Modulation” includes any change imparted on an existing immune response, a developing immune response, a potential immune response, or the capacity to induce, regulate, influence, or respond to an immune response. Modulation includes any alteration in the expression and/or function of genes, proteins and/or other molecules in immune cells as an immune response.

[0142] “Modulation of an immune response” includes, for example, the following: elimination, deletion, or sequestration of immune cells; induction or generation of immune cells that can modulate the functional capacity of other cells such as autoreactive lymphocytes, antigen presenting cells, or inflammatory cells; induction of an unresponsive state in immune cells (i.e., anergy); increasing, decreasing, or changing the activity or function of immune cells or the capacity to do so, including, but not limited to, altering the pattern of proteins expressed by these cells. Examples include altered production and/or secretion of certain classes of molecules such as cytokines, chemokines, growth factors, transcription factors, kinases, costimulatory molecules, or other cell surface receptors; or any combination of these modulatory events. In some embodiments, modulation includes suppression or immunosuppression of certain immune responses, e.g., in conditions or diseases where the immune system displays an excessive or overactive response such as autoimmune diseases, allergies, bone marrow or organ transplant, and other inflammatory diseases. Suppression or immunosuppression as used herein includes reduction, inhibition and lessening of an immune response.

[0143] “Sample” or “tissue sample” refers to a biological sample obtained from a tissue or bodily fluid of a subject or patient. The source of the tissue sample can be solid tissue as from a fresh, frozen and/or preserved organ, tissue sample, biopsy, or aspirate; blood or any blood constituents (e.g., serum, plasma); bone marrow or any bone marrow constituents; bodily fluids such as urine, cerebral spinal fluid, whole blood, plasma and serum. The sample can include a non-cellular fraction (e.g., urine, plasma, serum, or other non-cellular body fluid). In other embodiments, the body fluid from which the sample is obtained from an individual comprises blood (e.g., whole blood).

[0144] The term “subject” includes living organisms in which an immune response can be elicited (e.g., mammals, human). In one embodiment, the subject is a patient, e.g., a patient in need of immune cell therapy. In another embodiment, the subject is a donor, e.g. an allogenic donor of immune cells, e.g., intended for allogenic transplantation.

[0145] The compositions and methods of the present disclosure encompass polypeptides and nucleic acids having the sequences specified, or sequences substantially identical or similar thereto, e.g., sequences at least 85%, 90%, 95% identical or higher to the sequence specified. In the context of an amino acid sequence, the term “substantially identical” is used herein to refer to a first amino acid that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity. For example, amino acid sequences that contain a common structural domain having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, e.g., a sequence provided herein. In the context of nucleotide sequence, the term

“substantially identical” is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity. For example, nucleotide sequences having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence, e.g., a sequence provided herein. Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

[0146] To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid “identity” is equivalent to amino acid or nucleic acid “homology”).

[0147] The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch ((1970) *J. Mol. Biol.* 48:444-453) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used unless otherwise specified) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

[0148] The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller ((1989) *CABIOS*, 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

[0149] The nucleic acid and protein sequences described herein can be used as a “query sequence” to perform a search

against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) *J. Mol. Biol.* 215: 403-10. BLAST nucleotide searches can be performed with the NBLAST program, score =100, wordlength =12 to obtain nucleotide sequences homologous to a nucleic acid (e.g., SEQ ID NO: 1) molecules of the disclosure. BLAST protein searches can be performed with the XBLAST program, score =50, wordlength=3 to obtain amino acid sequences homologous to protein molecules of the disclosure. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>. It is understood that the molecules of the present disclosure may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on their functions.

[0150] The term “amino acid” is intended to embrace all molecules, whether natural or synthetic, which include both an amino functionality and an acid functionality and capable of being included in a polymer of naturally-occurring amino acids. Exemplary amino acids include naturally-occurring amino acids; analogs, derivatives and congeners thereof; amino acid analogs having variant side chains; and all stereoisomers of any of any of the foregoing. As used herein the term “amino acid” includes both the D- or L- optical isomers and peptidomimetics.

[0151] A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[0152] The term “functional variant” or “variant” or “variant form” in the context of a polypeptide refers to a polypeptide that is capable of having at least 10% of one or more activities of the naturally-occurring sequence. In some embodiments, the functional variant has substantial amino acid sequence identity to the naturally-occurring sequence, or is encoded by a substantially identical nucleotide sequence, such that the functional variant has one or more activities of the naturally-occurring sequence.

[0153] The term “molecule” as used herein can refer to a polypeptide or a nucleic acid encoding a polypeptide, as indicated by the context. This term includes full length, a fragment or a variant of a naturally-occurring, wild type polypeptide or nucleic acid encoding the same, e.g., a functional variant, thereof. In some embodiments, the variant is a derivative, e.g., a mutant, of a wild type polypeptide or nucleic acid encoding the same.

[0154] The terms “polypeptide”, “peptide” and “protein” (if single chain) are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be

linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component. The polypeptide can be isolated from natural sources, can be produced by recombinant techniques from a eukaryotic or prokaryotic host, or can be a product of synthetic procedures.

[0155] The terms “nucleic acid,” “nucleic acid sequence,” “nucleotide sequence,” or “polynucleotide sequence,” and “polynucleotide” are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. The polynucleotide may be either single-stranded or double-stranded, and if single-stranded may be the coding strand or non-coding (antisense) strand. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. The nucleic acid may be a recombinant polynucleotide, or a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which either does not occur in nature or is linked to another polynucleotide in a non-natural arrangement.

[0156] The term “parent polypeptide” refers to a wild-type polypeptide and the amino acid sequence or nucleotide sequence of the wild-type polypeptide is part of a publicly accessible protein database (e.g., EMBL Nucleotide Sequence Database, NCBI Entrez, ExPasy, Protein Data Bank and the like).

[0157] The term “mutant polypeptide” or “polypeptide variant” or “mutein” refers to a form of a polypeptide, wherein its amino acid sequence differs from the amino acid sequence of its corresponding wild-type (parent) form, naturally existing form or any other parent form. A mutant polypeptide can contain one or more mutations, e.g., replacement, insertion, deletion, etc. which result in the mutant polypeptide.

[0158] The term “corresponding to a parent polypeptide” (or grammatical variations of this term) is used to describe a polypeptide of the present disclosure, wherein the amino acid sequence of the polypeptide differs from the amino acid sequence of the corresponding parent polypeptide only by the presence of at least amino acid variation. Typically, the amino acid sequences of the variant polypeptide and the parent polypeptide exhibit a high percentage of identity. In one example, “corresponding to a parent polypeptide” means that the amino acid sequence of the variant polypeptide has at least about 50% identity, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 98% identity to the amino acid sequence of the parent polypeptide. In another example, the nucleic acid sequence that encodes the variant polypeptide has at least about 50% identity, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 98% identity to the nucleic acid sequence encoding the parent polypeptide.

[0159] The term “introducing (or adding etc.) a variation into a parent polypeptide” (or grammatical variations thereof), or “modifying a parent polypeptide” to include a variation (or grammatical variations thereof) do not neces-

sarily mean that the parent polypeptide is a physical starting material for such conversion, but rather that the parent polypeptide provides the guiding amino acid sequence for the making of a variant polypeptide. In one example, “introducing a variant into a parent polypeptide” means that the gene for the parent polypeptide is modified through appropriate mutations to create a nucleotide sequence that encodes a variant polypeptide. In another example, “introducing a variant into a parent polypeptide” means that the resulting polypeptide is theoretically designed using the parent polypeptide sequence as a guide. The designed polypeptide may then be generated by chemical or other means.

[0160] The term “isolated,” as used herein, refers to material that is removed from its original or native environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated by human intervention from some or all of the co-existing materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of the environment in which it is found in nature.

[0161] Various aspects of the disclosure are described in further detail below. Additional definitions are set out throughout the specification.

Modulatory Moiety

[0162] An modulatory moiety can be any agent or molecule capable of modulating, e.g., stimulating or suppressing, a function or activity of another agent, molecule or cell. In some embodiments, the modulatory moiety reduces, inhibits or suppresses a function or activity. In some embodiments, the modulatory moiety enhances, activates or stimulates a function or activity. In certain embodiments, the modulatory moiety activates or enhances proliferation of immune regulatory cells. In one embodiment, the modulatory moiety activates or enhances proliferation of Tregs, which in turn, regulates, reduces or suppresses certain immune response, thereby providing an immunosuppressive activity.

[0163] Various modulatory moieties can be used. In some embodiments, the modulatory moiety can be a cytokine or growth factor.

[0164] Cytokines are proteinaceous signaling compounds that are mediators of the immune response. They control many different cellular functions including proliferation, differentiation and cell survival/apoptosis; cytokines are also involved in several pathophysiological processes including viral infections and autoimmune diseases. Cytokines are synthesized under various stimuli by a variety of cells, including those of both the innate (monocytes, macrophages, dendritic cells) and adaptive (T- and B-cells) immune systems. Cytokines can be classified into two groups: pro- and anti-inflammatory. Pro-inflammatory cytokines, including IFN γ , IL-1 (IL-1 α , IL-1 β , IL-6 and TNF- α , are predominantly derived from the innate immune cells and Th1 cells. Anti-inflammatory cytokines, including IL-10, IL-4, IL-13 and IL-5, are synthesized from Th2 immune cells.

[0165] Growth factors are capable of stimulating cellular growth, proliferation, healing, and cellular differentiation. Usually it is a protein or a steroid hormone. Growth factor is sometimes used interchangeably with the term cytokine.

[0166] The present disclosure provides, inter alia, FMs (e.g., FM), that include or are engineered to contain one or more cytokine/growth factor molecules, e.g., cytokines, growth factors and/or functional variants thereof. Accordingly, in some embodiments, the cytokine molecule is an interleukin (IL) or a variant, e.g., a functional variant thereof. In some embodiments, the cytokine is a proinflammatory interleukin. In other embodiments, the cytokine is anti-inflammatory.

[0167] In embodiments, the cytokine molecule is full length, a fragment or a variant of a cytokine, e.g., a cytokine comprising one or more mutations. In some embodiments, the cytokine molecule of the FM includes an immunomodulatory cytokine, e.g., a pro-inflammatory cytokine or an anti-inflammatory cytokine. In some embodiments, the cytokine is a member of the common γ -chain(γ c) family of cytokines. In some embodiments, the cytokine molecule comprises a cytokine chosen from one or more of IL-15, IL-2, IL-4, IL-5, IL-7, IL-9, IL-10, IL-13, IL-18, IL-21, IL-27, IL-35, IFN γ , TNF β , IFN α , IFN β , Areg, GM-CSF, or GCSF, including variant forms thereof (e.g., a cytokine derivative, a complex comprising the cytokine molecule with a polypeptide, e.g., a cytokine receptor complex, and other agonist forms thereof). In some embodiments, the cytokine molecule is a pro-inflammatory cytokine molecule chosen from an IL-15, IL-2, IL-18, IL-27, IL-10, IL-35, Amphiregulin, IL-33, or TGF- β molecule. In certain embodiments, the cytokine is IL-7, IL-21, IL-6, IL-12, or IL-23. In some embodiments, the cytokine molecule is an anti-inflammatory cytokine molecule chosen from an IL-4, IL-10, IL-13, IL-35 cytokine molecule. In some embodiments, the cytokine molecule is a superagonist (SA). For example, the superagonist can have increased cytokine activity, e.g., by at least 10%, 20%, or 30%, compared to the naturally-occurring cytokine. In some embodiments, the cytokine molecule is a monomer or a dimer. In embodiments, the cytokine molecule further comprises a receptor or a fragment thereof, e.g., a cytokine receptor domain.

[0168] In one embodiment, the cytokine molecule comprises an amino acid sequence that is identical or substantially identical to the wild-type cytokine sequence, e.g., a human cytokine sequence. In some embodiments, the cytokine molecule comprises an amino acid sequence at least 95% to 100% identical, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to a wild-type cytokine sequence, e.g., a human cytokine sequence. In embodiments, the cytokine molecule comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to the wild-type cytokine sequence, e.g., the human cytokine sequence.

[0169] In some embodiments, the cytokine molecule comprises a complex of a cytokine and an anti-cytokine antibody molecule. For example, the cytokine can be IL-2 and the anti-cytokine antibody can be a non-neutralizing anti-IL-2 antibody molecule (see e.g., Spangler, J.B., et al. (2015) [dx.doi.org/10.1016/j.immuni.2015.04.015](https://doi.org/10.1016/j.immuni.2015.04.015); Letoumeau, S. et al. (2010) *PNAS* Vol 107(5): 2171-2176; Boyman, O. et al. (2006) *Science* 311, 1924; and Spangler, J.B. (2015) *Annu Rev Immunol* 33:139-167, the contents of which are entirely incorporated by reference).

[0170] In some embodiments, the cytokine molecule is an IL-15 molecule, e.g., a full length, a fragment or a variant of

IL-15, e.g., human IL-15. In embodiments, the IL-15 molecule is a wild-type, human IL-5, e.g., having the amino acid sequence of SEQ ID NO: 10. In other embodiments, the IL-15 molecule is a variant of human IL-5, e.g., having one or more amino acid modifications.

[0171] In some embodiments, the IL-15 variant comprises, or consists of, a mutation at position 45, 51, 52, or 72, e.g., as described in US 2016/0184399. In some embodiments, the IL-15 variant comprises, or consists of, an N, S or L to one of D, E, A Y or P substitution. In some embodiments, the mutation is chosen from L45D, L45E, S51D, L52D, N72D, N72E, N72A, N72S, N72Y, or N72P (in reference to the sequence of human IL-15, SEQ ID NO: 11). As those of skill will realize, any combination of the positions can be mutated. In some embodiments, the IL-15 variant comprises two or more mutations. In some embodiments, the IL-15 variant comprises three or more mutations. In some embodiments, the IL-15 variant comprises four, five, or six or more mutations.

[0172] In some embodiments, the IL-15 molecule comprises a mutation, e.g., an N72D point mutation as shown in SEQ ID NO: 11 herein. In some embodiments, the IL-15 molecule comprises a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 11, wherein the sequence comprises an N72D mutation relative to wild-type human IL-15, and having IL-15R α binding activity.

[0173] In some embodiments, the IL-15 variant comprises, or consists of, one or more mutations at amino acid position 8, 10, 61, 64, 65, 72, 101, or 108 (in reference to the sequence of human IL-15, SEQ ID NO: 11). In some embodiments the IL-15 variant possesses increased activity as compared with wild-type IL-15. In some embodiments the IL-15 variant possesses decreased activity as compared with wild-type IL-15. In some embodiments the IL-15 variant possesses approximately two-fold, four-fold, ten-fold, 20-fold, 40-fold, 60-fold, 100-fold, or more than 100-fold decreased activity as compared with wild-type IL-15. In some embodiments, the mutation is chosen from D8N, K10Q, D61N, D61H, E64H, N65H, N72A, N72H, Q101N, Q108N, or Q108H (in reference to the sequence of human IL-15, SEQ ID NO: 11). As those of ordinary skill in the art would realize, any combination of the positions can be mutated. In some embodiments, the IL-15 variant comprises two or more mutations. In some embodiments, the IL-15 variant comprises three or more mutations. In some embodiments, the IL-15 variant comprises four, five, or six or more mutations. In some embodiments the IL-15 variant comprises mutations at positions 61 and 64. In some embodiments the mutations at positions 61 and 64 are D61N or D61H and E64Q or E64H. In some embodiments the IL-15 variants comprises mutations at positions 61 and 108. In some embodiments the mutations at positions 61 and 108 are D61N or D61H and Q108N or Q108H.

[0174] In embodiments, the cytokine molecule further comprises a receptor domain, e.g., a cytokine receptor domain. In one embodiment, the cytokine molecule comprises an IL-15 receptor, or a fragment thereof (e.g., an IL-15 binding domain of an IL-15 receptor α) as described herein. In some embodiments, the cytokine molecule is an IL-15 molecule, e.g., IL-15 or an IL-15 superagonist as described herein. As used herein, a "superagonist" form of a cytokine molecule shows increased activity, e.g., by at least 10%, 20%, 30%, compared to the naturally-occurring

cytokine. An exemplary superagonist is an IL-15 SA. In some embodiments, the IL-15 SA comprises a complex of IL-15 and an IL-15 binding fragment of an IL-15 receptor, e.g., IL-15 receptor alpha or an IL-15 binding fragment thereof, e.g., as described herein. In other embodiments, the cytokine molecule further comprises a receptor domain, e.g., an extracellular domain of an IL-15R alpha, optionally, coupled to an immunoglobulin Fc or an antibody molecule. In embodiments, the cytokine molecule is an IL-15 superagonist (IL-15SA) as described in WO 2010/059253. In some embodiments, the cytokine molecule comprises IL-15 and a soluble IL-15 receptor alpha domain fused to an Fc (e.g., a sIL-15Ra-Fc fusion protein), e.g., as described in Rubinstein et al PNAS 103:24 p. 9166-9171 (2006).

[0175] The IL-15 molecule can further comprise a polypeptide, e.g., a cytokine receptor, e.g., a cytokine receptor domain, and a second, heterologous domain. In one embodiment, the heterologous domain is an immunoglobulin Fc region. In other embodiments, the heterologous domain is an antibody molecule, e.g., a Fab fragment, a FAB₂ fragment, a scFv fragment, or an affibody fragment or derivative, e.g. a sdAb (nanobody) fragment, a heavy chain antibody fragment. In some embodiments, the polypeptide also comprises a third heterologous domain. In some embodiments, the cytokine receptor domain is N-terminal of the second domain, and in other embodiments, the cytokine receptor domain is C-terminal of the second domain.

[0176] The wild-type IL-15 Receptor alpha sequence and fragment and variants of this sequence are set out below.

[0177] Wild-type IL-15 Receptor alpha sequence (Genbank Acc. No. AAI21141.1): SEQ ID NO: 41.

[0178] Wild-type IL-15 Receptor alpha extracellular domain (portion of accession number Q13261): SEQ ID NO: 63.

[0179] Isoform CRA_d IL-15 Receptor alpha extracellular domain (portion of accession number EAW86418): SEQ ID NO: 64.

[0180] The wild-type IL-15 Receptor alpha sequence is provided above as SEQ ID NO: 41. IL-15 receptor alpha contains an extracellular domain, a 23 amino acid transmembrane segment, and a 39 amino acid cytoplasmic tail. The extracellular domain of IL-15 Receptor alpha is provided as SEQ ID NO: 63.

[0181] In other embodiments, an IL-15 agonist can be used. For example, an agonist of an IL-15 receptor, e.g., an antibody molecule (e.g., an agonistic antibody) to an IL-15 receptor, that elicits at least one activity of a naturally-occurring cytokine. In embodiments, the IL-15 receptor or fragment thereof is from human or a non-human animal, e.g., mammal, e.g., non-human primate.

[0182] The wild-type IL-15 Receptor alpha sequence is provided above as SEQ ID NO: 64. IL-15 receptor alpha contains an extracellular domain, a 23 amino acid transmembrane segment, and a 39 amino acid cytoplasmic tail. The sushi domain has been described in the literature including, e.g., Bergamaschi et al. (2008), JBC VOL. 283, NO. 7, pp. 4189-4199; Wei et al. (2001), *Journal of Immunology* 167:277-282; Schluns et al. (2004) PNAS Vol 110 (15) 5616-5621; US 2016/0184399 (the contents of each of which is incorporated by reference herein).

[0183] The extracellular domain of IL-15 Receptor alpha is provided as SEQ ID NO: 63. The extracellular domain of IL-15 Receptor alpha comprises a domain referred to as the sushi domain, which binds IL-15. The general sushi domain,

also referred to as complement control protein (CCP) modules or short consensus repeats (SCR), is a protein domain found in several proteins, including multiple members of the complement system. The sushi domain adopts a beta-sandwich fold, which is bounded by the first and fourth cysteine of four highly conserved cysteine residues, comprising to a sequence stretch of approximately 60 amino acids (Norman, Barlow, et al. J Mol Biol. 1991 Jun 20;219(4):717-25). The amino acid residues bounded by the first and fourth cysteines of the sushi domain in IL-15Ralpha comprise a 62 amino acid polypeptide that we refer to as the minimal domain (SEQ ID NO: 52). Including additional amino acids of IL-15Ralpha at the N- and C-terminus of the minimal sushi domain, such as inclusion of N-terminal Ile and Thr and C-terminal Ile and Arg residues result in a 65 sushi amino acid domain (SEQ ID NO: 9).

[0184] A sushi domain as described herein may comprise one or more mutations relative to a wild-type sushi domain. For instance, residue 77 of IL-15Ra is leucine in the wild-type gene (and is underlined in SEQ ID NO: 41), but can be mutated to isoleucine (L77I). Accordingly, a minimal sushi domain comprising L77I (with the numbering referring to the wild-type IL-15Ra of SEQ ID NO: 41) is provided as SEQ ID NO: 65. An extended sushi domain comprising L77I (with the numbering referring to the wild-type IL-15Ra of SEQ ID NO: 41) is provided as SEQ ID NO: 66.

Minimal sushi domain, wild-type: (SEQ ID NO: 52)

CPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAGTSSLTECVLNKATN
VAHWTTPSLKC

Extended sushi domain, wild-type: (SEQ ID NO: 9)

ITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAGTSSLTECVLNKA
TNVAHWTTPSLKCIR

Minimal sushi domain, L77I: (SEQ ID NO: 65)

CPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAGTSSLTECVINKATN
VAHWTTPSLKCI

Extended sushi domain, L77I: (SEQ ID NO: 66)

ITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAGTSSLTECVINKA
TNVAHWTTPSLKCIR

[0185] In some embodiments, a sushi domain consists of 62-171 amino acids of SEQ ID NO: 63 or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, and having IL-15 binding activity. In some embodiments, a sushi domain consists of 65-171 amino acids of SEQ ID NO: 63 or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, and having IL-15 binding activity. In some embodiments, a sushi domain consists of up to 171 amino acids of SEQ ID NO: 63 or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, and having IL-15 binding activity. In some embodiments, a sushi domain consists of 62-171, 62-160, 62-150, 62-140, 62-130, 62-120, 62-110, 62-100, 62-90, 62-80, 62-70, 65-171, 65-160, 65-150, 65-140, 65-130, 65-120, 65-110, 65-100, 65-90, 65-80, or 65-70 amino acids of SEQ ID NO: 63 or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%,

98%, or 99% identity thereto, and having IL-15 binding activity. In some embodiments, a sushi domain consists of 62-171, 62-160, 62-150, 62-140, 62-130, 62-120, 62-110, 62-100, 62-90, 62-80, 62-70, 65-171, 65-160, 65-150, 65-140, 65-130, 65-120, 65-110, 65-100, 65-90, 65-80, or 65-70 amino acids of SEQ ID NO: 63. In some embodiments, the sushi domain comprises, or consists of, an amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 52.

[0186] In some embodiments, a sushi domain consists of 62-171 amino acids of SEQ ID NO: 63 or a sequence having up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or 40 modifications (e.g., substitutions) relative thereto, and having IL-15 binding activity. In some embodiments, a sushi domain consists of up to 171 amino acids of SEQ ID NO: 5 or a sequence having up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or 40 modifications (e.g., substitutions) relative thereto, and having IL-15 binding activity. In some embodiments, a sushi domain consists of 62-171, 62-160, 62-150, 62-140, 62-130, 62-120, 62-110, 62-100, 62-90, 62-80, 62-70, 65-171, 65-160, 65-150, 65-140, 65-130, 65-120, 65-110, 65-100, 65-90, 65-80, or 65-70 amino acids of SEQ ID NO: 63 or a sequence having up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or 40 modifications (e.g., substitutions) relative thereto, and having IL-15 binding activity.

[0187] In some embodiments, a sushi domain comprises at least 62 amino acids of SEQ ID NO: 63 or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, wherein the sequence comprises an L77I mutation relative to wild-type IL-15Ra, and having IL-15 binding activity. In some embodiments, a sushi domain comprises at least 65 amino acids of SEQ ID NO: 63 or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, wherein the sequence comprises an L77I mutation relative to wild-type IL-15Ra, and having IL-15 binding activity. In some embodiments, a sushi domain comprises a portion of SEQ ID NO: 63 or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, wherein the sequence comprises an L77I mutation relative to wild-type IL-15Ra, and having IL-15 binding activity. In some embodiments, the sushi domain comprises an amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 52.

[0188] In some embodiments, a sushi domain comprises at least 62 amino acids of SEQ ID NO: 63 or a sequence having up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or 40 modifications (e.g., substitutions) relative thereto, wherein the sequence comprises an L77I mutation relative to wild-type IL-15Ra, and having IL-15 binding activity. In some embodiments, a sushi domain comprises a portion of SEQ ID NO: 66 or a sequence having up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or 40 modifications (e.g., substitutions) relative thereto, wherein the sequence comprises an L77I mutation relative to wild-type IL-15Ra, and having IL-15 binding activity.

[0189] In embodiments, the sushi domain comprises at least 10, 20, 30, 40, 50, 60, 62, 65, 70, 80, 90, 100, 110, 120, 130, 140, 150, or 160 consecutive amino acids of SEQ ID NO: 63, or a sequence having an L77I mutation relative thereto. In embodiments, the sushi domain consists of 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130, 130-140, 140-150, 150-160, or 160-170 consecutive amino acids of SEQ ID NO: 63, or a sequence having an L77I mutation relative thereto.

[0190] In embodiments, the sushi domain is a sushi domain from human or a non-human animal, e.g., mammal, e.g., non-human primate.

[0191] In some embodiments, the polypeptide can have a second, heterologous domain, e.g., an Fc domain or a Fab domain.

[0192] In some embodiments, the polypeptide comprising the IL-15 receptor or fragment thereof comprises an Fc domain. In embodiments, the Fc domain is an effector-attenuated Fc domain, e.g., a human IgG2 Fc domain, e.g., a human IgG2 Fc domain of SEQ ID NO: 54 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

[0193] In embodiments, the effector-attenuated Fc domain has reduced effector activity, e.g., compared to a wild-type IgG1 Fc domain, e.g., compared to a wild-type IgG1 Fc domain of SEQ ID NO: 67. In some embodiments, effector activity comprises antibody-dependent cellular toxicity (ADCC). In embodiments, the effector activity is reduced by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% in an ADCC assay, e.g., compared to a wild-type IgG1 Fc domain of SEQ ID NO: 67. In some embodiments, effector activity comprises complement dependent cytotoxicity (CDC). In embodiments, the effector activity is reduced by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% in a CDC assay such as a CDC assay described in Armour et al., "Recombinant human IgG molecules lacking Fc gamma receptor I binding and monocyte triggering activities." *Eur J Immunol* (1999) 29:2613-24" e.g., compared to a wild-type IgG1 Fc domain of SEQ ID NO: 67.

[0194] In some embodiments, the Fc domain comprises an IgG1 Fc domain of SEQ ID NO: 67 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

[0195] In some embodiments, the Fc domain comprises an IgG2 constant region of SEQ ID NO: 68 or fragment thereof, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

[0196] In some embodiments, the Fc domain comprises an IgG2Da Fc domain of SEQ ID NO: 55 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto. In embodiments, the Fc domain comprises one or both of A330S and P331S mutations using Kabat numbering system. In embodiments, the Fc domain is one described in Armour et al. "Recombinant human IgG molecules lacking Fc gamma receptor I binding and monocyte triggering activities." *Eur J Immunol* (1999) 29:2613-24.

[0197] In some embodiments, the Fc domain has dimerization activity.

[0198] In some embodiments, the Fc domain is an IgG domain, e.g., an IgG1, IgG2, IgG3, or IgG4 Fc domain. In some embodiments, the Fc domain comprises a CH2 domain and a CH3 domain.

[0199] In some embodiments, the nanoparticle comprises a protein having a sequence of SEQ ID NO: 56 (sushi-IgG2Da-Fc) or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

[0200] In some embodiments, the FM that comprises a sushi domain described herein (e.g., in SEQ ID NO: 9) and an Fc domain described herein, e.g., an IgG2 Fc domain (e.g., SEQ ID NO: 54 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto). In some embodiments, the FM comprises

a sushi domain of SEQ ID NO: 9 and an Fc domain described herein, e.g., an IgG1 Fc domain, e.g., an Fc domain of SEQ ID NO: 67 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto. In some embodiments, the FM comprises a sushi domain of SEQ ID NO: 9 and an IgG2 Fc domain, e.g., an Fc domain of SEQ ID NO: 54 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto. In some embodiments, the FM comprises a sushi domain of SEQ ID NO: 9 and an IgG1 Fc domain, e.g., an Fc domain of SEQ ID NO: 67 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

[0201] In some embodiments, the FM comprises a sushi domain of SEQ ID NO: 9 and an IgG2Da Fc domain of SEQ ID NO: 56 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto. In some embodiments, the FM comprises a sushi domain of SEQ ID NO: 9 and an IgG2Da Fc domain of SEQ ID NO: 56 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto. In some embodiments, the FM comprises a sushi-IgG2Da-Fc protein having a sequence of SEQ ID NO: 56 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

[0202] In embodiments, the IL-15 molecule is a molecule described in PCT International Application Publication No. WO2017/027843, which is herein incorporated by reference in its entirety.

[0203] In some embodiments, the cytokine molecule is interleukin 2 (IL-2). IL-2 is chiefly secreted by T cells in response to antigenic stimuli, and is the main cytokine for T cell proliferation. IL-2 receptor is expressed by T lymphocytes, NK cells, B cells, macrophages, and monocytes; however, only T lymphocytes are capable of producing this cytokine.

[0204] The high affinity IL-2 receptor (IL-2R) is a heterotrimeric cell surface receptor composed of α , β , γ_c -polypeptide chains (K_D 10^{-11} M). The 55 kDa α -chain, also known as IL-2R α , CD25, p55, and Tac (T cell activation) antigen, is unique to the IL-2R. The β (CD122; 75) and γ_c (CD132) chains are part of a cytokine receptor superfamily (hematopoietin receptors) and are functional components of other cytokine receptors, such as IL-15R. The intermediate affinity receptor is a dimer composed of a β - and a γ_c -chain (K_D 10^{-9} M) while the low affinity receptor consists of a monomeric α -subunit that has no signal transduction capacity (K_D 10^{-8} M).

[0205] Treg cells constitutively express high levels of IL-2 α -chain, having thus a higher affinity to IL-2, and compete for this growth factor with proliferating cells. By depriving proliferating effector cells from IL-2, Treg cells do not only prevent them from continuing the proliferative process but also leave them without a vital cytokine, causing metabolic interruption and cell death.

[0206] In one embodiment, the IL-2 molecule (e.g., IL-2 polypeptide molecule) comprises a wild-type IL-2 amino acid sequence, e.g., a human IL-2 amino acid sequence, e.g., including the amino acid sequence of SEQ ID NO: 80. In some embodiments, the cytokine molecule is an IL-2 molecule, e.g., a full length, a fragment or a variant of IL-2, e.g., human IL-2 that retains at least some IL-2 activity such as binding to IL-2R. In other embodiments, the IL-2 molecule is a variant of human IL-2, e.g., having one or more amino

acid alterations, e.g., substitutions, to the human IL-2 amino acid sequence. In some embodiments, the IL-2 variant comprises, or consists of, one or more mutations while maintaining the IL-2R binding activity.

[0207] In some embodiments, the IL-2 of the present disclosure can have the following sequence (or a functional variant thereof):

(SEQ ID NO: 80)

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APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFPMPKKA
TELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE
TTFMCEYADETATIVEFLNRWITFCQSIISTLT

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[0208] In certain embodiments, variant forms of IL-2 can be made by introducing one or more mutations into the wild-type form, so as to fine tune its binding affinity to IL-2R. For example, a library of IL-2 mutants or variants can be made to test their affinity to IL-2R. These variants can also be conjugated or coupled to various targeting moieties, and the resulting FMs can be tested for targeting. In some embodiments, the IL-2 variant comprises, or consists of, one or more mutations.

[0209] In some embodiments, the IL-2 variants can be IL-2 $\alpha\beta\gamma$ selective agonists. Functionally they selectively activate the IL-2 $\alpha\beta\gamma$ receptor complex relative to the IL-2R $\beta\gamma$ receptor complex. It is derived from a wild type IL-2 protein structurally defined as having at least 95% sequence identity to the wild type IL-2 (e.g., SEQ ID NO: 80) and functionally defined by the ability to preferentially activate Treg cells. The variants can also be functionally defined by its ability to selectively activate IL-2 receptor signaling in Tregs, as measured by the levels of phosphorylated STATs protein in Treg cells compared to CD4⁺ CD25⁻/low T cells or NK cells, or by the selective activation of Phytohemagglutinin-stimulated T cells versus NK cells.

[0210] In some embodiments, modifications to the cytokine or growth factor amino acid sequence can be made to improve persistence and/or selectivity for an immune regulatory cell such as a regulatory T cell. One of the obstacles for prolonged surface persistence and signaling of cytokine fusions is cytokine/receptor internalization and degradation through the endosomal/lysosomal pathway. Extending the persistence of cytokine fusions can be achieved, in some embodiments, by altering the affinity of cytokines in the altered-pH environment of the endosome. By decreasing the affinity of cytokine for its receptor in the endosome, there is decreased lysosomal degradation and increased recycling to the cell surface. To promote endosomal dissociation of IL-2 from its receptor, histidine mutants can be engineered in receptor-binding motifs of IL-2. Histidine changes protonation state in the altered pH of the endosome, and this reduces the affinity of IL-2/receptor interactions. A number of different substitutions can be tested to find ones that increase recycling and persistence without decreasing surface affinity for the receptor. Alternatively, reduced surface affinity for the receptor can be desirable for enhancing Treg-selective IL-2 signaling. In embodiments, histidine substitutions can be focused in motifs that are involved in IL-2R β interaction in order to enhance IL-2R α binding and Treg selectivity.

[0211] In certain embodiments, altered receptor affinity for increased Treg selectivity can also be engineered. One of the hallmarks of Tregs is increased requirement and sensitivity to IL-2 signaling. This is mediated in part by significant

induction of the IL-2 receptor alpha subunit (IL2R α , CD25). This increased CD25 expression can be used in order to promote selectivity of IL-2 signaling on Treg cells. IL-2 signaling is mediated by hetero-trimers of IL2R α / β / γ or IL2R β / β / γ . Due to increased CD25 (IL2R α) expression, IL-2 signaling on Tregs is driven primarily by the α / β / γ trimer. Antibodies can be used that disrupt IL-2 binding to IL2R α or IL2 β bias IL-2 signaling and expansion towards T-effector cells (Teffs) or Tregs respectively. Instead of antibody-based approaches, disruption of IL-2 binding to IL2R β can be achieved via amino acid substitution. This can be tested using the histidine substitution approach detailed above, and through standard binding interface modulation substitutions (sterics, charge, etc.).

[0212] In some embodiments, the cytokine is IL-15 and its variants such as those disclosed in PCT International Application No. PCT/US2017/037249 filed Jun. 13, 2017, e.g., on pages 46-57, which is incorporated herein by reference in its entirety.

[0213] Amphiregulin (Areg) is a polypeptide growth factor that belongs to the epidermal growth factor (EGF) family. Areg is synthesized as a type 1 transmembrane protein precursor (proAR) and expressed on the cell surface. EGFR may be activated by Areg in several ways: autocrine or paracrine activation by the soluble form of Areg, a juxtacrine mode enabling the un-cleaved transmembrane form to activate EGFR, or by a newly described mode of signaling entailing Areg containing exosomes, that better enhance invasion of recipient cells in comparison to exosomes containing high affinity ligands.

[0214] TGF- β is a multifunctional cytokine belonging to the transforming growth factor superfamily that includes four different isoforms (TGF- β 1 to 4) and many other signaling proteins produced by all white blood cell lineages. Activated TGF- β complexes with other factors to form a serine/threonine kinase complex that binds to TGF- β receptors, which is composed of both type 1 and type 2 receptor subunits. After the binding of TGF- β , the type 2 receptor kinase phosphorylates and activates the type 1 receptor kinase that activates a signaling cascade. This leads to the activation of different downstream substrates and regulatory proteins, inducing transcription of different target genes that function in differentiation, chemotaxis, proliferation, and activation of many immune cells.

Targeting Moieties

[0215] Fusion molecules according to the present disclosure disclosed herein include a targeting moiety to target a regulatory immune cell, such as a regulatory T cell or regulatory B cell. In some embodiments, the targeting moiety may be an antibody molecule (e.g., an antigen binding domain as described herein), a receptor or a receptor fragment, or a ligand or a ligand fragment, or a combination thereof. In certain embodiments, the targeting moiety associates with or binds to a molecule, e.g., surface marker or receptor, present on the surface of a cell (a "targeting moiety"). According to some embodiments, the FM may be selectively targeted to a regulatory T cell by the binding of the targeting moiety to the cell surface. For example, the FM can be targeted to general Tregs by binding to general Treg surface markers such as CD4 and/or CD25 (FIG. 1G). The FM can also be specifically targeted to subsets of functional Tregs by binding to subset-specific surface markers such as

CD29 and/or NRP1 (FIG. 1G). In some embodiments, combinatorial targeting of two or more FMs can be achieved **[0216]** In some embodiments, the targeting moiety is chosen from an antibody molecule (e.g., a full antibody (e.g., an antibody that includes at least one, and preferably two, complete heavy chains, and at least one, and preferably two, complete light chains), or an antigen-binding fragment (e.g., a Fab, F(ab')₂, Fv, a single chain Fv, a single domain antibody, a diabody (dAb), a bivalent antibody, or bispecific antibody or fragment thereof, a single domain variant thereof, or a camelid antibody)), non-antibody scaffold, or ligand that binds to the CD45 receptor.

[0217] In some embodiments, the targeting moiety targets the FM to markers, cell surface receptors, or other molecules present on the surface of a regulatory T cell, including, for example, CD45 (via, e.g., BC8 (ACCT: HB-10507), 9.4 (ATTC: HB-10508)), CD4, CD3, CD2, CD25, CD127, CD197 (CCR7), CXCR3, CXCR4, CXCR5, CD38, CD27, CD8, CCR4, CCR5, CD137, CCR6 (CD196), CCR8, CCR10, OX40, GITR, NRP1, CTLA4, LAG3, CD73, CD103, CD62L, CCR2, CCR9, CD11a (via, for example, MHM24 monoclonal antibody), CD18 (via, for example, chimeric1B4 monoclonal antibody). In other embodiments, the targeting moiety targets CD19 on a regulatory B cell. In some embodiments, a targeting moiety is chosen from an antibody molecule, e.g., an antigen binding domain, non-antibody scaffold, or ligand that binds to CD45, CD4, CD3, CD2, CD25, CD127, CD197 (CCR7), CXCR3, CXCR4, CXCR5, CD38, CD27, CD8, CCR4, CCR5, CD137, CCR6 (CD196), CCR8, CCR10, OX40, GITR, NRP1, CTLA4, LAG3, CD73, CD103, CD62L, CCR2, CCR9, CD11a, or CD18.

[0218] According to some embodiments, the targeting moiety selectively targets the FM to regulatory T cells over other immune cells or other cell types. In some embodiments, the targeting moiety directs the FM to regulatory T cells by targeting cell surface receptors or other molecules that are expressed on the surface of a regulatory cell but may also be present on other immune cells or other cell types. In certain embodiments, the targeting moiety targets the FM to cell surface receptors or other molecules that are present in higher abundance on the surface of regulatory T cells as compared with other immune cells or other cell types that may be co-located with the regulatory T cells of interest.

[0219] In some embodiments, the targeting moiety can be a bispecific moiety that binds to more than one target. Exemplary bispecific Treg targeting moieties include CD4:CD45, CD8:CD45, CD4:CD39, CD4:NRP1, and CD4:CD25. In some embodiments, while CD127 is a negative surface marker for Tregs, the pairing of CD127 with a positive Treg surface marker (e.g., CD4:CD127, CD25:CD127) can be used to negatively enrich and/or sort Tregs.

[0220] "CD45," also known as leukocyte common antigen, refers to human CD45 protein and species, isoforms, and other sequence variants thereof. Thus, CD45 can be the native, full-length protein or can be a truncated fragment or a sequence variant (e.g., a naturally occurring isoform, or recombinant variant) that retains at least one biological activity of the native protein. CD45 is a receptor-linked protein tyrosine phosphatase that is expressed on leukocytes, and which plays an important role in the function of these cells (reviewed in Altin, JG (1997) Immunol Cell Biol. 75(5):430-45, incorporated herein by reference). For example, the extracellular domain of CD45 is expressed in

several different isoforms on T cells, and the particular isoform(s) expressed depends on the particular subpopulation of cell, their state of maturation, and antigen exposure. Expression of CD45 is important for the activation of T cells via the TCR, and that different CD45 isoforms display a different ability to support T cell activation.

[0221] “CD4” is a co-receptor for MHC Class II (with TCR, T-cell receptor); found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells. CD4+ T cells are crucial in achieving a regulated effective immune response to pathogens. Naive CD4+ T cells are activated after interaction with antigen-MHC complex and differentiate into specific subtypes depending mainly on the cytokine milieu of the microenvironment. Besides the classical T-helper 1 and T-helper 2, other subsets have been identified, including T-helper 17, regulatory T cell, follicular helper T cell, and T-helper 9, each with a characteristic cytokine profile. CD4+ T cells carry out multiple functions, ranging from activation of the cells of the innate immune system, B-lymphocytes, cytotoxic T cells, as well as nonimmune cells, and also play critical role in the suppression of immune reaction. See e.g., Rishi Vishal et al. “CD4+ T Cells: Differentiation and Functions,” *Clinical and Developmental Immunology*, vol. 2012, Article ID 925135, 12 pages, 2012. doi:10.1155/2012/925135.

[0222] “CD25” (also called interleukin-2 receptor alpha chain) is a type I transmembrane protein present on Tregs as well as activated T cells, activated B cells, some thymocytes, myeloid precursors, and oligodendrocytes.

[0223] “CD127” is part of the heterodimeric IL-7 receptor that is composed of CD127 and the common γ chain, which is shared by other cytokine receptors (IL-2R, IL-4R, IL-9R, IL-15R, and IL-21R). CD127 is expressed on thymocytes, T- and B-cell progenitors, mature T cells, monocytes, and some other lymphoid and myeloid cells. CD127 expression is down-modulated on the Treg cells, inversely correlating with the expression of Treg marker FoxP3. Studies have shown that IL-7R plays an important role in the proliferation and differentiation of mature T cells, and in vitro experiments show that the expression of CD127 is down-regulated following T cell activation. It is believed that FoxP3 interacts with the CD127 promoter and might contribute to reduced expression of CD127 in Tregs.

[0224] “CD8” is a transmembrane glycoprotein that serves as a co-receptor for the T cell receptor (TCR). Like the TCR, CD8 binds to a major histocompatibility complex (MHC) molecule, but is specific for the class I MHC protein. There are two isoforms of the protein, alpha and beta, each encoded by a different gene. In humans, both genes are located on chromosome 2 in position 2p12. The CD8 co-receptor is predominantly expressed on the surface of cytotoxic T cells, but can also be found on natural killer cells, cortical thymocytes, and dendritic cells. It is expressed in T cell lymphoblastic lymphoma and hypo-pigmented mycosis fungoides. To function, CD8 forms a dimer, consisting of a pair of CD8 chains. The most common form of CD8 is composed of a CD8- α and CD8- β chain, both members of the immunoglobulin superfamily with an immunoglobulin variable (IgV)-like extracellular domain connected to the membrane by a thin stalk, and an intracellular tail. Less-common homodimers of the CD8- α chain are also expressed on some cells. The extracellular IgV-like domain of CD8- α interacts with the $\alpha 3$ portion of the Class I MHC molecule. This affinity keeps the T cell receptor of the

cytotoxic T cell and the target cell bound closely together during antigen-specific activation. Cytotoxic T cells with CD8 surface protein are called CD8+ T cells. See e.g., Leahy DJ et al., (March 1992) “Crystal structure of a soluble form of the human T cell coreceptor CD8 at 2.6 Å resolution” *Cell* 68 (6): 1145-62; Gao G et al., (2000) “Molecular interactions of coreceptor CD8 and MHC class I: the molecular basis for functional coordination with the T-cell receptor” *Immunol Today*, 21 (12): 630-6; and Devine L et al. (1999) “Orientation of the Ig domains of CD8 alpha beta relative to MHC class I” *J Immunol.*, 162 (2): 846-51.

[0225] “CD39” is an integral membrane protein with two transmembrane domains and a large extracellular region with nucleoside triphosphate diphosphohydrolase activity. In humans, CD39 is mainly expressed by regulatory T cells but also other leukocytes. It is also named as ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1). CD39 is an ectoenzyme that hydrolyses ATP/UTP and ADP/UDP to the respective nucleosides such as AMP. A recent study demonstrates that CD39 is a cell surface marker of Foxp3 Treg cells which may regulate immune T cell suppression by the downstream production of adenosine and thus, might represent a target for the development of novel therapeutic methods useful for treating diseases associated with Treg activity (Deaglio et al., *J Exp Med.* 2007 Jun. 11; 204(6): 1257-65).

[0226] “NRP1” is a multi-functional receptor that contributes to the development of the nervous and vascular systems. NRP1 was described as a receptor that binds the semaphorin 3A ligand, acting with plexin co-receptors to regulate axon guidance (He and Tessier-Lavigne, *Cell* (1997) 90:739-51). It was later shown that NRP1 also binds members of the vascular endothelial growth factor (VEGF) ligand family to mediate vascular development (Soker et al, *Cell* (1998) 92:735-45; Kawasaki et al, *Development* (1999) 126:4895-902). Recent studies showed that NRP1 is expressed at high levels on nTreg cells and can be used to separate nTreg versus iTreg cells in certain physiological settings (Yadav et al., *J Exp Med.* 2012 Sep. 24;209(10):1713-22, S1-19). In addition, iTreg cells generated through antigen delivery or converted under homeostatic conditions lack NRP1 expression. NRP1(lo) iTreg cells show similar suppressive activity to nTreg cells in controlling ongoing autoimmune responses under homeostatic conditions. In contrast, their activity might be compromised in certain lymphopenic settings. Thus, NRP1 provides a marker to distinguish distinct Treg subsets and can be useful in studying the role of nTreg versus iTreg cells in different disease settings.

Antibody Molecules

[0227] The fusion proteins described herein may comprise one or more antibody molecule. For example, the targeting moiety may comprise an antibody molecule. In embodiments, the antibody molecule binds to a cell surface protein on a regulatory T cell, e.g., a human Treg antigen. For example, the antibody molecule binds specifically to an epitope, e.g., linear or conformational epitope, on the Treg antigen.

[0228] In an embodiment, an antibody molecule is a monospecific antibody molecule and binds a single epitope, e.g., a monospecific antibody molecule having a plurality of immunoglobulin variable domain sequences, each of which binds the same epitope.

[0229] In another embodiment, an antibody molecule is a multispecific antibody molecule, e.g., it comprises a plurality of immunoglobulin variable domains sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In an embodiment, the first and second epitopes are on the same antigen, e.g., the same protein (or subunit of a multimeric protein). In an embodiment, the first and second epitopes overlap. In an embodiment, the first and second epitopes do not overlap. In an embodiment, the first and second epitopes are on different antigens, e.g., the different proteins (or different subunits of a multimeric protein). In an embodiment, a multispecific antibody molecule comprises a third, fourth or fifth immunoglobulin variable domain. In an embodiment, a multispecific antibody molecule is a bispecific antibody molecule, a trispecific antibody molecule, or a tetraspecific antibody molecule.

[0230] In an embodiment, a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In an embodiment, the first and second epitopes are on the same antigen, e.g., the same protein (or subunit of a multimeric protein). In an embodiment, the first and second epitopes overlap. In an embodiment, the first and second epitopes do not overlap. In an embodiment, the first and second epitopes are on different antigens, e.g., the different proteins (or different subunits of a multimeric protein). In an embodiment, a bispecific antibody molecule comprises a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a first epitope and a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a second epitope. In an embodiment, a bispecific antibody molecule comprises a half antibody having binding specificity for a first epitope and a half antibody having binding specificity for a second epitope. In an embodiment, a bispecific antibody molecule comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment, a bispecific antibody molecule comprises a scFv or a Fab, or fragment thereof, have binding specificity for a first epitope and a scFv or a Fab, or fragment thereof, have binding specificity for a second epitope.

[0231] In an embodiment, an antibody molecule comprises a diabody, and a single-chain molecule, as well as an antigen-binding fragment of an antibody (e.g., Fab, F(ab')₂, and Fv). For example, an antibody molecule can include a heavy (H) chain variable domain sequence (abbreviated herein as VH), and a light (L) chain variable domain sequence (abbreviated herein as VL). In an embodiment, an antibody molecule comprises or consists of a heavy chain and a light chain (referred to herein as a half antibody). In another example, an antibody molecule includes two heavy (H) chain variable domain sequences and two light (L) chain variable domain sequence, thereby forming two antigen binding sites, such as Fab, Fab', F(ab')₂, Fc, Fd, Fd', Fv,

single chain antibodies (scFv for example), single variable domain antibodies, diabodies (Dab) (bivalent and bispecific), and chimeric (e.g., humanized) antibodies, which may be produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA technologies. These functional antibody fragments retain the ability to selectively bind with their respective antigen or receptor. Antibodies and antibody fragments can be from any class of antibodies including, but not limited to, IgG, IgA, IgM, IgD, and IgE, and from any subclass (e.g., IgG1, IgG2, IgG3, and IgG4) of antibodies. A preparation of antibody molecules can be monoclonal or polyclonal. An antibody molecule can also be a human, humanized, CDR-grafted, or in vitro generated antibody. The antibody can have a heavy chain constant region chosen from, e.g., IgG1, IgG2, IgG3, or IgG4. The antibody can also have a light chain chosen from, e.g., kappa or lambda. The term "immunoglobulin" (Ig) is used interchangeably with the term "antibody" herein.

[0232] Examples of antigen-binding fragments of an antibody molecule include: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a diabody (dAb) fragment, which consists of a VH domain; (vi) a camelid or camelized variable domain; (vii) a single chain Fv (scFv), see e.g., Bird et al. (1988) *Science* 242:423-426; and Huston et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; (viii) a single domain antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[0233] Antibody molecules include intact molecules as well as functional fragments thereof. Constant regions of the antibody molecules can be altered, e.g., mutated, to modify the properties of the antibody (e.g., to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, target cell function, or complement function).

[0234] Antibody molecules can also be single domain antibodies. Single domain antibodies can include antibodies whose complementary determining regions are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain antibodies, antibodies naturally devoid of light chains, single domain antibodies derived from conventional 4-chain antibodies, engineered antibodies and single domain scaffolds other than those derived from antibodies. Single domain antibodies may be any of the art, or any future single domain antibodies. Single domain antibodies may be derived from any species including, but not limited to mouse, human, camel, llama, fish, shark, goat, rabbit, and bovine. According to another aspect of the disclosure, a single domain antibody is a naturally occurring single domain antibody known as heavy chain antibody devoid of light chains. Such single domain antibodies are disclosed in WO 9404678, for example. For clarity reasons, this variable domain derived from a heavy chain antibody naturally devoid of light chain is known herein as a VHH or nanobody to distinguish it from the conventional VH of four chain immunoglobulins. Such a VHH molecule can be derived from antibodies raised in *Camelidae* species, for

example in camel, llama, dromedary, alpaca and guanaco. Other species besides *Camelidae* may produce heavy chain antibodies naturally devoid of light chain; such VHHs are within the scope of the present disclosure.

[0235] Antibody molecules can include non-antibody scaffolds and antibody mimetics. Exemplary non-antibody scaffolds include: lipocalins (e.g. anticalins), affibodies, fibronectin (e.g. monobodies or Adnectins), knottins, ankyrin repeats (e.g. DARPin), and A domains (e.g. avimers).

[0236] The VH and VL regions can be subdivided into regions of hypervariability, termed “complementarity determining regions” (CDR), interspersed with regions that are more conserved, termed “framework regions” (FR or FW).

[0237] The extent of the framework region and CDRs has been precisely defined by a number of methods (see, Kabat, E. A., et al. (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Chothia, C. et al. (1987) *Mol. Biol.* 196:901-917; and the AbM definition used by Oxford Molecular’s AbM antibody modeling software. See, generally, e.g., *Protein Sequence and Structure Analysis of Antibody Variable Domains*. In: *Antibody Engineering Lab Manual* (Ed.: Duebel, S. and Kontermann, R., Springer-Verlag, Heidelberg).

[0238] The terms “complementarity determining region,” and “CDR,” as used herein refer to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. In general, there are three CDRs in each heavy chain variable region (HCDR1, HCDR2, HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, LCDR3).

[0239] The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of known schemes, including those described by Kabat et al. (1991), “*Sequences of Proteins of Immunological Interest*,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (“Kabat” numbering scheme), Al-Lazikani et al., (1997) *JMB* 273,927-948 (“Chothia” numbering scheme). As used herein, the CDRs defined according to the “Chothia” number scheme are also sometimes referred to as “hypervariable loops.”

[0240] For example, under Kabat, the CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the light chain variable domain (VL) are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). Under Chothia, the CDR amino acids in the VH are numbered 26-32 (HCDR1), 52-56 (HCDR2), and 95-102 (HCDR3); and the amino acid residues in VL are numbered 26-32 (LCDR1), 50-52 (LCDR2), and 91-96 (LCDR3).

[0241] Each VH and VL typically includes three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[0242] The antibody molecule can be a polyclonal or a monoclonal antibody.

[0243] The terms “monoclonal antibody” or “monoclonal antibody composition” as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. A monoclonal

antibody can be made by hybridoma technology or by methods that do not use hybridoma technology (e.g., recombinant methods).

[0244] The antibody can be recombinantly produced, e.g., produced by phage display or by combinatorial methods.

[0245] Phage display and combinatorial methods for generating antibodies are known in the art (as described in, e.g., Ladner et al. U.S. Pat. No. 5,223,409; Kang et al. International Publication No. WO 92/18619; Dower et al. International Publication No. WO 91/17271; Winter et al. International Publication WO 92/20791; Markland et al. International Publication No. WO 92/15679; Breitling et al. International Publication WO 93/01288; McCafferty et al. International Publication No. WO 92/01047; Garrard et al. International Publication No. WO 92/09690; Ladner et al. International Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum Antibod Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths et al. (1993) *EMBO J* 12:725-734; Hawkins et al. (1992) *J Mol Biol* 226:889-896; Clackson et al. (1991) *Nature* 352:624-628; Gram et al. (1992) *PNAS* 89:3576-3580; Garrard et al. (1991) *Bio/Technology* 9:1373-1377; Hoogenboom et al. (1991) *Nuc Acid Res* 19:4133-4137; and Barbas et al. (1991) *PNAS* 88:7978-7982, the contents of all of which are incorporated by reference herein).

[0246] In one exemplary phage display technique, antibody repertoires can be displayed on the surface of filamentous bacteriophage, and the constructed library may be screened for phages that bind to the immunogen (e.g., CD4, CD25, CD39, NRP1). Antibody phage is based on genetic engineering of bacteriophages and repeated rounds of antigen-guided selection and phage propagation. This technique allows in vitro selection of monoclonal antibodies. The phage display process begins with antibody-library preparation followed by ligation of the variable heavy (VH) and variable light (VL) PCR products into a phage display vector, culminating in analysis of clones of monoclonal antibodies. The VH and VL PCR products, representing the antibody repertoire, are ligated into a phage display vector (e.g., the phagemid pComb3X) that is engineered to express the VH and VL as an scFv fused to the pIII minor capsid protein of a filamentous bacteriophage of *Escherichia coli* that was originally derived from the M13 bacteriophage. However, the phage display vector pComb3X does not have all the other genes necessary to encode a full bacteriophage in *E. coli*. For those genes, a helper phage is added to the *E. coli* that are transformed with the phage display vector library. The result is a library of phages, each expressing on its surface a monoclonal antibody and harboring the vector with the respective nucleotide sequence within. The phage display can also be used to produce the monoclonal antibody itself (not attached to phage capsid proteins) in certain strains of *E. coli*. Additional cDNA is engineered, in the phage display vector, after the VL and VH sequences to allow characterization and purification of the mAb produced. Specifically, the recombinant antibody may have a hemagglutinin (HA) epitope tag and a polyhistidine to allow easy purification from solution.

[0247] In one embodiment, the antibody is a fully human antibody (e.g., an antibody made in a mouse which has been genetically engineered to produce an antibody from a human immunoglobulin sequence), or a non-human antibody, e.g., a rodent (mouse or rat), goat, primate (e.g., monkey), camel

antibody. Preferably, the non-human antibody is a rodent (mouse or rat antibody). Methods of producing rodent antibodies are known in the art.

[0248] Human monoclonal antibodies can be generated using transgenic mice carrying the human immunoglobulin genes rather than the mouse system. Splenocytes from these transgenic mice immunized with the antigen of interest are used to produce hybridomas that secrete human mAbs with specific affinities for epitopes from a human protein (see, e.g., Wood et al. International Application WO 91/00906, Kuchlerapati et al. PCT publication WO 91/10741; Lonberg et al. International Application WO 92/03918; Kay et al. International Application 92/03917; Lonberg, N. et al. 1994 *Nature* 368:856-859; Green, L.L. et al. 1994 *Nature Genet.* 7:13-21; Morrison, S.L. et al. 1994 Proc. Natl. Acad. Sci. USA 81:6851-6855; Bruggeman et al. 1993 *Year Immunol* 7:33-40; Tuaillon et al. 1993 PNAS 90:3720-3724; Bruggeman et al. 1991 *Eur J Immunol* 21:1323-1326).

[0249] An antibody molecule can be one in which the variable region, or a portion thereof, e.g., the CDRs, are generated in a non-human organism, e.g., a rat or mouse. Chimeric, CDR-grafted, and humanized antibodies are within the present disclosure. Antibody molecules generated in a non-human organism, e.g., a rat or mouse, and then modified, e.g., in the variable framework or constant region, to decrease antigenicity in a human are within the present disclosure.

[0250] Chimeric antibodies can be produced by recombinant DNA techniques known in the art (see Robinson et al., International Patent Publication PCT/US86/02269; Akira, et al., European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., International Application WO 86/01533; Cabilly et al. U.S. Pat. No. 4,816,567; Cabilly et al., European Patent Application 125, 023; Better et al. (1988 *Science* 240:1041-1043); Liu et al. (1987) PNAS 84:3439-3443; Liu et al., 1987, *J. Immunol.* 139:3521-3526; Sun et al. (1987) PNAS 84:214-218; Nishimura et al., 1987, *Canc. Res.* 47:999-1005; Wood et al. (1985) *Nature* 314:446-449; and Shaw et al., 1988, *J. Natl Cancer Inst.* 80:1553-1559).

[0251] A humanized or CDR-grafted antibody will have at least one or two but generally all three recipient CDRs (of heavy and or light immunoglobulin chains) replaced with a donor CDR. The antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding to the antigen. Preferably, the donor will be a rodent antibody, e.g., a rat or mouse antibody, and the recipient will be a human framework or a human consensus framework. Typically, the immunoglobulin providing the CDRs is called the “donor” and the immunoglobulin providing the framework is called the “acceptor.” In one embodiment, the donor immunoglobulin is a non-human (e.g., rodent). The acceptor framework is a naturally-occurring (e.g., a human) framework or a consensus framework, or a sequence about 85% or higher, preferably 90%, 95%, 99% or higher identical thereto.

[0252] As used herein, the term “consensus sequence” refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related sequences (See e.g., Winnaker, *From Genes to Clones* (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of proteins, each position in the consensus sequence is

occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence. A “consensus framework” refers to the framework region in the consensus immunoglobulin sequence.

[0253] An antibody molecule can be humanized by methods known in the art (see e.g., Morrison, S. L., 1985, *Science* 229:1202-1207, by Oi et al., 1986, *BioTechniques* 4:214, and by Queen et al. US 5,585,089, US 5,693,761 and US 5,693,762, the contents of all of which are hereby incorporated by reference).

[0254] Humanized or CDR-grafted antibody molecules can be produced by CDR-grafting or CDR substitution, wherein one, two, or all CDRs of an immunoglobulin chain can be replaced. See e.g., U.S. Pat. 5,225,539; Jones et al. 1986 *Nature* 321:552-525; Verhoeyan et al. 1988 *Science* 239:1534; Beidler et al. 19881 *J. Immunol.* 141:4053-4060; Winter US 5,225,539, the contents of all of which are hereby expressly incorporated by reference. Winter describes a CDR-grafting method which may be used to prepare the humanized antibodies of the present present disclosure (UK Patent Application GB 2188638A, filed on Mar. 26, 1987; Winter U.S. Pat. No. 5,225,539), the contents of which is expressly incorporated by reference.

[0255] Also within the scope of the present disclosure are humanized antibody molecules in which specific amino acids have been substituted, deleted or added. Criteria for selecting amino acids from the donor are described in U.S. Pat. No. 5,585,089, e.g., columns 12-16 of U.S. Pat. No. 5,585,089, e.g., columns 12-16 of U.S. Pat. No. 5,585,089, the contents of which are hereby incorporated by reference. Other techniques for humanizing antibodies are described in Padlan et al. EP 519596 A1, published on Dec. 23, 1992.

[0256] The antibody molecule can be a single chain antibody. A single-chain antibody (scFv) may be engineered (see, for example, Colcher, D. et al. (1999) *Ann N Y Acad Sci* 880:263-80; and Reiter, Y. (1996) *Clin Cancer Res* 2:245-52). The single chain antibody can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target protein.

[0257] In yet other embodiments, the antibody molecule has a heavy chain constant region chosen from, e.g., the heavy chain constant regions of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE; particularly, chosen from, e.g., the (e.g., human) heavy chain constant regions of IgG1, IgG2, IgG3, and IgG4. In another embodiment, the antibody molecule has a light chain constant region chosen from, e.g., the (e.g., human) light chain constant regions of kappa or lambda. The constant region can be altered, e.g., mutated, to modify the properties of the antibody (e.g., to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, cell function, and/or complement function). For example, it is an isotype or subtype, fragment or other mutant, which does not support binding to an Fc receptor, e.g., it has a mutagenized or deleted Fc receptor binding region.

[0258] Methods for altering an antibody constant region are known in the art. Antibodies with altered function, e.g. altered affinity for an effector ligand, such as FcR on a cell, or the C1 component of complement can be produced by replacing at least one amino acid residue in the constant portion of the antibody with a different residue (see e.g., EP 388,151 A1, U.S. Pat. Nos. 5,624,821 and 5,648,260, the contents of all of which are hereby incorporated by refer-

ence). Similar type of alterations could be described which if applied to the murine, or other species immunoglobulin would reduce or eliminate these functions.

[0259] An antibody molecule can be derivatized or linked to another functional molecule (e.g., a cytokine molecule as described herein or other chemical or proteinaceous groups). As used herein, a “derivatized” antibody molecule is one that has been modified. Methods of derivatization include but are not limited to the addition of a fluorescent moiety, a radionucleotide, a toxin, an enzyme or an affinity ligand such as biotin. Accordingly, the antibody molecules of the present disclosure are intended to include derivatized and otherwise modified forms of the antibodies described herein, including immunoadhesion molecules. For example, an antibody molecule can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as a cytokine molecule, another antibody (e.g., a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

[0260] One type of derivatized antibody molecule is produced by crosslinking an antibody molecule to one or more proteins, e.g., a cytokine molecule, another antibody molecule (of the same type or of different types, e.g., to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (e.g., m-maleimido-benzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Ill.

[0261] In some embodiments, the targeting moiety of the FM includes an antibody molecule or a ligand that selectively binds to an immune regulatory cell surface target, e.g., an immune cell surface receptor. In some embodiments, the immune regulatory cell surface target or receptor can have one, two, three or more of the following properties: (i) is abundantly present on the surface of an immune cell (e.g., outnumbers the number of receptors for the cytokine molecule present on the immune cell surface); (ii) shows a slow downregulation, internalization, and/or cell surface turnover, e.g., relative to the receptors activated by the cytokine of the FM; (iii) is present on the surface of the immune regulatory cell for a prolonged period of time, e.g., relative to the receptors activated by the cytokine of the FM; or (iv) once internalized is substantially recycled back to the cell surface, e.g., at least 25%, 50%, 60%, 70%, 80%, 90% or more of the immune regulatory cell surface target is recycled back to the cell surface. In some embodiments, the targeting moiety of the FM binds to a recycling cell surface receptor. In some embodiments, the targeting moiety of the FM binds to a receptor expressed on a cell (e.g., an immune regulatory cell), e.g. the surface membrane of the cell, and further the cell also expresses a cytokine receptor (e.g., a receptor to the cytokine molecule of the FM).

[0262] In some embodiments, the targeting moiety of the FM can be chosen from an antibody molecule or a ligand molecule that binds to an immune regulatory cell surface target, e.g., a target chosen from CD45, CD4, CD3, CD2, CD25, CD127, CD197 (CCR7), CXCR3, CXCR4, CXCR5, CD38, CD27, CD8, CCR4, CCR5, CD137, CCR6 (CD196), CCR8, CCR10, OX40, GITR, NRP1, CTLA4, LAG3,

CD73, CD103, CD62L, CCR2, CCR9, CD11a, or CD18. In some embodiments, the immune regulatory cell surface target is chosen from CD4, CD25, CD39, CD45, and/or NRP1.

[0263] In one embodiment, the targeting moiety comprises an antibody molecule or a ligand molecule that binds to CD4. In one embodiment, the targeting moiety comprises an antibody molecule or a ligand molecule that binds to CD25. In one embodiment, the targeting moiety comprises an antibody molecule or a ligand molecule that binds to CD127. In one embodiment, the targeting moiety comprises a bispecific antibody molecule or a ligand molecule that binds to CD4 and CD25. In one embodiment, the targeting moiety comprises a bispecific antibody molecule or a ligand molecule that binds to CD127 and CD25 (e.g., for negative enrichment of Tregs). In one embodiment, the targeting moiety comprises a bispecific antibody molecule or a ligand molecule that binds to CD127 and CD4 (e.g., for negative enrichment of Tregs). The antibodies can be selected to fine tune the selective binding to the immune cell such as Treg.

[0264] In one embodiment, the targeting moiety comprises an antibody molecule or a ligand molecule that binds to CD45 (also interchangeably referred to herein as “CD45 receptor” or “CD45R”). In some embodiments, the target is CD45 (e.g., a CD45 isoform chosen from CD45RA, CD45RB, CD45RC or CD45RO). In embodiments, CD45 is primarily expressed on T cells. For example, CD45RA is primarily expressed on naïve T cells; CD45RO is primarily expressed on activated and memory T cells. In one embodiment, the targeting moiety comprises an antibody molecule or a ligand molecule that binds to CD8. In one embodiment, the targeting moiety comprises a bispecific antibody molecule or a ligand molecule that binds to CD8 and CD45.

[0265] In other embodiments, the targeting moiety of the FM comprises an antibody molecule (e.g., an antigen binding domain), a receptor molecule (e.g., a receptor, a receptor fragment or functional variant thereof), or a ligand molecule (e.g., a ligand, a ligand fragment or functional variant thereof), or a combination thereof, that binds to the Treg target or receptor.

[0266] In some embodiments, the antibody molecule of the targeting moiety of the FM comprises a full antibody (e.g., an antibody that includes at least one, and preferably two, complete heavy chains, and at least one, and preferably two, complete light chains), or an antigen-binding fragment (e.g., a Fab, F(ab')₂, Fv, a single chain Fv, a single domain antibody, a diabody (dAb), a bivalent antibody, or bispecific antibody or fragment thereof, a single domain variant thereof, or a camelid antibody)) that binds to the Treg target or receptor.

[0267] The heavy chain constant region of the antibody molecule can be chosen from IgG1, IgG2, IgG3, or IgG4, or a fragment thereof, and more typically, IgG1, IgG2 or IgG4. In some embodiments, the Fc region of the heavy chain can include one or more alterations, e.g., substitutions, to increase or decrease one or more of: Fc receptor binding, neonatal-Fc receptor binding, antibody glycosylation, the number of cysteine residues, cell function, complement function, or stabilize antibody formation (e.g., stabilize IgG4). For example, the heavy chain constant region for an IgG4, e.g., a human IgG4, can include a substitution at position 228 (e.g., a Ser to Pro substitution), as shown in, e.g., SEQ ID NO: 67 (see e.g., Angal, S, King, DJ, et al. (1993) *Mol Immunol* 30:105-108 (initially described as

S241P using a different numbering system); Owens, R, Ball, E, et al. (1997) *Immunotechnology* 3:107-116).

[0268] The light chain constant region of the antibody molecule can be chosen from the light chain constant regions of kappa or lambda, or a fragment thereof.

[0269] The antibody molecule of the targeting moiety of the FM can bind to the target antigen with a dissociation constant of less than about 100 nM, 50 nM, 25 nM, 10 nM, e.g., less than 1 nM (e.g., about 10-100 pM). In embodiments, the antibody molecule binds to a conformational or a linear epitope on the antigen. In certain embodiments, the antigen bound by the antibody molecule of the targeting moiety is stably expressed on the surface of the immune cell. In embodiments, the antigen is a cell surface receptor that is more abundant on the cell surface relative to a receptor for the cytokine molecule of the FM on the cell surface.

[0270] In some embodiments, the targeting moiety is chosen from an antibody molecule (e.g., a full antibody (e.g., an antibody that includes at least one, and preferably two, complete heavy chains, and at least one, and preferably two, complete light chains), or an antigen-binding fragment (e.g., a Fab, F(ab')₂, Fv, a single chain Fv, a single domain antibody, a diabody (dAb), a bivalent antibody, or bispecific antibody or fragment thereof, a single domain variant thereof, or a camelid antibody)), or a non-antibody scaffold, or a ligand that binds to an immune cell surface target or ligand. In some embodiments, the targeting moiety is an antibody molecule or a ligand that binds to CD45, CD4, CD3, CD2, CD25, CD127, CD197 (CCR7), CXCR3, CXCR4, CXCR5, CD38, CD27, CD8, CCR4, CCR5, CD137, CCR6 (CD196), CCR8, CCR10, OX40, GITR, NRP1, CTLA4, LAG3, CD73, CD103, CD62L, CCR2, CCR9, CD11a, or CD18, e.g., a bispecific antibody that binds to two or more of the aforesaid targets.

[0271] In some embodiments, the antibody molecule (e.g., mono- or bi-specific antibodies) binds to one or more of CD45, CD4, CD25, CD39, CD137, or NRP1, e.g., it is an IgG, e.g., human IgG4, or an antigen binding domain, e.g., a Fab, a F(ab')₂, Fv, a single chain Fv, that binds to CD45, CD4, CD25, CD39, CD137, or NRP1.

[0272] In some embodiments, the antibody molecule is a human, a humanized or a chimeric antibody. In embodiments, the antibody molecule is a recombinant antibody.

[0273] Also encompassed by the present disclosure are antibody molecules having the amino acid sequences disclosed herein, or an amino acid sequence substantially identical thereof), nucleic acid molecules encoding the same, host cells and vectors comprising the nucleic acid molecules.

[0274] In certain embodiments, the targeting moiety can be a binding agent for CD45, such as an antibody or antigen-binding fragment thereof. In some embodiments, the anti-CD45 antibody is a human anti-CD45 antibody, a humanized anti-CD45 antibody, or a chimeric anti-CD45 antibody. In some embodiments, the anti-CD45 antibody is an anti-CD45 monoclonal antibody. Exemplary anti-CD45 antibodies include antibodies BC8, 4B2, GAP8.3 or 9.4. Antibodies against other immune cell surface targets are also disclosed, e.g., anti-CD8 antibodies, such as OKT8 monoclonal antibodies, anti-CD18 antibodies, such as 1B4 monoclonal antibodies, and anti-CD11a antibodies, such as MHM24 antibodies.

[0275] In one embodiment, the antibody molecule that binds to CD45 is specific to one CD45 isoform or binds to

more than on CD45 isoforms, e.g., is a pan-CD45 antibody. In some embodiments, the anti-CD45 antibody molecule binds to CD45RA and CD45RO. In one embodiment, the anti-CD45 antibody molecule is a BC8 antibody. In some embodiments, the BC8 antibody binds to CD45RA and CD45RO. In other embodiments, the anti-CD45 antibody molecule is CD45RO-specific or is a pan-CD45 antibody molecule, e.g., it binds to activated and memory T cells. Additional examples of anti-CD45 antibody molecules include, but is not limited to, GAP8.3, 4B2, and 9.4.

Exemplary Fusion Proteins

[0276] Exemplary formats for the FM include a cytokine molecule (e.g., one or more cytokine molecules) coupled to an antibody molecule that binds to an immune cell surface target (e.g., immunoglobulin moiety (Ig), for example an antibody (e.g., a full antibody, IgG) or antibody fragment (Fab, scFv, a half antibody, or a single domain antibody and the like). In embodiments, the FM includes a fusion to the amino-terminus (N-terminus) or carboxy-terminus (C-terminus) of the antibody molecule, typically, the C-terminus. The cytokine molecule can be coupled to the antibody molecule, optionally, via a linker. In some embodiments, the cytokine or cytokine receptor, e.g., receptor fragment (e.g., sushi domain) is coupled to the N-terminus or the C-terminus of the antibody molecule. In embodiments, the cytokine or cytokine receptor, e.g., receptor fragment (e.g., sushi domain) is coupled to the N-terminus or the C-terminus of the light chain of the antibody molecule (e.g., a full antibody or fragment thereof, e.g., a Fab, a scFv, or a half antibody). Alternatively or in combination, the cytokine or cytokine receptor, e.g., receptor fragment (e.g., sushi domain) is coupled to the N-terminus or the C-terminus of the heavy chain of the antibody molecule (e.g., a full antibody or fragment thereof, e.g., a Fab, a scFv, a half antibody, or a single domain antibody (VH)). Examples of the formats are provided in, e.g., FIGS. 1, 2A, 3A-3D, 6A, 11A and 12A.

[0277] In some embodiments, the FM includes an antibody molecule or fragment thereof, e.g., anti-CD45 (e.g., BC8 antibody), anti-CD4, anti-CD25, anti-CD8, anti-CD39, or anti-NRP1, that is coupled to, e.g., covalently linked, to a cytokine molecule, e.g., IL-15, IL-2, IL-7, IL-21, IL-27, or a cytokine receptor, e.g., IL-15Ralpha sushi domain, at either the N-terminal region or the C-terminal region of the light chain or heavy chain (as depicted in, e.g., FIGS. 1B, 1G, 2A, 3A-3D, 6A, 11A and 12A). Any combination of orientation of light chain- or heavy chain-cytokine molecule can be present in the FM.

[0278] For example, the FM can include a full antibody or a tetramer of two identical half-antibodies, e.g., a first and second antibody, each having a light chain and a heavy chain (e.g., a scFv) of the antibody molecule, e.g., anti-CD45 (e.g., BC8 antibody), anti-CD8, anti-CD4, anti-CD25, anti-CD39, or anti-NRP1, that is coupled to, e.g., covalently linked, to a cytokine molecule, e.g., IL-2 or a variant thereof, IL-15 or IL-15 sushi domain, at the N-terminal region of the light chain (e.g., as depicted in FIGS. 3A and 3C).

[0279] In other embodiments, the FM can include a full antibody or a tetramer of two identical half-antibodies, e.g., a first and second antibodies, each having a light chain and a heavy chain of the targeting antibody molecule, e.g., anti-CD45 (e.g., BC8 antibody), anti-CD8, anti-CD4, anti-CD25, anti-CD39, or anti-NRP1, that is coupled to, e.g., covalently linked, to a cytokine molecule, e.g., IL-2 or its

variant, IL-15 or an IL-15 sushi domain, at the C-terminal region of the light chain (e.g., as depicted in FIGS. 2A, 3A, 3C and 6A).

[0280] Alternatively, or in combination with the aforesaid formats, the FM can include a full antibody or a tetramer of two identical half-antibodies, e.g., a first and second antibodies, each having a light chain and a heavy chain of the targeting antibody molecule, e.g., anti-CD45 (e.g., BC8 antibody), anti-CD8, anti-CD4, anti-CD25, anti-CD39, or anti-NRP1, that is coupled to, e.g., covalently linked, to a cytokine molecule, e.g., IL-2 or its variant, IL-15 or an IL-15 sushi domain, at the C-terminal region of the heavy chain (e.g., as depicted in FIG. 6A).

[0281] Alternatively, or in combination with the aforesaid format, the FM can include a full antibody or a tetramer of two identical half-antibodies, e.g., a first and second antibodies, each having a light chain and a heavy chain of the targeting antibody molecule, e.g., anti-CD45 (e.g., BC8 antibody), anti-CD8, anti-CD4, anti-CD25, anti-CD39, or anti-NRP1, that is coupled to, e.g., covalently linked, to a cytokine molecule, e.g., IL-2 or its variant, IL-15 or an IL-15 sushi domain, at the N-terminal region of the heavy chain.

[0282] Alternatively, or in combination with the aforesaid formats, the FM can include an antibody fragment (e.g., a scFv, a Fab) having a light chain variable domain and a heavy chain variable domain of the targeting antibody molecule, e.g., anti-CD45 (e.g., BC8 antibody), anti-CD8, anti-CD4, anti-CD25, anti-CD39, or anti-NRP1, that is coupled to, e.g., covalently linked, to a cytokine molecule, e.g., IL-2 or its variants, IL-15 or an IL-15 sushi domain, at the N- or C-terminal region of the heavy chain variable domain (e.g., as depicted in FIGS. 2A, 3B, 3D, 11A, and 12A).

[0283] Alternatively, or in combination with the aforesaid formats, the FM can include an antibody fragment (e.g., a scFv, a Fab) having a light chain variable domain and a heavy chain variable domain of the targeting antibody molecule, e.g., anti-CD45 (e.g., BC8 antibody), anti-CD8, anti-CD4, anti-CD25, anti-CD39, or anti-NRP1, that is coupled to, e.g., covalently linked, to a cytokine molecule, e.g., IL-2 or its variants, IL-15 or an IL-15 sushi domain, at the N- or C-terminal region of the light chain variable domain.

[0284] Alternatively, the FM includes a tetramer of two different half-antibodies, e.g., a first and second antibodies, wherein the first antibody has a light chain and a heavy chain (or an fragment thereof, e.g., scFv or Fab) of the targeting antibody molecule, e.g., anti-CD45 (e.g., BC8 antibody), anti-CD8, anti-CD4, anti-CD25, anti-CD39, or anti-NRP1, that is coupled to, e.g., covalently linked, to a cytokine molecule, e.g., IL-2 or its variants, IL-15 or an IL-15 sushi domain, at the N-terminal region of the light chain; and the second antibody has a light chain and a heavy chain (or an fragment thereof, e.g., scFv or Fab) of the targeting antibody molecule, e.g., anti-CD45 (e.g., BC8 antibody), anti-CD8, anti-CD4, anti-CD25, anti-CD39, or anti-NRP1, that is coupled to, e.g., covalently linked, to a cytokine molecule, e.g., IL-2 or its variants, IL-15 or an IL-15 sushi domain, at the C-terminal region of the light chain or the heavy chain. Any pairing of antibodies to the same or different targets can be used, such as CD4:CD45, CD8:CD45, CD4:CD39, CD4:NRP1, and CD4:CD25. In some embodiments, while CD127 is a negative surface marker for Tregs, the pairing of CD127 with a positive Treg surface marker (e.g., CD4:

CD127, CD25:CD127) can be used to negatively enrich and/or sort Tregs. For example, a blocking antibody can be targeted to non-Treg cells (for example activated Tcons having CD25). The CD127 can target the antibody to the Tcons and the CD25 can then block binding of the IL-2 tethered fusion. Then, when a CD4/CD25-IL2 molecule is introduced, the binding sites for the CD25 targeting portion of the antibody can be blocked on Tcons. Alternatively, an anti-CD127 antibody can be attached to an inhibitor of CD25, such that even if an IL-2 tethered fusion bound the Tcon, it would not activate it. This improves enrichment of IL-2 on Tregs in mixed-cell populations.

[0285] In certain embodiments, the FM can be represented with the following formula in an N to C terminal orientation: R1-(optionally L1)-R2 or R2-(optionally L1)-R1; wherein R1 comprises a targeting moiety, L1 comprises a linker (e.g., a peptide linker described herein), and R2 comprises a modulatory moiety, e.g., a cytokine molecule.

[0286] In some embodiments, the modulatory moiety, e.g., the cytokine molecule, is connected to, e.g., covalently linked to, the targeting moiety.

[0287] In some embodiments, the modulatory moiety, e.g., the cytokine molecule, is functionally linked, e.g., covalently linked (e.g., by chemical coupling, fusion, noncovalent association or otherwise) to the targeting moiety. For example, the modulatory moiety can be covalently coupled indirectly, e.g., via a linker to the targeting moiety.

[0288] In embodiments, the linker is chosen from: a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, or a non-helical linker. In some embodiments, the linker is a peptide linker. The peptide linker can be 5-20, 8-18, 10-15, or about 8, 9, 10, 11, 12, 13, 14, or 15 amino acids long. In some embodiments, the peptide linker comprises Gly and Ser, e.g., a linker comprising the amino acid sequence (Gly₄Ser)_n, wherein n indicates the number of repeats of the motif, e.g., n=1, 2, 3, 4 or 5 (e.g., a (Gly₄Ser)₂ or a (Gly₄Ser)₃ linker). In some embodiments, the linker comprises the amino acid sequence of SEQ ID NO: 36, 37, 38, or 39, or an amino acid sequence substantially identical thereto (e.g., having 1, 2, 3, 4, or 5 amino acid substitutions). In one embodiment, the linker comprises an amino acid sequence GGGSGGGS (SEQ ID NO: 37). In another embodiment, the linker comprises amino acids from an IgG4 hinge region, e.g., amino acids DKTHTSPSPAP (SEQ ID NO: 38).

[0289] In some embodiments, the linker can be a human serum albumin (HSA) linker. It has been surprisingly discovered that the HSA linker can improve IL-2 expression efficiency and increase overall stability of the FM. In some embodiments, the FM can have the formula R1-L1-R2 and can be constructed as a single genetic molecule for expression in cells.

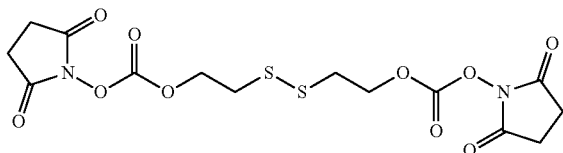
[0290] In other embodiments, the linker is a non-peptide, chemical linker. For example, the modulatory moiety is covalently coupled to the targeting moiety by crosslinking. Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). In yet other embodiments, the modulatory moiety is directly covalently coupled to the targeting moiety, without a linker.

[0291] In some embodiments, the linker can be a biodegradable or cleavable linker. A cleavable linker allows for

cleavage of the FM such that the immune stimulating moiety, e.g., the cytokine molecule, can be released from the immune targeting moiety. The cleavage of the linker may be caused by biological activation within the relevant tissue or, alternatively, by external stimuli such as, e.g., electromagnetic radiation e.g., UV-radiation.

[0292] In one embodiment, the cleavable linker is configured for cleavage exterior to a cell, e.g., to be cleaved in conditions associated with cell or tissue damage or disease. Such conditions include, for example, acidosis; the presence of intracellular enzymes (that are normally confined within cells), including necrotic conditions (e.g., cleaved by calpains or other proteases that spill out of necrotic cells); hypoxic conditions such as a reducing environment; thrombosis (e.g., a linker may be cleavable by thrombin or by another enzyme associated with the blood clotting cascade); immune system activation (e.g., a linker may be cleavable by action of an activated complement protein); or other condition associated with disease or injury.

[0293] In one embodiment, a cleavable linker may include an S-S linkage (disulfide bond), or may include a transition metal complex that falls apart when the metal is reduced. One embodiment of the S-S linker may have the following structure (as disclosed in U.S. Pat. No. 9,603,944, incorporated herein by reference in its entirety:



[0294] Another example pH sensitive linkers which are cleaved upon a change in pH, e.g., at low pH, which will facilitate hydrolysis of acid (or base) labile moieties, e.g. acid labile ester groups etc. Such conditions may be found in the extracellular environment, such as acidic conditions which may be found near cancerous cells and tissues or a reducing environment, as may be found near hypoxic or ischemic cells and tissues; by proteases or other enzymes found on the surface of cells or released near cells having a condition to be treated, such as diseased, apoptotic or necrotic cells and tissues; or by other conditions or factors. An acid-labile linker may be, for example, a cis-aconitic acid linker. Other examples of pH-sensitive linkages include acetals, ketals, activated amides such as amides of 2,3-dimethylmaleamic acid, vinyl ether, other activated ethers and esters such as enol or silyl ethers or esters, imines, iminiums, orthoesters, enamines, carbamates, hydrazones, and other linkages known in the art (see, e.g., PCT Publication No. WO 2012/155920 and Franco et al. AIMS Materials Science, 3(1): 289-323, incorporated herein by reference). The linkers disclosed in U.S. Provisional Application Nos. 62/554,067 filed Sep. 5, 2017 and 62/616,221 filed Jan. 11, 2018 can also be used and are incorporated herein by reference. The expression “pH sensitive” refers to the fact that the cleavable linker in question is substantially cleaved at an acidic pH (e.g., a pH below 6.0, such as in the range of 4.0-6.0).

[0295] In still another embodiment, the cleavable linker is configured for cleavage by an enzyme, such as a protease (e.g., pepsin, trypsin, thermolysine, matrix metalloprotei-

nase (MMP), a disintegrin and metalloprotease (ADAM; e.g. ADAM-10 or ADAM-17)), a glycosidase (e.g., α -, β -, γ -amylase, α -, β -glucosidase or lactase) or an esterase (e.g. acetyl cholinesterase, pseudo cholinesterase or acetyl esterase). Other enzymes which may cleave the cleavable linker include urokinase plasminogen activator (uPA), tissue plasminogen activator (tPA), granzyme A, granzyme B, lysosomal enzymes, cathepsins, prostate-specific antigen, Herpes simplex virus protease, cytomegalovirus protease, thrombin, caspase, and interleukin 1 beta converting enzyme.

[0296] Still another example is over-expression of an enzyme, e.g., proteases (e.g., pepsin, trypsin), in the tissue of interest, whereby a specifically designed peptide linker will be cleaved in upon arrival at the tissue of interest. Illustrative examples of suitable linkers in this respect are Gly-Phe-Ser-Gly (SEQ ID NO: 97), Gly-Lys-Val-Ser (SEQ ID NO: 98), Gly-Trp-Ile-Gly (SEQ ID NO: 99), Gly-Lys-Lys-Trp (SEQ ID NO: 100), Gly-Ala-Tyr-Met (SEQ ID NO: 101).

[0297] In still another example, over-expression of an enzyme, e.g. of glycosidases (e.g. α -amylase), in the tissue of interest, causes a specifically designed carbohydrate linker to be cleaved upon arrival at the tissue of interest. Illustrative examples of suitable linkers in this respect are—(α -1-4-D-Glucose)_n—where $n \geq 4$.

[0298] In still another example, the cleavable linker is configured for cleavage by electromagnetic radiation, e.g., UV-radiation. UV-exposure of the tissue of interest resulting in cleavage of the linker B can facilitate drug release or facilitate nanoparticles uptake in the desired tissue.

[0299] The cleavable linker may include a total of from 2 to 60 atoms, such as from 2 to 20 atoms. The cleavable linker may include amino acid residues, and may be a peptide linkage, e.g., of from 1 to 30, or from 2 to 10, amino acid residues. In one variant, the cleavable linker B consists of from 1 to 30, such as from 2 to 10, or from 2 to 8, or from 3 to 9, or from 4-10, amino acids. For pH sensitive linkers, the number of atoms is typically from 2 to 50, such as from 2-30.

[0300] In some embodiments of the invention, the linker includes an aminocaproic acid (also termed aminohexanoic acid) linkage or a linkage composed of from 1 to 30, or from 2 to 10 carbohydrate residues.

[0301] In one embodiment, the linker includes a peptide that can serve as a substrate of a matrix metalloproteinase. As the matrix metalloproteinase, for example, MMP-1 (interstitial collagenase), MMP-2 (gelatinase A), MMP-3, MMP-7, MMP-9 (gelatinase B), and the like are known, and a substrate peptide that can serve as a substrate of one or more kinds of matrix metalloproteinases among those mentioned above can be used. For matrix metalloproteinases, see for example, “Molecular mechanism of cancer metastasis”, Ed. by Tsuruo T., pp. 92-107, Medical View Co., Ltd., published in 1993. As for the substrate peptide that can serve as a substrate of a matrix metalloproteinase, for example, the matrix metalloproteinases of particular types and substrate peptides specifically recognized thereby are explained in Nature Biotechnology, 19, pp. 661-667, 2001. Therefore, by referring to this publication, a substrate peptide specifically cleaved by a particular type of matrix metalloproteinase can be chosen. For example, Val-Pro-Leu-Ser-Leu-Tyr-Ser-Gly (SEQ ID NO: 102) is known as a specific substrate for MMP-9, and it is preferable to use the aforementioned octapeptide as a substrate peptide that can serve as a

substrate of MMP-9. Illustrative examples of the substrate peptide that can serve as a substrate of a matrix metalloproteinase include Gly-Pro-Gln-Gly-Ile-Ala-Gly-Gln (SEQ ID NO: 103), Val-Pro-Met-Ser-Met-Arg-Gly-Gly (SEQ ID NO: 104), Ile-Pro-Val-Ser-Leu-Arg-Ser-Gly (SEQ ID NO: 105), Arg-Pro-Phe-Ser-Met-Ile-Met-Gly (SEQ ID NO: 106), Val-Pro-Leu-Ser-Leu-Thr-Met-Gly (SEQ ID NO: 107), Ile-Pro-Glu-Ser-Leu-Arg-Ala-Gly (SEQ ID NO: 108), Arg-His-Asp, Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys (SEQ ID NO: 109), Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys (SEQ ID NO: 110), Pro-Gln-Gly-Ile-Ala-Gly-Gln-Arg (SEQ ID NO: 111), Pro-Leu-Gly-Ile-Ala-Gly-Arg (SEQ ID NO: 112), Gly-Pro-Leu-Gly-Pro (SEQ ID NO: 113), Gly-Pro-Ile-Gly-Pro (SEQ ID NO: 114), and the like.

[0302] In another embodiment, the linker includes a peptide that can serve as a substrate of a disintegrinase and metalloproteases (ADAM), an MMP, or a granzyme. For example, without limitation the linker could comprise a peptide substrate of ADAM-8, ADAM-10, ADAM-12, ADAM-17, MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-13, granzyme A, or granzyme B. Substrates of ADAMs, MMPs, and granzymes have been well-described (e.g. in Miller, MA, et al. *Integrative Biology* (2011) 3:422-438, Van Damme et al. *Biol Chem* (2010) 391:983-997, and Casciola-Rosen, L, et al. *J Biol Chem* (2007) 282:4545-4552) and one skilled in the art would readily be able to incorporate such peptides, or variants thereof, into a linker of interest.

[0303] The linker may, besides the substrate peptide, contain connectors, involved in the bond or bonds with the therapeutic protein. Such connectors may each consist of one amino acid residue or of an oligopeptide containing from 2 to 10, such as from 3 to 9, or from 4 to 8, or from 2 to 8, amino acid residues. The amino acid residue or oligopeptide as the connectors may, if present, bind to both ends of the substrate peptide, or may bind only to one end of the substrate peptide so as to represent one of the structures. Types of one amino acid usable as the connector (s), and amino acid residues constituting an oligopeptide usable as the connector(s) are not particularly limited, and one amino acid residue of an arbitrary type, or an arbitrary oligopeptide containing, e.g., from 2 to 8 of the same or different amino acid residues of arbitrary types can be used. Examples of the oligopeptide usable as the connector(s) include, for example, connectors that are rich in Gly amino acids. Other organic moieties can also be used as connectors.

[0304] In yet other embodiments, the modulatory moiety and the targeting moiety of the FM are not covalently linked, e.g., are non-covalently associated.

[0305] Exemplary formats for fusion of a cytokine molecule to an antibody molecule, e.g., an immunoglobulin moiety (Ig), for example an antibody (IgG) or antibody fragment (Fab, scFv and the like) can include a fusion to the amino-terminus (N-terminus) or carboxy-terminus (C-terminus) of the antibody molecule, typically, the C-terminus of the antibody molecule. In one embodiment, a cytokine-Ig moiety fusion molecule comprising a cytokine polypeptide, cytokine-receptor complex, or a cytokine -receptor Fc complex joined to an Ig polypeptide, a suitable junction between the cytokine polypeptide chain and an Ig polypeptide chain includes a direct polypeptide bond, a junction having a polypeptide linker between the two chains; and, a chemical linkage between the chains. A typical junction is a flexible linker composed of small Gly4Ser linker (Gly-Gly-Gly-Gly-

Ser)_N, where _N indicates the number of repeats of the motif (Gly4Ser)₂ and (Gly4Ser)₃ are preferred embodiments of linkers for use in the fusion constructs of the present disclosure.

[0306] Exemplary fusion proteins described herein can comprise the amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to any of the aforesaid amino acid sequences).

[0307] Described herein are exemplary fusion proteins (or portion thereof) of the present disclosure. It should be noted that in certain scFv the arrangement is VH-linker-VL. However, the VL-linker-VH arrangement can also be used without affecting functionality. In addition, while specific linkers (Linker-1, Linker-2, etc.) were used in various constructs, other linkers disclosed herein can also be used interchangeably.

SEQ ID NO: 1

[0308] Name: LC-chBC8-sushi

[0309] Light-chain of chimeric BC8 anti-CD45 antibody; contains variable domain from parental BC8 mouse monoclonal antibody and human constant kappa domain; contains wild-type IL-15R α -sushi domain genetically fused to antibody light-chain C-terminus using a flexible linker.

DIVLTQSPASLAVSLGQRATISCRASKSVSTSGSYLHWYQQKPGQPPK
LLIYLASNLSEGVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHSREL
PFTFGSGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVLNNFYPRE
AKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKVY
ACEVTHQGLSPVTKSFNRGECGGGGSGGGSGGGGSITCPPPMVSVEHA
DIWVKSYSLSYRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSL
KCIR

[0310] From N- to C-terminus, a light chain portion of a chimeric BC8 Fab is shown fused via a peptide linker to an IL-15-binding sushi domain. The sequence of the light chain is shown in normal font; the location of the peptide linker is shown by italics and single underline; and the sushi domain is shown by the double underline. The kappa constant region is shown as SEQ ID NO: 74.

[0311] In some embodiments, the chimeric BC8 antibody comprises the light chain variable amino acid sequence (optionally, further including a kappa light chain sequence) shown in SEQ ID NO:1, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 95% or higher identical to SEQ ID NO:1). In embodiments, the chimeric BC8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the light chain variable region of the BC8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO: 1.

[0312] In embodiments, the FMs described herein can comprise the amino acid sequence selected from SEQ ID NO: 1, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO: 1, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 1. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 1.

SEQ ID NO: 2

[0313] Name: sushi-LC-chBC8

[0314] Light-chain of chimeric BC8 anti-CD45 antibody; contains variable domain from parental BC8 mouse monoclonal antibody and human constant kappa domain; contains wild-type IL-15R α -sushi domain genetically fused to antibody light-chain N-terminus using a flexible linker.

ITCPPPMSVEHADIWVKSYSLSYRERYICNSGFKRKAGTSSLTECVLNK
ATNVAHWTTPLSKCIRGGGGSGGGSGGGSDIVLTQSPASLAVSLGQR
 ATISCRASKSVSTSGSYSLHWYQQKPGQPPKLLIYLASNLESGVPARFS
 GSGSGTDFTLNHPVEEEDAATYYCQHSRELPTFGSGTKLEIKRTVA
 APSVFIPPPSDEQLKSGTASVCLLNFPYPAKRVQWKVDNALQSGNSQE
 SVTEQDSKSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
 RGEC

[0315] From N- to C-terminus, an IL-15-binding wild type sushi domain is fused via a peptide linker to a light chain portion of a chimeric BC8 Fab is shown. The sequence of the light chain is shown in normal font; the location of the peptide linker is shown by italics and single underline; and the sushi domain is shown by the double underline. The kappa constant region is shown as SEQ ID NO: 74.

[0316] In some embodiments, the chimeric BC8 antibody comprises the light chain variable amino acid sequence (optionally, further including a kappa light chain sequence) shown in SEQ ID NO:2, or an amino acid sequence substantially identical thereof (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to SEQ ID NO:2). In embodiments, the BC8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the light chain variable region of the BC8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have

at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO: 2.

[0317] In embodiments, the FMs described herein can comprise the amino acid sequence selected from SEQ ID NO: 2, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO: 2, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 2. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 2.

SEQ ID NO: 3

Name: LC-chBC8-IL15 (Also Referred to as LC-chBC8-L1-IL15)

[0318] Light-chain of chimeric BC8 anti-CD45 antibody; contains variable domain from parental mouse monoclonal antibody and human constant kappa domain; contains wild-type IL-15 genetically fused to antibody light-chain C-terminus using a flexible linker.

DIVLTQSPASLAVSLGQRATISCRASKSVSTSGSYSLHWYQQKPGQPPK
 LLIYLASNLESGVPARFSGSGSGTDFTLNHPVEEEDAATYYCQHSREL
 PFTFGSGTKLEIKRTVAAPSVPFI~~PPSDEQLKSGTASVCLLNFPY~~PRE
 AKVQWKVDNALQSGNSQESVTEQDSKSTYSLSTLTLSKADYEKHKVY
 ACEVTHQGLSSPVTKSFNRGECGGGGSGGGSGGGSNWNVISDLKKI
EDLIQSMHIDATLYTESDVHPSCVKTMKCFLLLEQLVLSLESGDASIHD
TVENLIILANNSLSSNGNVTESGCKECELEEKNIKEFLQSFVHIVQMF
 INTS

[0319] From N- to C-terminus, a light chain portion of a chimeric BC8 Fab is shown fused via a peptide linker to an IL-15 cytokine. The sequence of the light chain is shown in normal font; the location of the peptide linker is shown by italics and single underline; and the cytokine molecule is shown by the double underline. The kappa constant region is shown as SEQ ID NO: 74.

[0320] In some embodiments, the chimeric BC8 antibody comprises the light chain variable amino acid sequence (optionally, further including a kappa light chain sequence) shown in SEQ ID NO:3, or an amino acid sequence substantially identical thereof (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to SEQ ID NO:3). In embodiments, the BC8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the light chain variable domain of the BC8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO: 3.

[0321] In embodiments, the FMs described herein can comprise the amino acid sequence selected from SEQ ID

NO: 3, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO: 3, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 3. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 3.

SEQ ID NO: 4

Name: IL15-LC-chBC8

[0322] Light-chain of chimeric BC8 anti-CD45 antibody; contains variable domain from parental mouse monoclonal antibody and human constant kappa domain; contains wild-type IL-15 genetically fused to antibody light-chain N-terminus using a flexible linker.

NWNVNISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQV
ISLESGDASIHDVTENLIILANNSLSSNGNVTESGCKECEELEEKNIKE
FLQSFVHVQMFINTSGGGGSGGGGSDIVLTQSPASLAVSLGQR
 ATISCRASKSVSTSGSYLHWYQQKPGQPPLLIYLASNLESGVPARFS
 GSGSGTDFTLNINHPVEEEDAATYYCQHSRELPTFGSGTKLEIKRTVAA
 PSVFIFPPSPDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQE
 SVTEQDSKDSYSTLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
 RGE

[0323] From N- to C-terminus, an IL-15 cytokine fused via a peptide linker to a light chain portion of a chimeric BC8 Fab is shown. The sequence of the light chain is shown in normal font; the location of the peptide linker is shown by italics and underline; and the cytokine molecule is shown by the double underline. The kappa constant region is shown as SEQ ID NO: 74.

[0324] In some embodiments, the chimeric BC8 antibody comprises the light chain variable amino acid sequence (optionally, further including a kappa light chain sequence) shown in SEQ ID NO:4, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 95% or higher identical to SEQ ID NO:4). In embodiments, the BC8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the light chain variable domain of the BC8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from the any of the CDR sequences of SEQ ID NO: 4.

[0325] In embodiments, the FMs described herein can comprise the amino acid sequence selected from SEQ ID NO: 4, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO: 2, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 4. In embodiments, the FM comprises no more than

five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 4.

[0326] An exemplary corresponding heavy chain of the chimeric BC8 antibody molecule, e.g., a Fab or IgG, is shown below as SEQ ID NO:5 or SEQ ID NO:6, respectively.

SEQ ID NO: 5

Name: HC-chBC8-Fab

[0327] Fab heavy-chain of chimeric BC8 anti-CD45 antibody; contains variable domain from parental BC8 mouse monoclonal antibody and CH1 domain from human IgG1.

[0328] In some embodiments, the chimeric BC8 antibody comprises the heavy chain variable amino acid sequence (optionally, further including a CH1 domain sequence from human IgG1) shown in SEQ ID NO:5, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to SEQ ID NO:5). In embodiments, the BC8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the heavy chain variable domain of the BC8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from the CDR sequence of SEQ ID NO: 5.

SEQ ID NO: 6

Name: HC-chBC8-IgG4S228P

[0329] Heavy-chain of chimeric BC8 anti-CD45 antibody; contains variable domain from parental BC8 mouse monoclonal antibody and constant region from human IgG4 containing S228P mutation (IgG4-S228P).

[0330] In some embodiments, the chimeric BC8 antibody comprises the heavy chain variable amino acid sequence (optionally, further including a constant domain sequence from human IgG4 containing S228P mutation (IgG4-S228P)) shown in SEQ ID NO:6, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to SEQ ID NO:6). In embodiments, the chimeric BC8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the heavy chain variable domain of the BC8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO: 6.

SEQ ID NO: 7

Name: LC-chBC8

[0331] Light-chain of chimeric BC8 anti-CD45 antibody; contains variable domain from parental BC8 mouse monoclonal antibody and human constant kappa domain.

[0332] In some embodiments, the BC8 antibody comprises the light chain variable amino acid sequence (optionally, further including a constant domain sequence from human kappa) shown in SEQ ID NO:7, or an amino acid sequence substantially identical thereto (e.g., an amino acid

sequence at least 85%, 90%, 95% or higher identical to SEQ ID NO:7). In embodiments, the BC8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the light chain variable domain of the BC8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO: 7.

SEQ ID NO: 8

Name: HC-chBC8-IgG4S228P-IL15

[0333] Heavy-chain of chimeric BC8 anti-CD45 antibody; contains variable domain from parental BC8 mouse monoclonal antibody and constant region from human IgG4 containing S228P mutation (IgG4-S228P); contains wild-type IL-15 genetically fused to antibody heavy-chain C-terminus using a flexible linker.

QVQLVESGGGLVQPGGSLKLSCAASGFDfsRYWMSWRQAPGKLEWIG
EINPTSTSTINFTPSLKDKVFI SRDNAKNTLYLQMSKVRSEDTALYYCAR
GNYRYRGDAMDYWGQTSVTVSSASTKGPSVFPLAPCSRSTSESTAALG
CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSST
LGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVQSEDPEVQFNWYVDGVGVHNAKTKPRE
EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQ
PREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTTPPVLSDSGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKS
LSLSLGKGGGGGGGGGGGGSNWVNVI SDLKKI EDLIQSMHIDATLYT
ESDVHPSCKV TAMKCFLEELQVISLES GDASIHDTVENLI I LANNSLS

[0334] From N- to C-terminus, a heavy chain portion of a chimeric BC8 IgG4 fused to an IL-15 cytokine fused via a peptide linker is shown. The sequence of the heavy chain is shown in normal font; the location of the peptide linker is shown by italics and underline; and the cytokine molecule is shown by the double underline.

[0335] In some embodiments, the BC8 antibody comprises the heavy chain variable amino acid sequence (optionally, further including human IgG4 constant sequence) shown in SEQ ID NO:8, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 95% or higher identical to SEQ ID NO:8). In embodiments, the BC8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the heavy chain variable domain of the BC8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from the any of the CDR sequences of SEQ ID NO: 8.

[0336] In embodiments, the FMs described herein can comprise the amino acid sequence of SEQ ID NO: 8, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%,

98%, 99% or higher identity to SEQ ID NO: 8, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 8. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 8.

SEQ ID NO: 14

Name: HC-ch9.4-Fab

[0337] Fab heavy-chain of chimeric 9.4 anti-CD45 antibody; contains variable domain from parental 9.4 mouse monoclonal antibody and CH1 domain from human IgG1.

[0338] In some embodiments, the chimeric 9.4 antibody comprises the heavy chain variable amino acid sequence (optionally, further including a human IgG1 heavy chain sequence) shown in SEQ ID NO:14, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 95% or higher identical to SEQ ID NO:14). In embodiments, the 9.4 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the heavy chain variable domain of the 9.4 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO:14.

SEQ ID NO: 15

Name: LC-ch9.4-IL15

[0339] Light-chain of chimeric 9.4 anti-CD45 antibody; contains variable domain from parental 9.4 mouse monoclonal antibody and human constant kappa domain; contains wild-type IL-15 genetically fused to antibody light-chain C-terminus using a flexible linker.

DIVMTQAAPSVPTPGESLSISCRSSKSLHSSGITYLYWFLQRPGQSP
 QLLIYRMSNLAGVDPDRFSGSGSGTAFTLRISRVEADVGVVYCMQHLE
 YPFTFGGGTKLEIKRTVAAPSVFIFPPSPDEQLKSGTASVVCLLNNFYPR
 EAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKV
 YACEVTHQGLSSPVTKSFNRGECGGGSGGGSGGGSGGNWNVINISDLKK
IEDLIQSMHIDATLYTESDVHPSPCKVTAMKCFLLLEQVIVLSLESGDASIH
DTVENLIILANNSLSNGNVTESGCKECELEEKNIKEFLQSFVHIVQM
 FINTS

[0340] From N- to C-terminus, a light chain portion of a chimeric 9.4 IgG1 fused to an IL-15 cytokine fused via a peptide linker is shown. The sequence of the light-chain is shown in normal font; the location of the peptide linker is shown by italics and underline; and the cytokine molecule is shown by the double underline. The kappa constant region is shown as SEQ ID NO: 74.

[0341] In some embodiments, the light chain variable amino acid sequence of the chimeric 9.4 antibody (optionally, further including a kappa light chain sequence) corresponding to the antibody portion of the amino acid sequence

shown in SEQ ID NO:15, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to the antibody portion of SEQ ID NO:15). In embodiments, the 9.4 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the BC8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from the any of the CDR sequences of SEQ ID NO: 15.

[0342] In embodiments, the FMs described herein can comprise the amino acid sequence of SEQ ID NO: 15, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO: 15, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 15. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 15.

SEQ ID NO: 16

Name: HC-ch4B2-Fab

[0343] Fab heavy-chain of chimeric 4B2 anti-CD45 antibody; contains variable domain from parental 4B2 mouse monoclonal antibody and CH1 domain from human IgG1.

[0344] In some embodiments, the chimeric 4B2 antibody comprises the heavy chain variable amino acid sequence (optionally, further including a human IgG1 heavy chain sequence) shown in SEQ ID NO:16, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 95% or higher identical to SEQ ID NO:16). In embodiments, the 4B2 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the heavy chain variable domain of the 4B2 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO:16.

SEQ ID NO: 17

Name: LC-ch4B2-IL15

[0345] Light-chain of chimeric 4B2 anti-CD45 antibody; contains variable domain from parental 4B2 mouse monoclonal antibody and human constant kappa domain; contains wild-type IL-15 genetically fused to antibody light-chain C-terminus using a flexible linker.

DIVITQDELSNPVTSGESVVISCRSSKSLLYKDGKTYLNWFLQRPQGSF
QLLIYLMSTRASGVSDRFSGSGSGTDFTLEISRVAEDVGVIYCCQQLVE
YPFTFGGGTKLEVKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR
EAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKV
YACEVTHQGLSPVTKSFNRGECGGGGSGGGSGGGGNNWVNVISDLKK

-continued

IEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLLEQVISLESQDASIH

DTVENLIILANNLSLSSNGNVTESGCKECEELEEKNIKEFLQSPFVHIVQM

FINTS

[0346] From N- to C-terminus, a light chain portion of a chimeric 4B2 IgG1 fused to an IL-15 cytokine via a peptide linker is shown. The sequence of the light chain is shown in normal font; the location of the peptide linker is shown by italics and underline; and the cytokine molecule is shown by the double underline. The kappa constant region is shown as SEQ ID NO: 74.

[0347] In some embodiments, the light chain variable amino acid sequence of the chimeric 4B2 antibody (optionally, further including a kappa light chain sequence) corresponding to the antibody portion of the amino acid sequence shown in SEQ ID NO:17, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to the antibody portion of SEQ ID NO:17). In embodiments, the 4B2 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the 4B2 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from the any of the CDR sequences of SEQ ID NO: 17.

[0348] In embodiments, the FMs described herein can comprise the amino acid sequence of SEQ ID NO: 17, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO: 17, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 17. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 17.

SEQ ID NO: 18

Name: HC-hBC8(23)-Fab

[0349] Fab heavy-chain of humanized BC8 anti-CD45 antibody; contains humanized variable domain and CH1 domain from human IgG1.

[0350] In some embodiments, the heavy chain variable amino acid sequence of a humanized BC8 antibody (optionally, further including a CH1 domain sequence from human IgG1) has the amino acid sequence shown in SEQ ID NO:18, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to the antibody portion of SEQ ID NO:18). In embodiments, the BC8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the heavy chain variable domain of the BC8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO: 18.

SEQ ID NO: 19

Name: LC-hBC8(23)-IL15

[0351] Light-chain of humanized BC8 anti-CD45 antibody; contains humanized variable domain and human constant kappa domain; contains wild-type IL-15 genetically fused to antibody light-chain C-terminus using a flexible linker.

EIVLTQSPATLSLGLGERATISCRASKSVSTSGYSYLHWYQQKPGQAPK
 LLIYLASNRATGVPARFSGSGPGTDFTLTISSELEPEDFATYYCQHSREL
 PFTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE
 AKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVY
 ACEVTHQGLSSPVTKSFNRGECGGGGSGGGSGGGGSNVVNVISDLKK
IEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLESGDASIH
DTVENLIILANNSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQM
FINTS

[0352] From N- to C-terminus, a light chain portion of a humanized BC8 IgG1 fused to an IL-15 cytokine via a peptide linker is shown. The sequence of the light chain is shown in normal font; the location of the peptide linker is shown by italics and underline; and the cytokine molecule is shown by the double underline. The kappa constant region is shown as SEQ ID NO: 74.

[0353] In some embodiments, the light chain variable amino acid sequence of the humanized BC8 antibody (optionally, further including a kappa light chain sequence) corresponding to the antibody portion of the amino acid sequence shown in SEQ ID NO:19, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to the antibody portion of SEQ ID NO:19). In embodiments, the BC8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the light chain variable domain of the BC8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any the CDR sequences of SEQ ID NO: 19.

[0354] In embodiments, the FMs described herein can comprise the amino acid sequence of SEQ ID NO: 19, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO: 19, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 19. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 19.

SEQ ID NO: 20

[0355] Name: HC-hBC8(23)-null-Fab

[0356] Fab heavy-chain of humanized BC8 anti-CD45 antibody; contains humanized variable domain, an SGGGS (SEQ ID NO: 90) substitution in CDR-H3, and CH1 domain from human IgG1.

SEQ ID NO: 21

Name: LC-chBC8-L2-IL15

[0357] Light-chain of chimeric BC8 anti-CD45 antibody; contains variable domain from parental mouse monoclonal antibody and human constant kappa domain; contains wild-type IL-15 genetically fused to antibody light-chain C-terminus using a flexible linker with sequence GGGSGGGS (SEQ ID NO: 37).

DIVLTQSPASLAVSLGQRATISCRASKSVSTSGYSYLHWYQQKPGQPPK
 LLIYLASNLESGVPAARFSGSGSGTDFTLNHPVEEDAATYYCQHSREL
 PFTFGSGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE
 AKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVY
 ACEVTHQGLSSPVTKSFNRGECGGGGSGGGGSNVVNVISDLKKIEDLIQSM
HIDATLYTESDVHPSCKVTAMKCFLELQVISLESGDASIHDTVENLI
LANNSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

[0358] From N- to C-terminus, a light chain portion of a chimeric BC8 IgG1 fused to an IL-15 cytokine via a peptide linker (L2) is shown. The sequence of the light chain is shown in normal font; the location of the peptide linker is shown by italics and underline; and the cytokine molecule is shown by the double underline. The kappa constant region is shown as SEQ ID NO: 74.

[0359] In some embodiments, the light chain variable amino acid sequence of the chimeric BC8 antibody (optionally, further including a kappa light chain sequence) corresponding to the antibody portion of the amino acid sequence shown in SEQ ID NO:21, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to the antibody portion of SEQ ID NO:21). In embodiments, the BC8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the light chain variable domain of the BC8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any the CDR sequences of SEQ ID NO: 21.

[0360] In embodiments, the FMs described herein can comprise the amino acid sequence of SEQ ID NO: 21, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO:21, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 21. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 21.

SEQ ID NO: 22

Name: LC-chBC8-L3-IL15

[0361] Light-chain of chimeric BC8 anti-CD45 antibody; contains variable domain from parental mouse monoclonal antibody and human constant kappa domain; contains wild-type IL-15 genetically fused to antibody light-chain C-terminus using a linker related to the human IgG1 hinge (SEQ ID NO: 38).

DIVLTQSPASLAVSLGQRATISCRASKSVSTSGYSYLHWYQQKPGQPPK
 LLIIYASNLSESGVPARFSGSGSGTDFTLNIHPVEEDAATYYCQHSREL
 PFTFGSGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE
 AKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKVY
 ACEVTHQGLSSPVTKSFNRGECDKTHTSPFPAPNWNVISDLKKIEDL
IQSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLESQDASIHDTVE
NLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN
S

[0362] From N- to C-terminus, a light chain portion of a chimeric BC8 IgG1 fused to an IL-15 cytokine via a peptide linker (L3) is shown. The sequence of the light chain is shown in normal font; the location of the peptide linker is shown by italics and underline; and the cytokine molecule is shown by the double underline. The kappa constant region is shown as SEQ ID NO: 74.

[0363] In some embodiments, the light chain variable amino acid sequence of the chimeric BC8 antibody (optionally, further including a kappa light chain sequence) corresponding to the antibody portion of the amino acid sequence shown in SEQ ID NO:22, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to the antibody portion of SEQ ID NO:22). In embodiments, the BC8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the light chain variable domain of the BC8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO: 22.

[0364] In embodiments, the FMs described herein can comprise the amino acid sequence of SEQ ID NO: 22, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO:22, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 22. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 22.

SEQ ID NO: 25

[0365] Name: hBC8(23)-Null Heavy-Chain Variable Domain

[0366] Heavy-chain variable domain of humanized BC8 anti-CD45 antibody; contains an SGGGS substitution in CDR-H3.

[0367] In embodiments, the FMs described herein can comprise the amino acid sequence of SEQ ID NO: 25, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO:25, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 25. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 25.

SEQ ID NO: 26

Name: HC-chMHM24-Fab

[0368] Fab heavy-chain of chimeric MHM24 anti-CD11a antibody; contains variable domain from parental mouse monoclonal antibody and CH1 domain from human IgG1.

[0369] In some embodiments, the heavy chain variable amino acid sequence of a chimeric MHM24 antibody (optionally, further including a CH1 domain sequence from human IgG1) has the amino acid sequence shown in SEQ ID NO:26, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to the antibody portion of SEQ ID NO:26). In embodiments, the MHM24 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the heavy chain variable domain of the MHM24 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO: 26.

SEQ ID NO: 69

[0370] Name: HC-hMHM24-Fab

[0371] Fab heavy-chain of humanized MHM24 anti-CD11a antibody; contains variable domain from a humanized MHM24 heavy-chain variable domain and CH1 domain from human IgG1.

[0372] In some embodiments, the heavy chain variable amino acid sequence of a humanized MHM24 antibody (optionally, further including a CH1 domain sequence from human IgG1) has the amino acid sequence shown in SEQ ID NO: 27, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to the antibody portion of SEQ ID NO: 27). In embodiments, the MHM24 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the heavy chain variable domain of the MHM24 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO: 27.

SEQ ID NO: 27

Name: LC-chMHM24-IL15

[0373] Light-chain of chimeric MHM24 anti-CD11a antibody; contains variable domain from parental mouse mono-

clonal antibody and human constant kappa domain; contains wild-type IL-15 genetically fused to antibody light-chain C-terminus using a flexible linker.

DVQITQSPSYLAASPGETISINCRASKTISKYLAWYQEKPGKTNKLLIY
SGSTLQSGIPSRFSGSGSGTDFTLTITSSLEPEDFAMYCYQQHNEYPLTF
GTGKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYREAKVQ
WKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGECGGGGSGGGSGGGGSNWNVNSDLKKIEDLI
QSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLESSGDASIHDTVEN
LIILANNSLSSNGNVTESGCKECELEEKNIKEFLQSFVHIVQMFINTS

[0374] From N- to C-terminus, a light chain portion of a chimeric MHM24 IgG1 fused to an IL-15 cytokine via a peptide linker is shown. The sequence of the light chain is shown in normal font; the location of the peptide linker is shown by italics and underline; and the cytokine molecule is shown by the double underline. The kappa constant region is shown as SEQ ID NO: 74.

[0375] In some embodiments, the light chain variable amino acid sequence of the chimeric MHM24 antibody (optionally, further including a kappa light chain sequence) corresponding to the antibody portion of the amino acid sequence shown in SEQ ID NO:27, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to the antibody portion of SEQ ID NO:27). In embodiments, the chimeric MHM24 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the light chain variable domain of the antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO: 27.

[0376] In embodiments, the FMs described herein can comprise the amino acid sequence of SEQ ID NO: 27, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO:27, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 27. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 27.

SEQ ID NO: 28

Name: HC-ch1B4-Fab

[0377] Fab heavy-chain of chimeric 1B4 anti-CD18 antibody; contains variable domain from parental mouse monoclonal antibody and CH1 domain from human IgG1.

[0378] In some embodiments, the heavy chain variable amino acid sequence of a chimeric 1B4 antibody (optionally, further including a CH1 domain sequence from human IgG1) has the amino acid sequence shown in SEQ ID NO:28, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%,

95% or higher identical to the antibody portion of SEQ ID NO:28). In embodiments, the chimeric 1B4 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the heavy chain variable domain of the 1B4 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO: 28.

SEQ ID NO: 29

Name: LC-ch1B4-IL15

[0379] Light-chain of chimeric 1B4 anti-CD18 antibody; contains variable domain from parental mouse monoclonal antibody and human constant kappa domain; contains wild-type IL-15 genetically fused to antibody light-chain C-terminus using a flexible linker.

DIVLTQSPASLAVSLGQRATISCRASESVDSYGNFMHWYQKPGQPPK
LLIYRASNLESGIPARFSGSGSRDFTLTINPVEADVDATYCYQQSNED
PLTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYRE
AKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGECGGGGSGGGSGGGGSNWNVNSDLKKI
EDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLESSGDASIHD
TVENLIILANNSLSSNGNVTESGCKECELEEKNIKEFLQSFVHIVQMFIN
TS

[0380] From N- to C-terminus, a light chain portion of a chimeric 1B4 IgG1 fused to an IL-15 cytokine via a peptide linker is shown. The sequence of the light chain is shown in normal font; the location of the peptide linker is shown by italics and underline; and the cytokine molecule is shown by the double underline. The kappa constant region is shown as SEQ ID NO: 74.

[0381] In some embodiments, the light chain variable amino acid sequence of the chimeric 1B4 antibody (optionally, further including a kappa light chain sequence) corresponding to the antibody portion of the amino acid sequence shown in SEQ ID NO:29, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to the antibody portion of SEQ ID NO:29). In embodiments, the chimeric 1B4 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the light chain variable domain of the antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO: 29.

[0382] In embodiments, the FMs described herein can comprise the amino acid sequence of SEQ ID NO: 29, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO:29, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 29. In

embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 29.

SEQ ID NO: 30

Name: HC-chOKT8-Fab

[0383] Fab heavy-chain of chimeric OKT8 anti-CD8 antibody; contains variable domain from parental mouse monoclonal antibody and CH1 domain from human IgG1.

[0384] In some embodiments, the heavy chain variable amino acid sequence of a chimeric OKT8 anti-CD8 antibody (optionally, further including a CH1 domain sequence from human IgG1) has the amino acid sequence shown in SEQ ID NO:30, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to the antibody portion of SEQ ID NO:30). In embodiments, the chimeric OKT8 anti-CD8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the heavy chain variable domain of the OKT8 anti-CD8 antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from the CDR sequence of SEQ ID NO: 30.

SEQ ID NO: 31

Name: LC-chOKT8-IL15

[0385] Light-chain of chimeric OKT8 anti-CD8 antibody; contains variable domain from parental mouse monoclonal antibody and human constant kappa domain; contains wild-type IL-15 genetically fused to antibody light-chain C-terminus using a flexible linker.

DVQINQSPSFLAASPGETITINCRTSRISQYLAWYQEKPGKTNKLLIY
SGSTLQSGIPSRFSGSGSGTDFTLTISGLEPEDFAMYQCQHNENPLTF
GAGTKLELKRVTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSPVTKSFNRGECGGGGSGGGSGGGSNWNVNSDLKKIEDLI
QSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLES GDASIHDTVEN
LIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHVQMFINTS

[0386] From N- to C-terminus, a light chain portion of a chimeric OKT8 IgG1 fused to an IL-15 cytokine via a peptide linker is shown. The sequence of the light chain is shown in normal font; the location of the peptide linker is shown by italics and underline; and the cytokine molecule is shown by the double underline. The kappa constant region is shown as SEQ ID NO: 74.

[0387] In some embodiments, the light chain variable amino acid sequence of the chimeric OKT8 antibody (optionally, further including a kappa light chain sequence) corresponding to the antibody portion of the amino acid sequence shown in SEQ ID NO:31, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to the antibody portion of SEQ ID NO:31). In embodiments, the

chimeric OKT8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the light chain variable domain of the antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences of SEQ ID NO: 31.

[0388] In embodiments, the FMs described herein can comprise the amino acid sequence of SEQ ID NO: 31, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO:31, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 31. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 31.

SEQ ID NO: 32

Name: BC8scFv-IL15

[0389] IL-15 fused to the C-terminus of BC8-scFv using a short flexible linker and a hexahistidine tag.

QVQLVESGGGLVQPGGSLKLSCAASGPDFSRYSWMSWVRQAPGKGLEWIG
EINPTSSSTINFTPSLKDQVFISRDNAKNTLYLQMSKVRSEDTALYYCAR
GNYRYRGDAMDYWGQGTSTVTVSGGGSGGGSGGGSGGTGDIVLTQSPASLA
VSLGQRATISCRASKSVSTSGYSYLHWYQQKPGQPPKLLIYLASNLESG
VPAFSGSGSGTDFTLNIHPVEEEDAATYYCQHSRELPTFPFGSGTKLEI
KRSGSGGGSLQNWNVNSDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLELQVISLES GDASIHDTVENLIILANNSLSSNGNVTESGCK
ECEELEEKNIKEFLQSFVHVQMFINTSAAAHHHHHH

[0390] From N- to C-terminus, a light chain portion of a BC8 scFv fused to an IL-15 cytokine via a peptide linker is shown. The scFv sequence is shown in normal font; the location of the peptide linker is shown by italics and underline; and the cytokine molecule is shown by the double underline.

[0391] In some embodiments, the light chain variable amino acid sequence of the BC8 scFv antibody corresponding to the antibody portion of the amino acid sequence shown in SEQ ID NO:31, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95% or higher identical to the antibody portion of SEQ ID NO:32). In embodiments, the BC8 antibody comprises one, two, or all three CDR1, CDR2 or CDR3 of the light chain variable domain of the antibody, e.g., according to the Kabat definition, or a closely related CDR, e.g., CDRs which have at least one amino acid alteration, but not more than two, three or four alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) from any of the CDR sequences within SEQ ID NO: 32.

[0392] In embodiments, the FMs described herein can comprise the amino acid sequence of SEQ ID NO: 32, or an

amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to SEQ ID NO:32, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 32. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to SEQ ID NO: 32.

[0393] Additional sequences that can be included in the FM of the present disclosure are shown in Table 1 below. In some embodiments, the FM comprises a constant lambda or

lamda region. Exemplary constant lambda or lamda regions include SEQ ID NOS: 74-78. In various embodiments, the FMs described herein can comprise one or more of the amino acid sequences of SEQ ID NOS: 1-86, or an amino acid sequence substantially identical thereto (e.g., an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identity to any one of SEQ ID NOS: 1-86, or having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to any one of SEQ ID NOS: 1-86. In embodiments, the FM comprises no more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) relative to any one of SEQ ID NOS: 1-86.

TABLE 1

Additional Sequences		
SEQ ID NO:	Name	Description/Comments
9	IL-15R α -sushi (also referred as sushi)	Sushi domain from wild-type IL-15R α .
10	IL15-WT	Wild-type IL-15, corresponds to mature polypeptide from full IL-15 sequence described in SEQ ID NO: 40.
11	IL15-N72D	IL-15 containing N72D mutation
12	sushiL77I-Fc	Sushi domain from IL-15R α fused to IgG1-Fc; sushi domain contains L77I mutation
13	sushi-Fc	Sushi domain from IL-15R α fused to IgG1-Fc
23	hBC8(23) heavy-chain variable domain	Humanized BC8(23) heavy-chain variable domain.
24	hBC8(23) light-chain variable domain	Humanized BC8(23) light-chain variable domain.
33	Leader-1	Leader sequence (e.g. signal sequence, signal peptide) used for antibody light-chain, IL-15, N-terminal IL-15 fusions, and N-terminal fusion of sushi to Fab-light-chain.
34	Leader-2	Leader sequence (e.g. signal sequence, signal peptide) used for antibody heavy-chain.
35	IL-15R α leader sequence	Leader sequence used for sushi and sushi-Fc constructs.
36	Linker-1 (L1)	(G4S)3 linker
37	Linker-2 (L2)	(G3S)2 linker
38	Linker-3 (L3)	Linker derived from IgG1 hinge
39	Linker-4 (L4)	Linker for fusing IL-15 to C-terminus of BC8-scFy
40	Human IL-15 full sequence	Genbank Accession No. CAA62616.1
41	Human IL-15R α full sequence	Genbank Accession No. AAI21141.1
42	IL-7 full sequence	Genbank Accession No. AAA59156, AAC63047, and NP_000871, and UniProtKB/Swiss-Prot- P13232
43	IL-7 mature sequence	
44	IL-21 full sequence	Genbank Accession No. AAG29348, AAH66262, AAH69124, and EAX05226
45	IL-21 mature sequence	
46	IL-12A full sequence (also referred to as IL-12p35)	Genbank Accession No. P29459
47	IL-12A mature sequence	
48	IL-12B full sequence (also referred to as IL-12p40)	Genbank Accession No. P29460
49	IL-12B mature sequence	
50	scIL-12p70-BA	Synthetic sequence; IL-12B and IL-12A joined by flexible linker
51	scIL-12p70-AB	Synthetic sequence; IL-12A and IL-12B joined by flexible linker
52	Minimal sushi domain	
53	IgG1-Fc	Fc domain (CH2 and CH3 domains) from human IgG1
54	IgG2-Fc	Fc domain (CH2 and CH3 domains) from human IgG2
55	IgG2Da-Fc	Fc domain from human IgG2 containing two point mutations
56	sushi-IgG2Da-Fc (also referred to as sushi-Fc2Da)	Sushi domain from IL-15R α fused to IgG2Da-Fc

TABLE 1-continued

Additional Sequences		
SEQ ID NO:	Name	Description/Comments
57	BC8 heavy-chain variable domain	
58	BC8 light-chain variable domain	
59	9.4 heavy-chain variable domain	
60	9.4 light-chain variable domain	
61	4B2 heavy-chain variable domain	
62	4B2 light-chain variable domain	
70	Linker-5 (L5)	(G ₃ S) ₄ linker, e.g., as described above in SEQ ID NOs: 50 and 51
71	Linker-6 (L6)	(G ₄ S) ₄ linker
72	scIL-12p70-BA-L6	Synthetic sequence; IL-12B and IL-12A joined by linker L5.
73	scIL-12p70-AB-L5	Synthetic sequence; IL-12A and IL-12B joined by linker L5.
74	human immunoglobulin kappa constant domain	
75	human immunoglobulin lambda constant 1	
76	human immunoglobulin lambda constant 2	
77	human immunoglobulin lambda constant 3	
78	human immunoglobulin lambda constant 7	
79	LC-h9.4Fab	Fab light chain of a humanized anti-CD45 antibody; contains humanized 9.4 (h9.4) light-chain variable domain and human constant kappa domain mature
80	Human IL-2 sequence	
81	HC-h9.4Fab-IL2	Heavy-chain of a humanized anti-CD45 antibody; contains variable domain from h9.4 heavy-chain and the CH1 domain from human IgG1; contains a human IL-2 (SEQ ID NO: 80) genetically fused to antibody heavy- chain C-terminus using a flexible linker (Linker-1, SEQ ID NO: 36).
82	HSA	Human Serum Albumin (HSA)
83	scFv-h9.4-HSA-IL2	An scFv comprising a humanized 9.4 antibody; heavy- chain and light-chain variable domains are genetically fused using a flexible linker (Linker-6; SEQ ID NO: 101); contains an HSA peptide (SEQ ID NO: 82) genetically fused to the c-terminus of the scFv domain using a flexible linker (Linker-1, SEQ ID NO: 36); contains a human IL-2 (SEQ ID NO: 80) genetically fused to the HSA c-terminus with a flexible linker (GGGGS (SEQ ID NO: 88))
84	FcgR3B	
85	scFv-h9.4-FcgR3B-IL2	An scFv comprising a humanized 9.4 antibody; heavy- chain and light-chain variable domains are genetically fused using a flexible linker (Linker-6; SEQ ID NO: 101); contains an FcgR3B peptide (SEQ ID NO: 84) genetically fused to the c-terminus of the scFv domain using a flexible linker (Linker-1, SEQ ID NO: 36); contains a human IL-2 (SEQ ID NO: 80) genetically fused to the FcgR3B c-terminus with a flexible linker (GGGSGGGGS (SEQ ID NO: 89)).
86	HC-h9.4Fab-HSA-IL2	Heavy-chain of a humanized anti-CD45 antibody; contains variable domain from h9.4 heavy-chain and the CH1 domain from human IgG1; contains an HSA peptide (SEQ ID NO: 82) genetically fused to the antibody heavy-chain C-terminus using a flexible linker (Linker-1, SEQ ID NO: 36); contains a human IL-2 (SEQ ID NO: 80) genetically fused to the c-terminus of the HSA peptide using a flexible linker (GGGGS, SEQ ID NO: 88)

Protein variants

[0394] Full length polypeptides and variants thereof are described below. Full-length IL-15 sequence (SEQ ID NO: 40) is taken from Genbank Accession No. CAA62616.1; mature IL-15 is devoid of the signal sequence and is defined in SEQ ID NO: 10. Full-length IL-15R α (SEQ

[0395] ID NO: 41) is taken from Genbank Accession No. AAI21141.1. The sushi domain of IL-15R α (IL-15R α -sushi) is given by SEQ ID NO: 9. A minimal sushi domain encompassing the first and fourth cysteines and the intervening amino acids (SEQ ID NO: 52) have also been described elsewhere and are plausible substitutes. Similarly, optional N-terminal additions to the minimal sushi domain comprising the native Thr or Ile-Thr and/or optional C-terminal additions to the minimal sushi domain comprising Ile or Ile-Arg residues are also plausible.

[0396] Protein variants described below specify protein subunit names and SEQ ID NOs corresponding to the mature proteins. Each protein subunit was recombinantly expressed with an N-terminal signal peptide to facilitate secretion from the expressing cell. The native IL-15R α signal peptide (SEQ ID NO: 35) was used for sushi, sushi-L771-Fc, and sushi-Fc. The leader sequence in SEQ ID NO: 33 was used to support secretion of antibody light-chains, IL15-WT, N-terminal IL-15 fusions, IL15-N72D, and N-terminal fusion of sushi to Fab-light-chain. The leader sequence in SEQ ID NO: 34 was used to support secretion of antibody heavy chains.

[0397] For “heavy-chain” and “light-chain” nomenclature we use the standard naming system for antibodies. For example, in an antibody Fab fragment both chains have approximately the same molecular mass, but we refer to the heavy-chain as the chain of the Fab fragment corresponding to the heavy-chain in the full-length antibody (e.g. containing the variable heavy-chain and CH1 domains). Further, in the case of cytokine fusions to the light-chain of a Fab fragment, the light-chain would actually have a larger molecular mass than the heavy-chain due to the cytokine fusion; for consistency, however, we maintain the standard naming convention in which the variable light-chain domain and constant kappa domain and cytokine fusion comprise the “light-chain” while the variable heavy-chain domain and CH1 domain comprises the “heavy-chain”.

Protein Name: chBC8-IL15/sushi Fab (Also Referred to as chBC8-L1-IL15/sushi Fab).

[0398] This protein was made by coexpression of three subunits: HC-chBC8-Fab (SEQ ID NO: 5), LC-chBC8-IL15 (SEQ ID NO: 3), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of wild-type IL-15 to the C-terminus of the chimeric BC8 Fab fragment light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association with IL-15R α -sushi (via interaction with IL-15). The chimeric BC8 Fab is an anti-human CD45R antibody Fab fragment comprising variable-heavy and variable-light chain domains (VH and VL) from the parental mouse monoclonal antibody (mAb) and constant domains from human (human constant kappa domain and human IgG1-CH1 domain).

Protein Name: IL15-chBC8/sushi Fab

[0399] This protein was made by coexpression of three subunits: HC-chBC8-Fab (SEQ ID NO: 5), IL15-LC-chBC8 (SEQ ID NO: 4), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of IL-15 to the N-terminus of the chimeric BC8 Fab fragment light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association between IL15-N72D and IL-15R α -sushi.

minus of the chimeric BC8 Fab fragment light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association between IL-15 and IL-15R α -sushi.

Protein Name: chBC8-sushi/IL15-N72D Fab

[0400] This protein was made by coexpression of three subunits: HC-chBC8-Fab (SEQ ID NO: 5), LC-chBC8-sushi (SEQ ID NO: 1), and IL15-N72D (SEQ ID NO: 11). The resulting protein comprises a fusion of IL-15R α -sushi to the C-terminus of the chimeric BC8 Fab fragment light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association between IL15-N72D and IL-15R α -sushi.

Protein Name: sushi-chBC8/IL15-N72D Fab

[0401] This protein was made by coexpression of three subunits: HC-chBC8-Fab (SEQ ID NO: 5), sushi-LC-chBC8 (SEQ ID NO: 2), and IL15-N72D (SEQ ID NO: 11). The resulting protein comprises a fusion of IL-15R α -sushi to the N-terminus of the chimeric BC8 Fab fragment light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association between IL15-N72D and IL-15R α -sushi.

Protein Name: chBC8-IL15/sushi IgG

[0402] This protein was made by coexpression of three subunits: HC-chBC8-IgG4S228P (SEQ ID NO: 6), LC-chBC8-IL15 (SEQ ID NO: 3), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of wild-type IL-15 to the C-terminus of the chimeric BC8 IgG-S228P light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association with IL-15R α -sushi (via interaction with IL-15). The chimeric BC8 IgG4-S228P is an anti-human CD45R antibody comprising variable-heavy and variable-light chain domains (VH and VL) from the parental mouse mAb and constant domains from human (human constant kappa domain and human IgG4 containing an S228P point mutation, which reduces susceptibility of Fab-arm-exchange of IgG4).

Protein Name: IL15-chBC8/sushi IgG

[0403] This protein was made by coexpression of three subunits: HC-chBC8-IgG4S228P (SEQ ID NO: 6), IL15-LC-chBC8 (SEQ ID NO: 4), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of wild-type IL-15 to the N-terminus of the chimeric BC8 IgG4-S228P light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association between IL-15 and IL-15R α -sushi.

Protein Name: chBC8-sushi/IL15-N72D IgG

[0404] This protein was made by coexpression of three subunits: HC-chBC8-IgG4S228P (SEQ ID NO: 6), LC-chBC8-sushi (SEQ ID NO: 1), and IL15-N72D (SEQ ID NO: 11). The resulting protein comprises a fusion of IL-15R α -sushi to the C-terminus of the chimeric BC8 IgG4-S228P light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association with between IL-15R α -sushi and IL-15.

Protein Name: sushi-chBC8/IL15-N72D IgG

[0405] This protein was made by coexpression of three subunits: HC-chBC8-IgG4S228P (SEQ ID NO: 6), sushi-LC-chBC8 (SEQ ID NO: 2), and IL15-N72D (SEQ ID NO: 11). The resulting protein comprises a fusion of IL-15R α -sushi to the N-terminus of the chimeric BC8 IgG4-S228P light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association with between IL-15R α -sushi and IL-15.

Protein Name: chBC8-(HC)-IL15/sushi IgG

[0406] This protein was made by coexpression of three subunits: HC-chBC8-IgG4S228P-IL15 (SEQ ID NO: 8), LC-chBC8 (SEQ ID NO: 7), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of IL-15 to the C-terminus of the chimeric BC8 IgG4-S228P heavy-

chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association with between IL-15 and IL-15R α -sushi.

Protein Name: chBC8-IL15/sushi scFv

[0407] This protein was made by coexpression of two subunits: BC8scFv-IL15 (SEQ ID NO: 32) and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of IL-15 to the C-terminus of BC8-scFv using linker-L1 (SEQ ID NO: 36), and a noncovalent association with between IL-15 and IL-15R α -sushi. BC8-scFv comprises the variable domains from the parental mouse mAb joined by a flexible linker.

Protein Name: IL15-N72D/sushiL77I-Fc

[0408] This protein was made by coexpression of two subunits: IL15-N72D (SEQ ID NO: 11) and sushi-L77I-Fc (SEQ ID NO: 12). The resulting protein comprises IL-15R α -sushi containing an L77I mutation fused to the N-terminus of human IgG1-Fc region, and a noncovalent association with between IL15-N72D and IL-15R α -sushi.

Protein Name: IL15-WT/sushi-Fc

[0409] This protein was made by coexpression of two subunits: IL15-WT (SEQ ID NO: 10) and sushi-Fc (SEQ ID NO: 13). The resulting protein comprises a fusion of IL-15R α -sushi to the N-terminus of human IgG1-Fc region, and a noncovalent association with between IL15-WT and IL-15R α -sushi.

Protein Name: IL15-WT/sushi-IgG2Da-Fc

[0410] This protein was made by coexpression of two subunits: IL15-WT (SEQ ID NO: 10) and sushi-IgG2Da-Fc (SEQ ID NO: 56). The resulting protein comprises a fusion of IL-15R α -sushi to the N-terminus of human IgG2Da-Fc region, and a noncovalent association with IL15-WT (via interaction with IL-15R α -sushi).

Protein Name: ch9.4-IL15/sushi Fab

[0411] This protein was made by coexpression of three subunits: HC-ch9.4-Fab (SEQ ID NO: 14), LC-ch9.4-IL15 (SEQ ID NO: 15), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of wild-type IL-15 to the C-terminus of the chimeric 9.4 Fab fragment light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association between IL-15 and IL-15R α -sushi. The chimeric 9.4 Fab is an anti-CD45R antibody Fab fragment comprising variable-heavy and variable-light chain domains (VH and VL) from the parental mouse mAb (corresponding to ATCC hybridoma HB-10508) and constant domains from human (human constant kappa domain and human IgG1-CH1 domain).

Protein Name: ch4B2-IL15/sushi Fab

[0412] This protein was made by coexpression of three subunits: HC-ch4B2-IL15 (SEQ ID NO: 16), LC-ch4B2-IL15 (SEQ ID NO: 17), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of wild-type IL-15 to the C-terminus of the chimeric 4B2 Fab fragment light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association between IL-15 and IL-15R α -sushi. The chimeric 4B2 Fab is an anti-CD45R antibody Fab fragment comprising variable-heavy and variable-light chain domains (VH and VL) from the parental mouse mAb (corresponding

to ATCC hybridoma HB-11186) and constant domains from human (human constant kappa domain and human IgG1-CH1 domain).

Protein Name: hBC8(23)-IL15/sushi Fab

[0413] This protein was made by coexpression of three subunits: HC-hBC8(23)-Fab (SEQ ID NO: 18), LC-hBC8(23)-IL15 (SEQ ID NO: 19), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of wild-type IL-15 to the C-terminus of the humanized BC8 Fab fragment light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association between IL-15 and IL-15R α -sushi. The humanized BC8(23) Fab is an anti-CD45R antibody Fab fragment comprising variable-heavy and variable-light chain domains (VH and VL) humanized from the parental mouse mAb and constant domains from human (human constant kappa domain and human IgG1-CH1 domain).

Protein Name: hBC8-null-IL15/sushi Fab

[0414] This protein was made by coexpression of three subunits: HC-chBC8(23)-null-Fab (SEQ ID NO: 20), LC-hBC8(23)-IL15 (SEQ ID NO: 19), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of wild-type IL-15 to the C-terminus of the humanized BC8 Fab fragment light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association between IL-15 and IL-15R α -sushi. This protein differs from hBC8(23)-IL15/sushi Fab in that its heavy chain contains a GGGs substitution within the CDR-H3 loop, which results in ablated binding affinity towards CD45R (hence, the designation as "null" binding variant). This IL15 fusion protein thus serves as a negative control for the effect of CD45R engagement.

Protein Name: chBC8-L2-IL15/sushi Fab

[0415] This protein was made by coexpression of three subunits: HC-chBC8-Fab (SEQ ID NO: 5), LC-chBC8-L2-IL15 (SEQ ID NO: 21), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of IL-15 to the N-terminus of the chimeric BC8 Fab fragment light-chain using linker-L2 (SEQ ID NO: 37), and a noncovalent association between IL-15 and IL-15R α -sushi.

Protein Name: chBC8-L3-IL15/sushi Fab This protein was made by coexpression of three subunits: HC-chBC8-Fab (SEQ ID NO: 5), LC-chBC8-L3-IL15 (SEQ ID NO: 22), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of IL-15 to the N-terminus of the chimeric BC8 Fab fragment light-chain using linker-L3 (SEQ ID NO: 38), and a noncovalent association between IL-15 and IL-15R α -sushi.

Protein Name: chMHM24-IL15/sushi Fab

[0416] This protein was made by coexpression of three subunits: HC-chMHM24-Fab (SEQ ID NO: 26), LC-MHM24-IL15 (SEQ ID NO: 27), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of wild-type IL-15 to the C-terminus of the chimeric MHM24 Fab fragment light-chain using linker-L1 (SEQ ID NO: 36), and a noncovalent association with IL-15R α -sushi (via interaction with IL-15). The chimeric MHM24 Fab is an anti-human CD11a antibody Fab fragment comprising variable-heavy and variable-light chain domains (VH and VL) from the parental mouse mAb and constant domains from human (human constant kappa domain and human IgG1-CH1 domain).

Protein Name: ch1B4-IL15/sushi Fab

[0417] This protein was made by coexpression of three subunits: HC-ch1B4-Fab (SEQ ID NO: 28), LC-ch1B4-

IL15 (SEQ ID NO: 29), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of wild-type IL-15 to the C-terminus of the chimeric 1B4 Fab fragment light-chain using linker-L1 (SEQ ID NO: 36), and a non-covalent association with IL-15R α -sushi (via interaction with IL-15). The chimeric 1B4 Fab is an anti-human CD18 antibody Fab fragment comprising variable-heavy and variable-light chain domains (VH and VL) from the parental mouse mAb and constant domains from human (human constant kappa domain and human IgG1-CH1 domain). Protein Name: chOKT8-IL15/sushi Fab

[0418] This protein was made by coexpression of three subunits: HC-chOKT8-Fab (SEQ ID NO: 30), LC-chOKT8-IL15 (SEQ ID NO: 31), and IL-15R α -sushi (SEQ ID NO: 9). The resulting protein comprises a fusion of wild-type IL-15 to the C-terminus of the chimeric OKT8 Fab fragment light-chain using linker-L1 (SEQ ID NO: 36), and a non-covalent association with IL-15R α -sushi (via interaction with IL-15). The chimeric OKT8 Fab is an anti-human CD8 antibody Fab fragment comprising variable-heavy and variable-light chain domains (VH and VL) from the parental mouse mAb and constant domains from human (human constant kappa domain and human IgG1-CH1 domain).

Protein Name: Ab-HSA-Linker-IL-2

[0419] This protein was made by expression of a fusion protein comprising three subunits: the targeting antibody (e.g., anti-CD45, anti-CD4 and/or anti-CD25), human serum albumin (SEQ ID NO: 79, no underline), and IL-2 (SEQ ID NO: 80). The three subunits are operably linked together via a peptide linker comprising G and S (SEQ ID NO: 79, single underline).

Protein Name: h9.4Fab-IL2

[0420] This protein is made by coexpression of two subunits: LC-h9.4Fab (SEQ ID NO: 79), and HC-h9.4Fab-IL2 (SEQ ID NO: 81). The resulting protein comprises a fusion of a human IL-2 to the C-terminus of h9.4 Fab fragment heavy-chain using Linker-1 (SEQ ID NO: 36), and IL-2 to the C-terminus of HSA using a flexible linker (GGGGS) (SEQ ID NO: 88).

Protein Name: h9.4Fab-HSA-IL2

[0421] This protein is made by coexpression of two subunits: LC-h9.4Fab (SEQ ID NO: 79), and HC-h9.4Fab-HSA-IL2 (SEQ ID NO: 81). The resulting protein comprises a fusion of HSA to the C-terminus of h9.4 Fab fragment heavy-chain using Linker-1 (SEQ ID NO: 36), and IL-2 to the C-terminus of HSA using a flexible linker (GGGGS) (SEQ ID NO: 88).

Immune Regulatory Cells

[0422] In embodiments, the FMs disclosed herein can be used to target an immune cell such as a nucleated cell. In some embodiments, the targeting moiety is capable of binding to an immune cell surface target, thereby targeting the modulatory moiety (e.g., cytokine) to the immune cell, e.g., an immune regulatory cell (e.g., a regulatory T cell). In certain embodiments, the FMs, once targeted to an immune cell via binding between the targeting moiety and its corresponding immune regulatory cell surface target, can be transpresented to neighboring cells such as neighboring immune effector cells.

[0423] Without wishing to be bound by theory, binding of the immune targeting moiety to the immune cell surface

target is believed to increase the concentration, e.g., the concentration over time, of the immune modulatory moiety, e.g., cytokine molecule, with its corresponding receptor, e.g., a cytokine receptor, on the surface of the immune cell, e.g., relative to the association of the free cytokine molecule with its cytokine receptor. This can result in an immune effect on the immune cell itself bound by the FMs (autocrine signaling), or on a neighboring immune cell (paracrine signaling).

[0424] In some embodiments, the immune regulatory cell surface target is abundantly present on the surface of an immune regulatory cell (e.g., outnumbers the number of receptors for the cytokine molecule present on the immune cell surface). In some embodiments, the targeting moiety can be an antibody molecule or a ligand molecule that binds to an immune cell surface target, e.g., CD45, CD4, CD25, CD39, or Neuropilin 1 (NRP1) on Tregs. In some embodiments, the immune cell surface receptor is selected from one or more of CD4, CD45, CD3, CD2, CD25, CD127, CD197 (CCR7), CXCR3, CXCR4, CXCR5, CD38, CD27, CCR4, CCR5, CD137, CD39, CCR4, CCR5, CCR6 (CD196), CCR8, CCR10, OX40, GITR, CTLA4, LAG3, CD73, CD103, CD62L, CCR2, CCR9, Neuropilin 1 (NRP1), CD8, CD11a, or CD18, or variants thereof.

[0425] In one embodiment, the targeting moiety comprises an antibody molecule or a ligand molecule that binds to CD45. In other embodiments, the targeting moiety binds bispecifically to CD4 and CD25. In embodiments, the targeting moiety is believed to specifically deliver and/or increase the concentration of the cytokine molecule (e.g., IL-2) to the surface of an immune cell, thereby resulting in one or more of increased localization, altered distribution and/or enhanced cell surface availability of the cytokine molecule. In embodiments, the FM does not substantially interfere with the signaling function of the cytokine molecule. Such targeting effect results in localized and prolonged stimulation of proliferation and activation of the immune cells, thus inducing the controlled expansion and activation of an immune response.

[0426] In some embodiments, the immune cell is a regulatory T cells. In embodiments, the immune regulatory cell, e.g., a regulatory T cell acquired from a patient, e.g., a patient's blood. In other embodiments, the immune regulatory cell is acquired from a healthy donor. Regulatory T cells express the transcription factor Foxp3 and restrain immune responses to self and foreign antigens. Treg cells express abundant amounts of the interleukin 2 receptor α -chain (IL-2R α ; CD25), but are unable to produce IL-2. IL-2 binds with low affinity to IL-2R α or the common γ -chain (γ c)-IL-2R β heterodimers, but receptor affinity increases ~1,000 fold when these three subunits together interact with IL-2. IL-2 and STATS, a key IL-2R downstream target, are indispensable for Foxp3 induction and differentiation of Treg cells in the thymus. IL-2R β and γ c are shared with the IL-15 receptor, whose signaling can also contribute to the induction of Foxp3. IL-2, in cooperation with the cytokine TGF- β , is also required for extrathymic Treg cell differentiation.

[0427] CD4⁺ T cells are commonly divided into regulatory T (Treg) cells and conventional T helper (Th) cells. Th cells control adaptive immunity against pathogens and cancer by activating other effector immune cells. Treg cells are defined as CD4⁺T cells in charge of suppressing potentially deleterious activities of Th cells. This review briefly summa-

rizs the current knowledge in the Treg field and defines some key questions that remain to be answered. Functions for Treg cells include:

- [0428] 1. Prevention of autoimmune diseases by establishing and maintaining immunologic self-tolerance.
- [0429] 2. Suppression of allergy and asthma.
- [0430] 3. Induction of tolerance against dietary antigens, i.e. oral tolerance.
- [0431] 4. Induction of maternal tolerance to the fetus.
- [0432] 5. Suppression of pathogen-induced immunopathology.
- [0433] 6. Regulation of the effector class of the immune response.
- [0434] 7. Suppression of T-cell activation triggered by weak stimuli.
- [0435] 8. Feedback control of the magnitude of the immune response by effector Th cells.
- [0436] 9. Protection of commensal bacteria from elimination by the immune system.
- [0437] 10. Prevention of T cells that have been stimulated by their true high-affinity agonist ligand from killing cells that only express low-affinity T-cell receptor (TCR) ligands such as the self peptide-major histocompatibility complex (MHC) molecule that positively selected the T cell.
- [0438] The most widely used markers for Treg cells are:
 - [0439] CD25
 - [0440] cytotoxic T lymphocyte-associated antigen 4 (CTLA-4)
 - [0441] glucocorticoid-induced tumour necrosis factor receptor family-related gene (GITR)
 - [0442] lymphocyte activation gene-3 (LAG-3)
 - [0443] CD127 (negative marker)
 - [0444] forkhead/winged-helix transcription factor box P3 (Foxp3)
- [0445] Human Treg cells, in some embodiments, can be identified by the CD4+CD25+CD127-markers.
- [0446] Exemplary Treg includes:
 - [0447] rTreg (nTreg previously): FoxP3+; develop in the thymus; control central tolerance
 - [0448] pTreg (iTreg previously): FoxP3+; develop in the periphery; important for peripheral tolerance
 - [0449] iTreg: Foxp3+; induced in vitro via IL-10 and TGF- β stimulation
 - [0450] Tr1: FoxP3-, CD49B+, Lag3+, CD226+; functions primarily through IL-10 secretion
 - [0451] Th3: Involved in oral allergy tolerance, TGF- β mediated suppression
 - [0452] Tr35: FoxP3-; makes IL-35 inducing anergy in neighboring cells; induced by IL-35; target of infectious tolerance

[0453] In some embodiments, the Tregs can be ex vivo induced Tregs. For example, CD4 cells can be isolated and induced to become Tregs by activation and culturing with, e.g., TGF-B, IL-10. In some embodiments, the Tregs can be antigen trained (specific) Tregs, which can be used in, e.g., organ or cell transplant rejection. In some embodiments, the Tregs can be CAR-Tregs or transgenic TCR Tregs.

[0454] In some embodiments, the FMs can be used for the in vitro expansion of Treg cells. For example, the FMs can be used as a cell culturing agent, e.g., in place of free stocks of cytokines currently used in Treg manufacturing and expansion during ex vivo culturing prior to injection. FMs can provide greater persistence than natural form of cyto-

kine. In addition, cell specific targeting is also possible with FMs for ex vivo expansion of sorted Tregs as well as selective expansion of Tregs from a population of mixed PBMCs.

[0455] In certain embodiments, the FMs disclosed herein can be directly administered in vivo as an immune therapy such as immunosuppressive therapy. By targeting specific cell surface receptors (e.g., CD4, CD25, CD39, Neuropilin 1), the FMs can facilitate cell specific targeting, e.g., to Tregs. In some embodiments, the FMs can be designed to target to cell surface receptors nonselectively expressed (e.g., CD45, CD2, CD11a, CD18) and to increase the pharmacokinetics and/or alter cytokine biodistribution.

[0456] In some embodiments, the FMs disclosed herein can be pre-loaded to immune cells prior to a cell therapy, e.g., autologous cell therapy. For example, FMs can be pre-loaded onto a Treg or CAR-T cell.

Nucleic Acids/Vectors/Cells

[0457] The disclosure also features nucleic acids comprising nucleotide sequences that encode the fusion proteins described herein. Further provided herein are vectors comprising the nucleotide sequences encoding an FM described herein. The vectors include, but are not limited to, a virus, plasmid, cosmid, lambda phage or a yeast artificial chromosome (YAC). Numerous vector systems can be employed. For example, one class of vectors utilizes DNA elements which are derived from animal viruses such as, for example, bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (Rous Sarcoma Virus, MMTV or MOMLV) or SV40 virus. Another class of vectors utilizes RNA elements derived from RNA viruses such as Semliki Forest virus, Eastern Equine Encephalitis virus and Flaviviruses.

[0458] Additionally, cells which have stably integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow for the selection of transfected host cells. The marker may provide, for example, prototrophy to an auxotrophic host, biocide resistance (e.g., antibiotics), or resistance to heavy metals such as copper, or the like. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcriptional promoters, enhancers, and termination signals.

[0459] Once the expression vector or DNA sequence containing the constructs has been prepared for expression, the expression vectors may be transfected or introduced into an appropriate host cell. Various techniques may be employed to achieve this, such as, for example, protoplast fusion, calcium phosphate precipitation, electroporation, retroviral transduction, viral transfection, gene gun, lipid based transfection or other conventional techniques. In the case of protoplast fusion, the cells are grown in media and screened for the appropriate activity.

[0460] Methods and conditions for culturing the resulting transfected cells and for recovering the antibody molecule produced are known to those skilled in the art, and may be varied or optimized depending upon the specific expression vector and mammalian host cell employed, based upon the present description.

[0461] In another aspect, the application features host cells and vectors containing the nucleic acids described herein.

The nucleic acids may be present in a single vector or separate vectors present in the same host cell or separate host cell. The host cell can be a eukaryotic cell, e.g., a mammalian cell, an insect cell, a yeast cell, or a prokaryotic cell, e.g., *E. coli*. For example, the mammalian cell can be a cultured cell or a cell line. Exemplary mammalian cells include lymphocytic cell lines (e.g., NSO), Chinese hamster ovary cells (CHO), COS cells, oocyte cells, and cells from a transgenic animal, e.g., mammary epithelial cell.

[0462] The disclosure also provides host cells comprising a nucleic acid encoding an antibody molecule as described herein. In one embodiment, the host cells are genetically engineered to comprise nucleic acids encoding the antibody molecule. In one embodiment, the host cells are genetically engineered by using an expression cassette. The phrase “expression cassette,” refers to nucleotide sequences, which are capable of affecting expression of a gene in hosts compatible with such sequences. Such cassettes may include a promoter, an open reading frame with or without introns, and a termination signal. Additional factors necessary or helpful in effecting expression may also be used, such as, for example, an inducible promoter. The present disclosure also provides host cells comprising the vectors described herein. The cell can be, but is not limited to, a eukaryotic cell, a bacterial cell, an insect cell, or a human cell. Suitable eukaryotic cells include, but are not limited to, Vero cells, HeLa cells, COS cells, CHO cells, HEK293 cells, BHK cells and MDCKII cells. Suitable insect cells include, but are not limited to, Sf9 cells.

Compositions and Modified Cells

[0463] Compositions, including pharmaceutical compositions, comprising the fusion proteins are provided herein. A composition can be formulated in pharmaceutically-acceptable amounts and in pharmaceutically-acceptable compositions. The term “pharmaceutically acceptable” means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients (e.g., biologically-active proteins of the nanoparticles). Such compositions may, in some embodiments, contain salts, buffering agents, preservatives, and optionally other therapeutic agents. Pharmaceutical compositions also may contain, in some embodiments, suitable preservatives. Pharmaceutical compositions may, in some embodiments, be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. Pharmaceutical compositions suitable for parenteral administration, in some embodiments, comprise a sterile aqueous or non-aqueous preparation of the nanoparticles, which is, in some embodiments, isotonic with the blood of the recipient subject. This preparation may be formulated according to known methods. A sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent.

[0464] Additional compositions include modified cells, such as modified Tregs having a plurality of the fusion proteins tethered on cell surface. This can be useful for ex vivo Treg expansion as well as in vivo expansion.

[0465] For example, conventional Treg manufacturing additionally requires long and costly ex vivo expansion. In contrast, as shown in FIG. 1D, sorted Tregs or unsorted PBMC can be loaded with backpacks (e.g., CD3/CD28/IL-2) followed by direct infusion to patient, whereby the Tregs are expanded in vivo, without in vitro culturing. Further-

more, loaded Tregs (e.g., with IL-2 backpacks), prepared either from ex vivo expansion or without culturing, can provide pro-survival signal in vivo, while increasing expansion and engraftment (FIG. 1E).

Therapeutic Uses and Methods

[0466] The fusion proteins and compositions containing such fusions have numerous therapeutic utilities, including, e.g., the treatment of conditions or diseases where the immune system displays an excessive or overactive response. The present disclosure provides, inter alia, methods for reducing or suppressing an immune response in a subject with conditions in which the immune system is overactive, such as autoimmune diseases, allergies and/or bone marrow or organ transplant. Exemplary methods comprise administering to the subject a therapeutically effective amount of any of the fusion proteins and/or modified cells (e.g., Treg-targeted fusion proteins) described herein to provide, e.g., an immunosuppressive therapy.

[0467] Exemplary applications of immunosuppressive therapy include allo-immune diseases, auto-immune diseases, allergy, and other inflammatory diseases (FIG. 1H). Allo-immune diseases include organ transplant rejection, graft versus host disease (GVHD) (e.g., post allogeneic hematopoietic stem cell transplant, HSCT) and GVHD post allogeneic stem cell transplantation (SCT). Treg number has been shown to correlate with GVHD control and organ transplant tolerance.

[0468] For allergy or allergic diseases, expansion of antigen-specific Tregs has been shown to associate with loss of milk allergy, and Treg depletion enhances allergy. Allergic rhinitis is the most common of these diseases, affecting 15-20% of the population. The allergic reaction is triggered by allergen-mediated cross-linking of specific IgE on the surface of mast cells and basophils, leading to release of histamine and other mediators, thus causing an acute allergic reaction, followed by a late-phase reaction characterized by an influx of eosinophils, neutrophils and Th2 cells producing IL-4, IL-5 and IL-13.

[0469] Autoimmune diseases are diseases in which the immune system attacks its own proteins, cells, and tissues, or in which immune effector T cells are autoreactive to endogenous self peptides and cause destruction of tissue. Thus an immune response is mounted against a subject's own antigens, referred to as self antigens. A comprehensive listing and review of autoimmune diseases can be found in *The Autoimmune Diseases* (Rose and Mackay, 2014, Academic Press). Autoimmune diseases are generally considered to be Th1 biased. As a result, induction of a Th2 immune response or Th2 like cytokines can be beneficial. Such cytokines include IL-4, IL-5 and IL-10. Exemplary auto-immune diseases that can be treated include Type 1 diabetes, Multiple Sclerosis and alopecia. These diseases have functional defects in Tregs, and decreased number of Tregs. Autoimmune diseases include but are not limited to rheumatoid arthritis, Crohn's disease, multiple sclerosis, systemic lupus erythematosus (SLE), autoimmune encephalomyelitis, myasthenia gravis (MG), Hashimoto's thyroiditis, Goodpasture's syndrome, pemphigus (e.g., pemphigus vulgaris), Grave's disease, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, scleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis, pernicious anemia, idiopathic Addison's disease, autoimmune-associated infertility, glomerulonephritis

(e.g., crescentic glomerulonephritis, proliferative glomerulonephritis), bullous pemphigoid, Sjogren's syndrome, insulin resistance, and autoimmune diabetes mellitus.

[0470] Inflammatory diseases can be used to broadly define a vast array of disorders and conditions that are characterized by inflammation. Examples include allergy, asthma, autoimmune diseases, coeliac disease, glomerulonephritis, hepatitis, inflammatory bowel disease, preperfusion injury and transplant rejection.

[0471] More specifically, inflammatory diseases can include Inflammatory Bowel Disease, Rheumatoid Arthritis and Lupus. Inflammatory Bowel Disease (IBD) includes two major types, namely Crohn's Disease (CD) and Ulcerative Colitis (UC). These diseases have increased Treg number, but decreased Treg function. Tregs have been shown to control/prevent these diseases in mouse models. For example, both CD and UC as inflammatory disorders have long been considered as a breakdown in immunoregulation in the tissues of the intestinal mucosa, representing the most immunologically active sites of the human body. The interaction between luminal flora and the adaptive immune system is considered critical to disease pathogenesis. Exuberant Teff (proinflammatory) activity is observable in both animal models and human disease alike, and has been attributed in recent years to a breakdown in Treg-mediated (anti-inflammatory) homeostatic mechanisms. Thus, in certain embodiments, the compositions and cells (e.g., modified and/or expanded Tregs) disclosed herein can be used as an anti-inflammatory agent to treat various inflammatory diseases.

[0472] In embodiments, the fusion proteins or pharmaceutical composition can be administered to the subject parenterally. In embodiments, the modified cells can be administered to the subject intravenously, subcutaneously, intratumorally, intranodally, intramuscularly, intradermally, or intraperitoneally. In embodiments, the cells are administered, e.g., injected, directly into a tumor or lymph node. In embodiments, the cells are administered as an infusion (e.g., as described in Rosenberg et al., *New Eng. J. of Med.* 319:1676, 1988) or an intravenous push. In embodiments, the cells are administered as an injectable depot formulation.

[0473] In embodiments, the subject is a mammal. In embodiments, the subject is a human, monkey, pig, dog, cat, cow, sheep, goat, rabbit, rat, or mouse. In embodiments, the subject is a human. In embodiments, the subject is a pediatric subject, e.g., less than 18 years of age, e.g., less than 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or less years of age. In embodiments, the subject is an adult, e.g., at least 18 years of age, e.g., at least 19, 20, 21, 22, 23, 24, 25, 25-30, 30-35, 35-40, 40-50, 50-60, 60-70, 70-80, or 80-90 years of age.

[0474] The fusion proteins disclosed herein can be used in combination with a second therapeutic agent or procedure.

[0475] In some embodiments, the FM is administered in conjunction with a cell therapy, e.g., a cell therapy chosen from an adoptive cell therapy, CAR-T cell therapy, engineered TCR T cell therapy, a tumor infiltrating lymphocyte therapy, an antigen-trained T cell therapy, or an enriched antigen-specific T cell therapy.

[0476] In embodiments, the FM and the second therapeutic agent or procedure are administered/performed after a subject has been diagnosed with a condition in which the immune system is overactive, e.g., before the condition has been eliminated from the subject. In embodiments, the FM

and the second therapeutic agent or procedure are administered or performed simultaneously or concurrently. For example, the delivery of one treatment is still occurring when the delivery of the second commences, e.g., there is an overlap in administration of the treatments. In other embodiments, the FM and the second therapeutic agent or procedure are administered or performed sequentially. For example, the delivery of one treatment ceases before the delivery of the other treatment begins.

[0477] In embodiments, combination therapy can lead to more effective treatment than monotherapy with either agent alone. In embodiments, the combination of the first and second treatment is more effective (e.g., leads to a greater reduction in symptoms and/or cancer cells) than the first or second treatment alone. In embodiments, the combination therapy permits use of a lower dose of the first or the second treatment compared to the dose of the first or second treatment normally required to achieve similar effects when administered as a monotherapy. In embodiments, the combination therapy has a partially additive effect, wholly additive effect, or greater than additive effect.

EXAMPLES

Example 1. Constructing Antibody/IL-15 Fusion Molecules with Increased Biological Persistence

[0478] To demonstrate the ability to generate FMs with improved properties we constructed a series of FMs comprising multiple formats: (i) FMs comprising an IgG, Fab fragment, or scFv fragment, (ii) fusion to the N- or C-terminus of the antibody or antibody fragment; (iii) fusion of either IL-15 or the IL-15R α sushi domain to the antibody or antibody fragment (e.g., FIG. 1 and FIGS. 2A-2C); and (iv) FMs comprising varied linker composition between the antibody and cytokine.

A. IL-15 FMs Comprising Multiple Different Antibody Formats

[0479] To explore the potential for fusion molecules of IL-15 and an immune-targeted antibody to improve IL-15 biological activity we constructed FMs comprising IL-15 and mAb clone BC8, which targets human CD45, an abundant receptor on the surface of immune cells (Cyster et al., *EMBO Journal*, Vol 10, no4, 893-902, 1991). The FMs comprised three different antibody formats: scFv, Fab, and full-length IgG. Briefly, wild-type IL-15 was genetically fused to the C-terminus of the Fab or IgG light-chain using an amino acid linker consisting of (GGGGS)₃ (SEQ ID NO: 36) or to the C-terminus of the scFv using the amino acid linker RSGSGGGGSLQ (SEQ ID NO: 39). The Fab and IgG antibody formats comprised human constant regions and variable domains from the mouse monoclonal antibody BC8 (e.g. chBC8 Fab or chBC8 IgG). The scFv comprised BC8 heavy- and light-chain variable domains genetically fused with a (GGGGS)₃ linker. The IL-15/antibody fusions were co-expressed with IL-15R α -sushi; the high-affinity interaction between IL-15 and sushi resulted in an IL-15-antibody complex comprising a noncovalent association with IL-15R α -sushi. Together, this resulted in three FM variants: chBC8-IL15/sushi scFv, chBC8-IL15/sushi Fab, and chBC8-IL15/sushi IgG (FIG. 2A; see Protein Variants section for more details).

[0480] The effects of the FMs on CD8 T cell expansion were evaluated using a pulse bioassay in which the cytokine is incubated with cells for a fixed amount of time followed by removal of unbound cytokine by washing. As compared with a “static” stimulation assay, in which the cytokine is not washed away, the pulse bioassay provides better characterization of the persistence of a biological effect. It also accommodates confounding factors associated with ligand-depletion, which is a well-appreciated challenge for quantitative analysis of high-affinity receptor/ligand interactions (Hulme, Tevethick, Ligand binding assays at equilibrium: validation and interpretation *Br J Pharm* 2010, 161:1219-1237). The same principles apply to quantitative cell-based assays of cytokines and growth factors that mediate their effects by binding to cellular receptors. We compared the effects of the FMs to an IL15/sushi-Fc protein comprising a sushi domain containing a conservative L77I mutation fused to a human IgG1-Fc domain, and noncovalently associated (via interaction with the sushi domain) with an IL-15 variant containing an N72D mutation: IL15^{N72D}/sushiL77I-Fc. The N72D mutation is purported to improve agonistic properties of IL-15 (Zhu, Marcus, Xu, Lee, et al. Novel Human Interleukin-15 Agonists. *Journal of Immunology* 2009). As a negative control we also evaluated chBC8 alone (without fusion to IL-15). FMs and controls were incubated with CD8 T cells for 1 hr at 37° C., followed by three washes to remove unbound protein. Cells were then plated in full media, allowed to grow for three days, and then were assessed for proliferation using both CellTiter Blue (FIG. 2B) and flow cytometry counting beads (FIG. 2C). Both of these methods yielded consistent results, and demonstrated that all three antibody formats were capable of producing FMs with greater persistence than the IL-15^{N72D}/sushiL77I-Fc protein. There was a trend of increasing potency of FMs containing scFv, Fab, or IgG (i.e. potency: IgG FM>Fab FM>scFv FM). The negative control of the chBC8 antibody alone did not induce T cell expansion indicating the presence of IL-15 is necessary for the proliferative effects of the FMs. The foregoing provides a non-limiting example of a fusion of IL-15 to an anti-CD45 antibody or antibody fragment that improves biological persistence of IL-15. Multiple antibody formats, e.g., as described herein, are capable of producing such FMs with improved potency.

B. FMs Comprising Varied Fusion Strategies of IL-15 to Anti-CD45

[0481] To explore alternative fusion strategies between the cytokine and the antibody of the FM we constructed six additional FMs comprising fusion of IL-15 to the N- or C-terminus of chimeric BC8 Fab or IgG antibodies. The first two FMs were constructed by genetically fusing IL-15 to the N-terminus of chBC8 Fab or chBC8 IgG light-chains and the antibody fusions were co-expressed with IL-15R α to result in the FMs: IL15-chBC8/sushi Fab and IL15-chBC8/sushi IgG (FIGS. 3A-3B; see “Protein Variants” section for more details). We use a naming convention of chBC8-IL15 in Example 1A above and IL15-chBC8 here to refer to fusion of IL-15 to the antibody C- or N-terminus, respectively. The four additional FMs were constructed by fusing wild-type IL-15R α -sushi to the N- or C-terminus of the chBC8 Fab or IgG light-chain, and co-expressed these antibody fusions with IL-15^{N72D} (FIGS. 3C-3D): chBC8-sushi/IL15^{N72D} Fab, sushi-chBC8 /IL15^{N72D} Fab, chBC8-sushi/IL15^{N72D} IgG, sushi-chBC8 /IL15^{N72D} IgG (see Pro-

tein Variants section for more details). Along with the two Fab and IgG FMs in Example 1A (chBC8-IL15/sushi Fab and chBC8-IL15/sushi IgG) these eight FMs allowed us to test a variety of antibody fusion strategies, genetic fusion of IL-15 vs. genetic fusion of sushi to the immune-targeting moiety, and FMs comprising either wild-type or mutated IL-15 variants.

[0482] In a first experiment we evaluated the FMs comprising IL-15^{N72D} in the pulse bioassay format and found that for both the Fab and IgG fusions, as well as the N- and C-terminal fusions, the FMs exhibited improved activity as compared to the IL-15 format comprising a complex between IL-15^{N72D} and a sushi-Fc variant (FIGS. 4A-4B). In an additional experiment comparing all eight FM variants we observed similar potencies for either IL-15 or sushi fusion to the antibody, and also observed similar potencies for protein variants containing wild-type IL-15 and IL15-N72D (FIGS. 5A-5B). We observed a trend in which fusion of IL-15 or sushi to the C-terminus of the chBC8 IgG or Fab resulted in greater CD8 T cell expansion than fusion to the antibody N-terminus (FIGS. 4A-4B and FIGS. 5A-5B). However, each of the FM variants (including N-terminal fusion to the IgG or Fab) displayed greater potency than the control protein, IL15-N72D/sushiL77I-Fc, which does not contain CD45 receptor binding functionality (FIGS. 3C-3D and FIGS. 4A-4B). As a control, chimeric BC8 or the parental BC8 mouse mAb antibodies alone did not induce T cell expansion (FIGS. 4A-4B and FIGS. 5A-5B), consistent with our observations above. We also evaluated the eight FMs on CD8 T cell proliferation in a “static” assay format. After three days of incubation (without washing) each of the FMs resulted in similar activity as IL15^{N72D}/sushiL77I-Fc, with the exception of the FM comprising N-terminal fusion of IL-15 to the Fab fragment (IL15-chBC8/sushi Fab), which resulted in slightly weaker potency in this assay format (FIGS. 5C-5D). The IgG fusions displayed similar potency to the IL-15 Fab fusions (FIG. 3B). We conclude that multiple fusions strategies of the IL-15/sushi complex to an antibody targeting the CD45 receptor result in FMs with increased biological persistence, and that the FM fusion strategy improves activity of both wild-type and mutated forms of IL-15.

C. FMs Comprising IL-15 Fused to the C-Terminus of an Antibody Heavy Chain or Light Chain

[0483] We constructed an additional chBC8-cytokine fusion to evaluate the ability to fuse the cytokine to the antibody heavy- or light-chain C-terminus. Wild-type IL-15 was genetically fused to the C-terminus of the chBC8 heavy-chain (HC) and this construct was co-expressed with IL15R α -sushi to yield the FM variant chBC8(HC)-IL15/sushi IgG (FIG. 6A). We compared chBC8(HC)-IL15/sushi IgG to the chBC8-IL15/sushi IgG described in the previous example, which comprises IL-15 fused to the light-chain C-terminus; for clarity we refer to this latter protein here as chBC8(LC)-IL15/sushi IgG in order to differentiate from the FM comprising fusion to the heavy-chain C-terminus. Activated human CD8 T cells were pulsed with seven serial five-fold dilutions spanning 400 nM to 25.6 pM of chBC8(HC)-IL15/sushi IgG or chBC8-IL15(LC)/sushi IgG for 1 hr at 37° C., and then washed three times with full media and plated at 500,000 cells/mL. Serial dilution of media only, the parental BC8 antibody (which does not contain a fusion with IL-15), or IL-15^{WT}/sushi-Fc (which is devoid of immune-

targeting function) were used as controls. Following 5 days of culture we measured CD8 T cell expansion using CellTiter Blue. The chBC8(HC)-IL15/sushi IgG and chBC8-IL15(LC)/sushi IgG FMs, but not IL15^{WT}/sushi-Fc or the parental BC8 antibody, each induced T cell expansion and exhibited similar dose-response characteristics (FIG. 6B). We conclude that fusion of IL-15 to the C-terminus of the antibody light- or heavy-chain are each capable of producing FMs with increased persistence.

D. Functional Antibody Binding is Required for Improved Potency of FMs.

[0484] We have demonstrated above that binding to CD45 alone (e.g. with a BC8 antibody that does not comprise a fusion with IL-15) does not explain the increased activity of the FMs (FIGS. 3C-3D, FIGS. 4A-4B, and FIGS. 5A-5D). This supports the requirement of the cytokine for the potency of the FM. We conducted two additional controls to demonstrate the functional requirement of the antibody binding to its cognate cell surface receptor. In the first experiment we conducted a pulse assay on activated primary human CD8 T cells for each of the eight FMs described in Example 1A in the presence or absence of 1 μ M BC8 as a soluble competitor to the CD45 cell surface receptor. Following a 1 hr pulse incubation at 37° C. cells were washed three times and then plated in full RPMI medium at a density of 300,000 cells/mL. Following 5 days at 37° C. cell expansion was measured using CellTiter Blue; the presence of the BC8 competitor ablated the activity of all eight FMs in the pulse bioassay (FIGS. 7A-7B).

[0485] In a second experiment we generated two FMs comprising either a functional or non-functional BC8 binding region. The FM with the functional BC8 region comprised a Fab antibody fragment with humanized BC8 variable domain, in which CDRs from the parental BC8 antibody were grafted onto human variable domains, and a fusion of IL-15^{WT} to the humanized BC8 Fab light-chain. This complex was co-expressed with IL-15R α -sushi to result in the FM variant hBC8(23)-IL15/sushi Fab (see “Protein Variants” section for more details). The humanized BC8 antibody (hBC8(23)) possesses similar binding affinity as the chimeric BC8 antibody (FIG. 10A). The FM with the nonfunctional BC8 region (hBC8-null-IL15/sushi Fab) comprised the same Fab fragment, with the exception of five amino acids of CDR3 in the antibody heavy-chain, which were replaced with the residues SGGGS. These amino acid substitutions resulted in a humanized BC8 antibody that did not exhibit detectable binding to cellular CD45 for the concentrations tested (FIG. 10A). Each of these proteins were pulsed with activated human CD8 T cells (seven serial five-fold dilutions spanning a concentration range of 400 nM to 25.6 pM), cells were then washed and plated at 500,000 cells/mL in 96-well plates. Following three days at 37° C. hBC8(23)-IL15/sushi Fab, but not hBC8-null-IL15/sushi Fab, supported CD8 T cell expansion (FIG. 7C). Taken together, these data demonstrate functional cell surface receptor binding is required for the improved potency of the FMs.

E. Fusion to Anti-CD45 Increases Loading and Cell Surface Persistence of IL-15

[0486] To explore whether the improved biological persistence of the FMs correlates to increased loading and

persistence of IL-15 onto cells we analyzed cell surface FM levels over time. CD8 T cells at a density of 5×10^7 cells/mL were pulsed with 0.75 mg/mL hBC8(23)-IL15/sushi Fab, IL-15/sushi-Fc2Da, or IL-15^{N72D}/sushiL771-Fc for 1 hr in a 1:1 mixture of PBS and HBSS, and then washed three times with full media. Sushi-Fc2Da is a fusion of wild-type IL-15R α -sushi to a variant of the human IgG2-Fc domain. A mock pulse control in which cells were incubated in a 1:1 mixture of PBS and HBSS was used as a negative control. Cells were then stained for IL-15 using a fluorescently-labeled anti-IL-15 antibody or for IgG domains (Fab or Fc fragments) using a fluorescently-labeled polyclonal antibody that recognizes human IgG light and heavy chains. These antibodies provided detection of both IL-15 or the associated Fab or Fc fragments on the T cell surface. The hBC8(23)-IL15/sushi Fab protein yielded substantially higher levels of IL-15 staining than the IL15/sushi-Fc2Da or IL15-N72D/sushiL771-Fc proteins indicating a greater abundance of IL-15 on the cell surface (FIG. 8A). We observed consistent results for IgG staining in which the FMs resulted in a higher levels of staining than the IL15/sushi-Fc constructs (FIG. 8A). As a positive control for proliferative effects of IL-15 we added approximately 10 nM of IL15/sushi-Fc2Da to a mock pulse control (a “mock then saturating” condition); this corresponds to a concentration above the EC90 for IL-15/sushi-containing variants (see FIGS. 3A-3D) and thus serves as a positive control for a maximal proliferative effect by IL-15. The cells were propagated in culture and monitored for IL-15 surface levels for three days following the pulse incubation. IL-15 detection remained above background for hBC8(23)-IL15/sushi Fab, while both of the IL15/sushi-Fc variants were indistinguishable from background one day after the pulse incubation (FIG. 8B). The pulse of hBC8(23)-IL15/sushi Fab further resulted in similar cell expansion to the saturating amount of the IL15/sushi-Fc variant (FIG. 8C).

[0487] We reason that the IL-15 antibody that we used (clone no. 34559, R&D Systems) likely binds to an epitope on IL-15 that at least partially overlaps with IL-15 β , and thus may detect IL-15 that is not already productively engaged with IL-15 signaling receptors. This antibody has been described as a blocking/neutralizing antibody by several groups (see e.g., Neely, GG, Robbins, Amankwah, et al. (2001) J Imm 167:5011-5017; Krutzik, Hewison, Liu, Robles, et al (2008) J Imm 181:7115-7120; Schlaepfer, Speck (2008) PLoS ONE 3:e1999; and Correia, Cardoso, Pereira, Neves, et al., J Imm (2009) 182: 6149-6159). However, it does not appear to mediate its neutralizing effects through competitive IL-15 binding with IL-15R α : IL-15 is presented on the dendritic cell surface via its interaction with IL-15R α , and this antibody has been used previously to detect such a presentation of IL-15 on dendritic cells (Ferlazzo, Pack, Thomas, Paludan, et al PNAS 2004). Consistent with these observations, we are able to detect hBC8(23)-IL15/sushi Fab and IL15/sushi-Fc variants on the T cell surface using this antibody (FIGS. 8A-8B; each protein variant comprises an association between IL-15R α -sushi and IL-15). The antibody’s neutralizing activity may therefore derive from competitive binding with one of the other two cell surface receptors for IL-15, IL-2/IL-15R β or the common γ chain. As such, detection of IL-15 with this antibody may reflect an excess loading of IL-15 onto the T cell surface beyond the levels that saturate its cell surface receptors, e.g. IL-2/IL-15R β or the common γ chain. This may also explain the low

levels of cell surface IL-15 detected for the IL-15/sushi-Fc constructs (FIGS. 8A-8B), which would only interact with T cells through the IL-15 receptors, though the dimeric IL-15 that results from association with the sushi-Fc fusion could result in a slight abundance of the cytokine. Overall, we conclude that FMs both facilitate greater initial loading of IL-15 on the T cell surface and support elevated levels of biologically available IL-15 on the cell surface over time.

F. Multiple Linker Compositions Support Enhanced FM Potency

[0488] To evaluate flexibility around linkage between IL-15 and the antibody of the FM we explored multiple different polypeptide linker compositions. The FMs explored in Example 1 each comprised a flexible 15 amino acid linker between IL-15 or sushi and the antibody consisting of three tandem repeats of (GGGGS)₃; Linker-1, SEQ ID NO: 36). Here, we explored two additional linker compositions including a flexible eight amino acid linker ((GGGS)₂; Linker-2, SEQ ID NO: 37) and a moderately flexible 12 amino acid linker derived from the human IgG1 hinge region (DKTHTSPSPAP; Linker-3, SEQ ID NO: 38, underlined positions denote residues of the IgG1 hinge region that were mutated from cysteine to serine for this linker). IL-15^{WT} was fused to the C-terminus of the chBC8 Fab fragment light-chain using Linker-2 or Linker-3 and was co-expressed with IL-15R α -sushi to generate chBC8-L2-IL15/sushi Fab and chBC8-L3-IL15/sushi Fab, respectively (FIG. 9A). We previously described chBC8-IL15/sushi Fab in Example 1; this FM variant comprises a (GGGGS)₃ linker (Linker-1, SEQ ID NO: 36) between IL-15 and the chBC8 light-chain; for consistency we refer to this construct here as chBC8-L1-IL15/sushi Fab (FIG. 9A). These protein constructs were evaluated in the pulse bioassay with CD8 T cells as described in Example 1. Incubation with a mock serial dilution comprising media only or hBC8-null-IL15/sushi Fab, which comprises a BC8 binding domain mutated to ablate affinity to CD45 (FIG. 10A), were used as negative controls. Three days after the pulse incubation we analyzed cell proliferation using CellTiter Blue and found all three linker compositions were capable of inducing T cell expansion (FIG. 9B). We conclude that multiple linker compositions between the cytokine and the antibody are capable of generating FMs with improved potency.

Methods

[0489] Protein expression. Proteins were produced from suspension adapted HEK 293 cells in serum-free media. An exception was the parental mouse monoclonal antibody BC8, which was purified from the BC8 hybridoma culture obtained from American Type Cell Culture (ATCC, cat. no. HB-10507). Proteins were then purified by either protein A resin (for Fc fusions and full-length (IgG) antibodies), KappaSelect resin (GE Healthcare; for fusions to antibody Fab fragments), or histidine affinity resin (e.g. nickel-nitrilotriacetic acid, Ni-NTA; for 6-His-tagged scFv fusions) as appropriate. Purified proteins were buffer exchanged into phosphate buffered saline (PBS). Approximate molecular weights were confirmed by reducing and non-reducing SDS-PAGE and aggregation and multimeric status was determined by size-exclusion chromatography (SEC). Based on aggregation or multimeric status, proteins were optionally purified by SEC using a HiLoad Superdex 200 prep-grade

columns (GE Life Sciences) on an AKTA Pure chromatography system (GE Life Sciences).

[0490] Static bioassay. CD8 T-cells isolated from human blood (from Biospecialty Corp.) were activated with CD3/CD28 beads (Dynabeads cat. No. 11132D, ThermoFisher Scientific, Inc.) according to the manufacturer's instructions and in the presence of 10 ng/mL human IL-2 (cat. no. 202-IL-050/CF, R&D Systems) according to the manufacturer's instructions, plated in 6 well 9.5 cm² plates in full RPMI media containing RPMI 1640 (cat. No. 30-2001, American Type Cell Culture), supplemented with 10% heat inactivated fetal bovine serum (FBS-HI, ThermoFisher Scientific, Inc.), penicillin/streptomycin, and 1% Glutamax (cat. no. 35050-061, ThermoFisher Scientific, Inc.), and cultured at 37° C. and 5% CO₂. After 3 days of stimulation, the CD3/CD28 beads were removed, and cells were allowed to rest in full RPMI media for 24 hours. The T-cells were then seeded into 96-well plates at 0.5×10⁶ cells/mL in the presence of six serial four-fold dilution of FMs or control proteins spanning a concentration range of 20 nM to 4.9 pM, unless otherwise indicated, or with media only as a negative control. Following three days at 37° C. and 5% CO₂ cell expansion was analyzed via CellTiter-Blue (cat. no. G8081, Promega).

[0491] Pulse Bioassay. Human CD8 T-cells (from Biospecialty Corp.) were stimulated with CD3/CD28 beads (Dynabeads, ThermoFisher Scientific, Inc.) in the presence of 10 ng/mL IL-2 as described for the static bioassay. After 3 days of stimulation, the CD3/CD28 beads were removed, and cells allowed to rest in full RPMI media for 24 hours. Unless otherwise indicated, the activated T-cells were incubated at a cell density of 500,000 cells/mL with FMs or control proteins at concentration range of 80 nM to 5 nM (three serial four-fold dilutions), unless otherwise indicated, or with media only (0 nM) as a negative control. Following 1 hour at 37° C. and 5% CO₂ cells were washed three times with full RPMI media to remove unbound cytokine and then plated in full RPMI media (in the absence of additional cytokines) at 0.5×10⁶ cells/mL in 96-well plates unless otherwise indicated. Additional controls optionally included either the parental BC8 antibody or chBC8 IgG antibody. Following three days at 37° C. and 5% CO₂ T cell expansion was analyzed via CellTiter-Blue or Flow cytometry. For flow cytometry, 7-AAD (cat. no. A9400-5MG, Sigma) was used to stain for (and subsequently exclude dead cells during the analysis) and CountBright Absolute Counting Beads (cat. no. C36950, Thermo Fisher Scientific, Inc.) were used to quantify viable cell densities according the manufacturer's instructions.

[0492] Measurement of FM on cell surface. Cells were analyzed by immunofluorescent staining to detect cell surface FMs following 0, 1, and 3 days in culture following pulse incubation and washes. Briefly, cells were washed with 1× phosphate buffered saline (PBS) containing 1 mg/mL bovine serum albumin (PBS/BSA) and then stained with a 1:100 dilution of PE-conjugated anti-IL15 antibody (clone no. 34559; R&D Systems cat. no. IC2471P) and a 1:100 dilution of DyLight650-conjugated anti-human IgG H+L polyclonal antibody (ThermoFisher cat. no. Sa5-10129) in PBS/BSA. Following 20-30 min incubation at 4° C., cells were washed one time with PBS/BSA, and then resuspended in PBS/BSA for analysis on a FACSCelesta

using Diva software (BD Biosciences). Data were analyzed using Cytobank (Cytobank, Inc) and GraphPad Prism (GraphPad Software, Inc).

Example 2. Fusion of IL15 to Alternative Anti-CD45 Antibodies

[0493] To evaluate whether anti-CD45 antibodies other than BC8 are capable of generating FMs with improved potency, we evaluated two additional anti-CD45 monoclonal antibodies (mAb clones 9.4 and 4B2). We first evaluated the binding affinities of chimeric IgG forms of these antibodies to CD45 expressed on the surface of activated CD8 T cells. The chimeric antibodies each comprise mouse variable domains and human constant region (human constant kappa domain and human IgG4 containing an S228P mutation to stabilize the IgG4 hinge region). For comparison with previous results we evaluated these antibodies alongside chBC8, hBC8(23), and a non-binding variant hBC8-null. The humanized BC8 antibody consists of variable domains containing CDR regions grafted from BC8, and the hBC8-null variant contains an SGGGS substitution in the heavy-chain CDR3, which ablates binding to CD45 (FIG. 10A). The ch4B2 and ch9.4 antibodies exhibited the highest affinity to cellular CD45, with apparent dissociation constants ($K_{D,app}$) of 0.44 and 0.84 nM, respectively, while the chBC8 and hBC8(23) antibodies exhibited $K_{D,app}$ of 6.68 and 7.8 nM, respectively (FIG. 10A). The hBC8-null antibody did not exhibit significant binding at the highest concentration examined (500 nM; FIG. 10A). We use the term apparent dissociation constant ($K_{D,app}$) to distinguish from a true equilibrium dissociation constant: the bivalent nature of the IgG antibody introduces an avidity effect that complicates the calculation of a true equilibrium dissociation constant for these type of assays. Nevertheless, the $K_{D,app}$ is a useful metric for scoring relative affinities of the antibodies to the cellular receptor in its native context.

[0494] We next evaluated the potency of FMs comprising each of these antibodies. Briefly, IL-15 was fused to the C-terminus of ch9.4 or ch4B2 Fab fragment light-chain and co-expressed with IL-15R α -sushi to generate ch9.4-IL15/sushi Fab and ch4B2-IL15/sushi Fab, respectively. Construction of chBC8-IL15/sushi Fab, hBC8(23)-IL15/sushi Fab, and hBC8-null-IL15/sushi Fab were described in Example 1. We compared the potency of these FMs in the pulse bioassay, and included a series of negative controls: hBC8-null-IL15/sushi Fab (an FM with ablated CD45 binding), IL-15^{WT}/sushi-Fc, BC8 antibody, and media only. Each of the FMs induced greater T cell expansion than the negative controls as measured three days after the pulse incubation (FIG. 10B). The FMs comprising 4B2 or 9.4 possessed increased potency compared to FMs comprising chBC8 or hBC8(23). This is consistent with the increased affinity of 4B2 and 9.4 compared to chBC8 and hBC8(23), and is consistent with the idea that increased affinity of the antibody to the cell surface receptor results in increased potency of the FM. Overall, we conclude that generation of FMs with improved biological persistence is not restricted to the BC8 antibody, and that increased affinity to the cell surface receptor can improve the potency of the FM.

Methods

[0495] Cell binding assay. Primary human CD8 T-cells were activated with CD3/CD28 beads and 10 ng/mL IL-2 as

described in Example 1. Following activation and an overnight rest in full RPMI media (see Example 1 methods) the T-cells were incubated with seven serial five-fold dilutions of antibodies spanning 500 nM to 6.4 pM, or with media only control at 4° C. for 1 hr. Cells were washed with MACS buffer (PBS, 2% FBS, 1 mM EDTA) three times, and antibody binding was detected using a PE-conjugated anti-human IgG-Fc antibody (BioLegend, cat. no. HP6017) for 20 min. at 4° C. Cells were washed two times with MACS buffer, and the resuspended in MACS buffer with live-dead stain (7-AAD). Antibody binding was quantified using the median fluorescence intensity (MFI) of the live cell population (via 7-AAD exclusion) within the PE signal. Curves were generated by plotting mean fluorescent units against antibody concentration, and apparent dissociation constants ($K_{D,app}$) were determined by non-linear regression using a four-parameter logistic equation using GraphPad Prism (GraphPad Software, Inc.).

[0496] Pulse bioassay. The pulse bioassay was conducted as described in Example 1.

[0497] Protein expression. Proteins were produced as described in Example 1.

Example 3. FMs Targeting Additional Cell Surface Receptors Facilitate Improved Potency and Specificity

[0498] A. Additional Cell Surface Receptors Other than CD45 Facilitate Improved Cytokine Potency.

[0499] To explore whether FMs targeting cell surface receptors other than CD45 are capable of resulting in increased biological potency we constructed FMs comprising antibodies targeting additional cell surface receptors. The alternative receptors can either be abundant receptors on the cell surface or exhibit persistence at the protein level (e.g. display slow receptor internalization or strong recycling back to the cell surface upon receptor internalization). Ideally the receptor is both abundant and persistent. Based on these considerations we selected FMs targeting CD8, CD11a, or CD18 for evaluation. CD8 is a co-receptor for the T cell receptor that is expressed on cytotoxic T cells. CD8 can also be expressed in cortical thymocytes, dendritic cells, and natural killer cells. In cytotoxic T cells, CD8 helps maintain the activated T cell receptor/MHC complex on the cell surface (Wooldridge, van den berg, Glick, et al. JBC 2005), and once the T cell receptor is internalized the CD8 co-receptor is preferentially maintained on the cell surface in comparison to the T cell receptor (1989 J Imm Boyer, Auphan, Gabert, Schmitt-Verhulst et al Comparison of phosphorylation and internalization of the antigen receptor/CD3 complex, CD8, and Class I MHC-encoded proteins on T cells). Lymphocyte function-associated antigen 1 (LFA-1) is a heterodimeric cell surface receptor composed of integrin alpha-L (CD11a) and integrin beta-2 (CD18) and is involved in intercellular adhesion and lymphocyte costimulation. Integrins generally display persistence at the protein level: they typically either exhibit slow internalization from the cell surface or are recycled back to the cell surface upon internalization (2012 J Cell Sci Integrins at a Glance and 1989 EMBO J Endocytosis and recycling of the Fn receptor in CHO). LFA-1 is present at high levels on several different types of leukocytes and has been shown to exhibit slow internalization in multiple different cell types, including monocytic U937 cells, the promyelocytic leukemia cell line HL-60, and the lymphoblastoid cell line JY (1992 EMBO J

Circulating Integrins Bretscher). In addition, preclinical studies of the CD11a antibody efalizumab demonstrated that CD11a exhibits persistence at the protein level in human and mouse T cells isolated from blood; efalizumab can, however, induce receptor internalization and degradation in the presence of a secondary cross-linking antibody (2004 Coffey, Pippig et al J Pharm Exp Ther).

[0500] We constructed FMs targeting CD8 (antibody clone OKT8), CD11a (antibody clone MHM24), or CD18 (antibody clone 1B4) by genetically fusing IL-15 to the C-terminus of the respective antibody fragment light-chain. The IL-15-antibody fusions were co-expressed with IL-15 α -sushi to generate three FMs: chMHM24-IL15/sushi Fab, ch1B4-IL15/sushi Fab, and chOKT8-IL15/sushi Fab (FIG. 11A). Each Fab fragment was composed of chimeric human and mouse regions with mouse variable region and human constant domains (kappa and CH1). The potency of the FMs on CD8 T cells was analyzed in the pulse bioassay as described in Example 1; after three days in culture following the pulse incubation cells were analyzed for proliferation using CellTiter Blue. The three FMs each supported similar levels of T cell proliferation to that of chBC8-IL15/sushi Fab (FIG. 11B). We have demonstrated above that forms of IL-15 that do not contain an immune-targeting functionality exhibit minimal effects in the pulse bioassay (FIGS. 2A-2C, 4A-4B, 5A-5D, 6A-6B, and 8A-8C). Together this demonstrates that additional cell surface receptors are capable of generating FMs with improved biological persistence.

B. FMs that Facilitate Improve Potency Towards CD8 T Cells

[0501] To evaluate whether cell surface receptor targeting can facilitate improved potency towards individual cell types we compared the potency of CD8-targeted FM on CD4 and CD8 T cells. CD4 or CD8 cells were treated with chOKT8-IL15/sushi Fab in the pulse bioassay format, and cell proliferation was measured three days after the pulse incubation. The CD8-targeted FM more potently stimulated CD8 T cells compared to CD4 T cells (FIGS. 12A-12B), supporting the ability to target specific cell types based on the antibody of the FM.

Methods

[0502] Pulse bioassay on CD4 or CD8 T cells. Primary human CD3 T cells, which contain a mixture of cells expressing CD4, CD8, or CD4 and CD8 coreceptors, were activated using CD3/CD28 activation beads in the presence of 10 ng/mL IL-2 as described in Example 1. Following three days of activation the beads were removed and cells were rested overnight in full RPMI media. We then purified individual populations of CD4 and CD8 T cells using magnetic activated cell sorting (MACS) using (cat. no. 130-045-101, and no. 130-045-201, Miltenyi Biotec, Inc.) according to the manufacturer's protocol. CD4 or CD8 T cells were then analyzed in the pulse bioassay as described in Example 1.

Example 4. Protein Nanogels Comprising IL-15 FMs Support Long-Term Cell Expansion

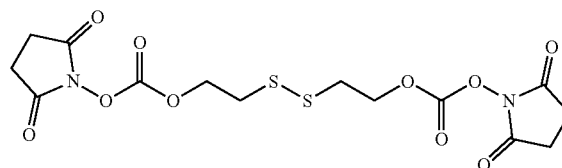
[0503] Drug-loaded nanoparticles (also referred to herein as "backpacks") hold potential for supporting cell viability and function. We therefore explored the ability to form nanoparticles comprising an FM and the effect of these

nanoparticles on T cell viability and expansion. We cross-linked hBC8(23)-IL15/sushi Fab into a protein nanogel using a reversible, amine-reactive, homobifunctional cross-linker. Protein nanogels comprising IL15^{N72D}/sushi^{L77L}-Fc were used as a comparator. Both of these proteins efficiently crosslinked into protein nanogels: by SEC analysis the protein nanogels traveled with a faster elution time than the free protein, consistent with a larger species (FIGS. 13A and 13B). Comparison of HPLC traces of the protein nanogel with the free (noncrosslinked) protein (FIGS. 13A-13B) revealed a minimal amount of free protein remaining in the cross-linked nanogels, suggesting high conversion of the proteins into nanogels. The protein nanogels were coated with a co-block polymer of polyethylene glycol and polylysine and incubated with CD8 T cells. Both protein nanogels supported long term cell expansion in comparison to non-crosslinked protein and unstimulated controls (FIG. 13C).

Methods

[0504] Protein nanogel synthesis. Protein nanogels comprising a crosslinked protein nanoparticle were formed as follows. The chBC8-IL15/sushi Fab fusion protein was crosslinked into protein nanogels by incubating the protein at a concentration of 17 mg/mL with a 27-fold molar excess of a degradable crosslinker (Formula I) in the presence of a final concentration of 2.5% polyethylene glycol with an average molecular weight of 400 Dalton (PEG-400, Spectrum Chemical Mfg. Corp.) and 10% glycerol (Sigma). The IL15^{N72D}/sushi^{L77L}-Fc protein was crosslinked into protein nanogels by incubating the protein at a concentration of 17 mg/mL with a 27-fold molar excess of a degradable crosslinker (Formula I). The cross-linker used in this study, Bis[2-(N-succinimidyl-oxycarbonyloxy)ethyl] disulfide, contains two N-hydroxysuccinimide (NHS) ester groups joined together by a flexible disulfide-containing linker as shown in Formula I.

Formula I



[0505] After 30 minutes incubation at room temperature, the reactions were diluted with Dulbecco's phosphate buffered saline (DPBS) to a final cytokine concentration of 1.5 mg/mL. Protein nanogels were then purified from linker leaving groups (which comprise molecular fragments of the linker that are removed as part of the cross-linking reaction) and unreacted linker by buffer exchange into DPBS using a Zeba column (40,000 MW cut-off, Thermo-Fisher). Zeba columns were used according to the manufacturer's instructions, including equilibrating the column in DPBS by three consecutive washes with DPBS to facilitate buffer exchange, followed by application of the reaction products. Buffer-exchanged protein nanogels were analyzed by size exclusion chromatography (SEC) using a BioSepTM SEC-s4000 column (Phenomenex Inc.) with PBS (pH 7.2) as eluent (flow rate 0.5 mL/min) on a Prominence HPLC system equipped with a photodiode array (Shimadzu Corp.).

[0506] Protein nanogels at a cytokine concentration of approximately 1-1.5 mg/mL were conjugated with a polyethylene glycol-polylysine (PEG-polyK) block co-polymer: PEGSk-polyK30 (Alamanda Polymers cat. no. 050-KC030), which is a block co-polymer comprising a polyethylene glycol polymer of 5 kiloDalton (kD) average molecular weight and a 30 amino acid polylysine polymer (polylysine30 or polyK30). PEG5k-polyK30 or were reconstituted to 1 mg/mL in DPBS and added to 1-1.5 mg/mL of protein nanogels at a final block copolymer concentration of 50 ug/mL and incubated at room temperature for 30 min.

[0507] T cell expansion analysis. Protein nanogels comprising PEG5k-polyK30 at a protein concentration of 1-1.5 mg/mL were mixed in equal volume with 1×10^6 CD8 T-cells in HBSS at a cell concentration of 100×10^6 cells/mL and incubated at 37° C. for 1 hr. T-cells were then washed three times with RPMI media containing 10% FBS, penicillin/streptomycin, and Glutamax (all from ThermoFisher Scientific, Inc.), seeded at a cell density of approximately 1×10^6 cells/mL, and cultured for 9 days in 24-well tissue culture plates. Cells were split into fresh medium at a ratio of 1:5 on three days after cell attachment of backpacks. Cell proliferation was measured by live-dead cell stain (7-AAD) and counting beads (CountBright Absolute Counting Beads, Thermo Fisher Scientific, Inc.) by flow cytometry on Days 0, 1, 2, 3, 6, and 9.

Example 5: Versatility of Immunotargeted Surface Receptor

[0508] FIG. 14 illustrates various combinations of immunotargeting moieties (e.g., antibodies against various cell surface receptors) and cytokine molecules. Multiple receptors can facilitate cytokine immunotargeting. For example, tethered fusions can be made with various cytokines tethered to antibodies against CD11a, CD18, CD45 and/or CD2 which may be present on multiple cell types. Cytokines can also be immunotargeted to specific cells via cell type-specific surface receptors such as CD4, CD8 or CD56.

Example 6: Tethered Fusion Platform Enables Selective Cell Targeting

[0509] CD8-targeted IL-7 delivers selective loading and expansion of CD8 T cells. FIGS. 15A-15B shows cell-specific targeting, in which CD8-targeted-IL-7 selectively targets CD8 T cells. FIG. 15C shows selective expansion of CD8 T cells, but not CD4 T cells, in cultures of activated total CD3 human T cells by pulse incubation with CD8-targeted IL-7. Briefly, activated human T cells were prepared by stimulating total CD3 human T cells (comprising both CD4 and CD8 T cells) for three days using CD3/CD28 Dynabead Activator beads (Thermo) according to the manufacturer's instructions and in the presence of in the presence of 20 ng/mL IL-7 and 100 ng/mL IL-21. The CD3/CD28 beads were then removed and cells were incubated in full medium (RPMI 1640 containing 10% FBS) overnight in the presence of 20 ng/mL IL-7 and 100 ng/mL IL-21 at 37 C and 5% CO₂. Cells were then washed and incubated with 500 nM of a CD8-targeted IL-7 construct (chOKT8Fab-IL7). After 1 hr at 37 C cells were washed two times with full medium, plated at a density of 200,000 cells/mL, and incubated at 37 C and 5% CO₂. Separately, cells were analyzed for loading onto CD8 and CD4 T cells by staining the T cells with DyLight 650-conjugated anti-human IgG

polyclonal antibody (Thermo cat. no. SA5-10129; DyLight 650 fluorophore can be read on the APC channel for flow cytometry, see FIG. 15B), FITC-conjugated anti-CD4 antibody (BioLegend cat. no. 344604), and APC/Cy7-conjugated anti-CD8 antibody (BioLegend cat. no. 344715). The anti-human IgG polyclonal antibody detects the Fab region of the CD8-targeted FM. The anti-CD4 and anti-CD8 antibodies can differentiate the CD4 and CD8 T cell populations (FIG. 15A). FIG. 15B shows that the CD8-targeted IL-7 construct selectively loaded to greater levels on CD8 T cells when incubated with a mixed population of CD4 and CD8 T cells. FIG. 15C shows selective expansion of the CD8 T cells by the CD8-targeted IL-7 construct.

[0510] To further explore selective cell loading by different antibody clones and antibody configurations we compared the loading onto CD4 and CD8 T cells by two different anti-CD8 monoclonal antibodies. Briefly, we recombinantly produced anti-human CD8 antibody clones OKT8 and 51.1 as Fab fragments (chOKT8Fab and ch51.1Fab). We additionally constructed two IL-15 FMs: one comprising the chOKT8Fab (chOKT8Fab-IL15/sushi) and a second comprising the chOKT8Fab and an anti-CD45 scFv (chOKT8Fab-BC8scFv-IL15/sushi). This latter construct contains antibody fragments against two different cell surface receptors, CD8 and CD45, and we reasoned it may give stronger cell loading of CD8 T cells through an avidity effect. We evaluated the ability of these four antibody and FM constructs to load onto CD4 and CD8 T cells using total CD3 T cells (comprising mixtures of CD4 and CD8 T cells). FIG. 15D shows strong selective loading onto CD8 T cells for each construct. In FIG. 15E we similarly show selective loading of IL-15 onto CD8 T cells using anti-IL15 staining (Fab constructs that do not contain IL-15, chOKT8Fab and ch51.1Fab, are of course negative in this assay). To compare relative affinities against CD8 T cells, we compiled the binding titrations against CD8 T cells from FIG. 15D into a single plot in FIG. 15F. The chOKT8Fab-BC8scFv antibody configuration delivered the tightest binding to the CD8 T cells. This FM also resulted in loading onto CD4 T cells when used at higher concentrations (FIG. 15D and FIG. 15E). We reason that this is due to the BC8scFv, which binds to the CD45 receptor, which is present on both CD4 and CD8 T cells. The relative affinity of chOKT8Fab-BC8scFv-IL15/sushi for CD4 T cells observed in FIG. 15D is similar to the affinity of monomeric BC8scFv against T cells (FIG. 15G, the

[0511] BC8scFv was constructed using a hexahistidine tag, which is used here as a handle for detection by flow cytometry); together with the minimal binding of chOKT8Fab and chOKT8Fab-IL15/sushi to CD4 T cells, we conclude that binding of chOKT8Fab-BC8scFv-IL15/sushi to CD4 T cells is driven by the BC8scFv. A dimeric form of BC8scFv results in higher affinity to T cells, likely due to the avidity affect from bivalent binding. We further reason that the higher binding affinity of chOKT8Fab-BC8scFv-IL15/sushi to CD8 vs CD4 T cells—as well as the higher binding to CD8 T cells as compared with the monovalent chOKT8Fab-IL15/sushi—is similarly due an avidity effects that results from bivalent binding to CD8 T cells. We thus conclude that FMs can be constructed using antibody clones that are specific for different cell surface receptors to improve cellular affinity and loading. Furthermore, one or more of the selected antibodies can be specific for a particular cell type in order to enable improved affinity while

still retaining cell-selective loading. Using such a method, we show that it is further feasible to construct heterospecific antibodies with varied loading efficiencies on different cell types (e.g. in this case, stronger loading onto CD8 T cells and weaker—but nonzero—loading onto CD4 T cells).

Example 7: Cytokine Engineering Improves Tethered Fusion Potency

[0512] We evaluated the ability to further augment FM potency by engineering cytokine mutants with modulated biological activity. Based on the structure-function understanding of the interaction between IL-15 and its cellular signaling receptors (Chirifu et al. *Nat Immunol.* 2007 Sep; 8(9):1001-7; Ring et al. *Nat Immunol.* 2012 Dec; 13(12): 1187-95), we constructed three IL-15 variants comprising mutations to amino acid side-chains believed to interact with the IL-15/IL-2 receptor beta (specifically, IL-15-D8N, IL-15-D61N, and IL-15N72A), and three additional IL-15 variants comprising mutations to amino acid side-chains believed to interact with the gamma chain (IL-15-K10Q, IL-15-Q101N, and IL-15-Q108N). We constructed the IL-15 mutants as fusions to the chBC8Fab antibody fragment and evaluated their binding and activity on human T cells. Briefly, primary human T cells were activated as described in Example 1, and then incubated with five-fold serial dilutions of FMs comprising the IL-15 variants (without washing after FM incubation, e.g. a ‘constant’ incubation assay format). An FM comprising wild-type IL-15 and an IL-15/sushi-Fc construct were used as controls for comparison. After incubation with the IL-15 constructs for three days at 37 C and 5% CO₂ cell density was analyzed using CountBright Absolute Counting Beads (Thermo) by flow cytometry following the manufacturer’s instructions. The FM containing wild-type IL-15 (chBC8Fab-IL15/sushi) exhibited similar cellular potency as the IL-15/sushi-Fc construct (FIG. 16A and FIG. 16B). In addition, FIG. 16A and FIG. 16B shows that three different IL-15 variants result in stronger cellular potency than wild-type IL-15 (IL-15-K10Q, IL-15-Q101N, and IL-15-N72A), two IL-15 variants resulted in reduced cellular potency (IL-15-D61N and IL-15-Q108N), and a single IL-15 variant did not display cellular activity under the range of concentrations evaluated (IL-15-D8N). In a separate experiment we evaluated the surface persistence of the two attenuated IL-15 variants (IL-15-D61N and IL-15-Q108N) in the pulse assay format as described in Example 1. We found that the FMs comprising the attenuated variants exhibited stronger persistence on the cell surface (FIG. 16C). Activation of growth factor and cytokine signaling receptors induces negative regulatory feedback mechanisms within the cell. One such mechanism involves internalization and degradation of activated cytokine and growth factor receptors (reviewed in Jones et al. *Trends Biotechnol.* 2008 Sep; 26(9):498-505).

[0513] Without wishing to be bound by theory, we reason that the elevated surface persistence from cytokine variants having attenuated activity may be due to weaker induction of such negative regulatory feedback mechanisms within the cell.

[0514] To further explore the relationship between cytokine activity and FM surface persistence and potency, we designed four additional IL-15 variants comprising mutations at the interface with IL-15 and its IL-15/IL-2 receptor beta. In particular, we constructed CD8-targeted FMs comprising the IL-15 variants IL-15-D61H, IL-15E64H, IL-15-

D65H, or IL-15-N72H. Activated human T cells were prepared by stimulating CD3 human T cells for three days using CD3/CD28 Dynabead Activator beads (Thermo) according to the manufacturer’s instructions, beads were then removed and cells were incubated in full medium (RPMI 1640 containing 10%

[0515] FBS) overnight in the presence of 20 ng/mL IL-7 and 100 ng/mL IL-21 at 37 C and 5% CO₂. The activated T cells were then incubated with a serial five-fold dilution of FMs spanning a concentration range of 10-0.0032 nM and plated at a density of 200,000 cells/mL into 96-well plates (without washing after FM incubation, e.g. a ‘constant’ incubation assay format). After three days at 37 C and 5% CO₂ cells were harvested and evaluated for cell expansion and FM surface levels. For analysis of FM surface levels, cells were stained with BV421-conjugated anti-CD4 antibody (BioLegend cat. no. 344632), BV786-conjugated anti-CD8 antibody (BioLegend cat. no. 344740), and Alexa-Fluor-647-conjugated anti-human kappa (light-chain) antibody. FIG. 16D demonstrates high loading of the CD8-targeted IL-15 constructs onto CD8 T cells, and minimal loading onto CD4 T cells. The limited loading of the CD8-targeted FMs onto

[0516] CD4 T cells enabled a controlled evaluation of the effects of IL-15 mutations on cellular responses. Expansion of CD4 T cells in response to incubation with CD8-targeted IL-15 constructs comprising D61H, E64H, N65H, or N72H point mutations demonstrated attenuating activity of D61H, E64H, and N65H mutations (FIG. 16E).

[0517] We further evaluated activity of these constructs on CD8 and CD4 T cells in the pulse assay format. Briefly, activated total CD3 human T cells were incubated with the CD8-targeted IL-15 variants for 1 hr at 37 C, washed to remove unbound FM, plated at a density of 200,000 cells/mL, and incubated at 37 C and 5% CO₂. To control for effects of induced cytokine secretion or tethered fusion unbinding and rebinding, each of which may support further T cell survival or expansion, we refreshed the cell culture medium one and three days after pulse incubation; cells were additionally split using a five-fold dilution ratio into fresh medium three days after pulse incubation to allow for further cell expansion. Each CD8-targeted construct selectively loaded to higher degrees onto CD8 T cells as compared with CD4 T cells (FIG. 17A). In addition, each of the histidine mutants comprising attenuated cellular activity (D61H, E64H, and N65H) exhibited higher surface persistence one day following the pulse incubation onto mixed CD4 and CD8 T cells (FIG. 17A). To evaluate the ability of the CD8-targeted constructs to selectively expand CD8 vs CD4 T cells we compared their activity with a nonselectively-loaded, CD45-targeted IL-15 FM constructed with the same antibody configuration as the CD8-targeted IL-15 FMs (h9.4Fab-BC8scFv-IL15/sushi, which contains a Fab-scFv format consistent with the antibody format for the CD8-targeted constructs). Each CD8-targeted IL-15 construct delivered selective expansion of CD8 T cells, and the CD8-targeted IL-15 constructs comprising the D61H and E64H mutations supported greater CD8 T cell expansion than the CD8-targeted construct comprising wild-type IL-15 (FIG. 17B). Notably, in this pulse assay format each of the CD8-targeted IL-15 constructs only weakly expanded the CD4 T cells (FIG. 17B), consistent with their design for CD8-specificity and consistent with their specificity for loading onto CD8 T cells. For comparison, a full expansion

time course is shown for the CD8-targeted constructs comprising wild-type IL-15, IL-15-D61H, or IL-15-E64H (FIG. 17C).

[0518] We additionally evaluated the attenuated IL-15 variant IL-15-D61H within the context of a CD45-targeted FM (h9.4Fab-BC8scFv-IL15-D61H). Consistent with the data observed for the CD8-targeted IL-15-D61H FM, the CD45-tethered construct delivered stronger cell surface persistence as compared both with a monovalent and bivalent CD45-tethered IL-15 FMs (FIG. 17D). Collectively, we conclude that FMs comprising attenuated IL-15 variants can result in higher cell surface persistence and induce stronger cell proliferation over time.

Example 8. Sorting of Tregs

[0519] FoxP3 positivity defines the Treg cell subset; however, because FoxP3 is an intracellular protein, it cannot be used to sort and isolate live cells using currently available technologies. CD25 is a surface marker for Tregs, but it is also expressed on activated T cells. The IL-7 receptor (CD127) can be used to distinguish between Tregs and activated T cells based on its higher expression on activated T cells. Magnetic and flow cytometry-based sorting methods that enrich for the surface markers CD4⁺CD25⁺ and deplete CD127⁺ cells are used to enrich and isolate Tregs from PBMCs or CD3 T cells.

[0520] Frozen CD3 T cells were sorted into Treg-enriched and Treg-depleted CD4 populations by magnetic sorting. The Treg-enriched fraction was positively selected for CD4 and CD25, and negatively selected for CD127 (CD4⁺/CD25⁺/CD127⁻) using magnetic sorting reagents from Miltenyi and following the manufacturer's protocols. Treg-enriched and -depleted cells were activated with CD3/CD28 beads and expanded with IL-2 for 5 days. After 5 days of culture, the Treg-enriched cells maintained significantly more FoxP3⁺ cells, and significantly fewer CD127⁺ cells (FIG. 18). As shown in FIG. 18, enrichment via CD4⁺/CD25⁺/CD127⁻ magnetic selection gives 30% FoxP3⁺ cells, a 2-fold enrichment over Treg-depleted.

Example 9. IL-2 Containing Fusion Molecules

[0521] An exemplary IL-2 containing FM can have the following sequence (SEQ ID NO: 79) that can be linked to an antibody or fragment thereof at one terminus:

```
GGGGGGGGGGGGGSDAHKSEVAHRFKDLGEENFKALVLIIFAQYLQQC
PFEDHVKLNVNEVTEFAKTCAVEDSAENCDKSLHTLFQDKLCTVATLRET
YGEMADCCAKQEPEERNECFQHKDDNPNLPRLVPEVDVMTAFHDNEE
TFLKKYLYEIAARRHPFYAPELLFFAKRYKAAFTCCQAADKAACLLPK
LDELRLDEGKASSAKQRLKCAQLQKFGERAFAKAWAVARLSQRFPKAEFAE
VSKLVTDLTQVHTECHGDLLECADRADLAKYICENQDSISSKLKECC
EKLPLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG
MFLYFYARRHPDYSVVLLRLAKTYETTLKCCAAADPHECYAKVFDEF
KPLVEEPQNLIKQNCLEFQEGYKFNALLVRYTKKVPQVSTPTLVEV
SRNLGKVGSKCKKHEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTK
CCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIK
```

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KQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCKCKADDKETCFAEEGK
KLVAASQAALGLGGGGSAPTSSSTKKTQLEHLLLDLQMLNGINNYK
NPKLTRMLTFKFPMPKATELKHLCLEEBELKPLEEVNLQAQSKNFHLR
PRDLISNINIVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIIS
```

TLT*

Double underline: IL-2

Single underline: Linker

No underline: HSA (human serum albumin)

[0522] This FM was made by expression of a fusion protein comprising three proteins: the targeting antibody (e.g., anti-CD45, anti-CD4 and/or anti-CD25), human serum albumin (SEQ ID NO: 79, no underline), and IL-2 (SEQ ID NO: 80). The three subunits are operably linked together via a peptide linker comprising G and S (SEQ ID NO: 79, single underline). Details of nucleic acid construct preparation, protein expression and use can be found in Examples 1-4.

Example 10: Suppressive Cell Therapy Using IL-2 Fusion

[0523] While Treg therapies have shown promise in clinical trials for Graft vs. Host Disease, Type 1 Diabetes, and Crohn's Disease, one common failure point in all of these trials has been a rapid loss of detectable Tregs. Multiple trials have suggested that 90-95% of injected Tregs are lost within the first two weeks after reinjection into the human subject. It is widely believed that a lack of cytokine support for the in vitro-expanded Tregs leads to Activation-Induced Cell Death (AICD) and a rapid decrease in cell numbers. To support Treg survival in vivo, some studies have incorporated systemic low-dose IL-2 into the Treg adoptive cell therapy treatment regimen. IL-2 is a pan-T cell pro-proliferative and survival cytokine. A form of IL-2 (aldesleukin) is FDA-approved for treatment of renal cell carcinoma; however, it possesses severe dose-limiting toxicities. Because Tregs express high levels of the IL-2 receptor alpha subunit (IL2R α or CD25), it is possible to obtain a degree of selective Treg expansion by using IL-2 doses well below toxic levels and with minimal effects on pro-inflammatory T cells. In this Example, we evaluate FMs comprising IL-2 on adoptively transferred Tregs. By directly tethering IL-2 to the surface of adoptively transferred Tregs, this approach can provide strong IL-2 signaling to the Tregs while minimizing effects on other pro-inflammatory cells. This approach can improve Treg survival and engraftment, and promote stronger and more durable treatment of various immune-related conditions, such as recovery of gut homeostasis in Inflammatory Bowel Disease (IBD), including refractory Crohn's Disease and Ulcerative Colitis.

[0524] The technology as disclosed in, e.g., U.S. Publication No. 2017/0080104, U.S. Pat. No. 9,603,944, U.S. Publication No. 2014/0081012, and PCT Application No. PCT/US2017/037249, each of which is incorporated herein by reference in its entirety, is built upon the concept of attaching protein nanogel and fusion molecules to the surface of immune cells immediately prior to adoptive transfer. These non-covalently attached proteins allow for non-genetic control of cell behavior after transfer into the patient. When

applied in Treg therapies, IL-2 loading improves on state-of-the-art cell therapy approaches by providing the cells with their own pro-survival/proliferation microenvironment that they carry with them into the patient. Because cytokine is attached directly to the target cells, delivery is non-systemic and a comparable on-target dose is given with far-lower total protein administration, leading to a significantly improved safety profile. Furthermore, because it is non-genetic, the cell production time is reduced, genome insertion related concerns are eliminated, and the cell process is significantly less expensive. Together this can provide to a cell product with improved efficacy and lower cost to the patient and insurers. The proposed mode of action (MOA), as compared to conventional Treg adoptive cellular therapies (ACT), is shown in FIG. 19.

[0525] We first evaluated the effects of an FM comprising IL-2 on Treg-enriched CD4 T cells using the pulse assay format. Briefly, Treg-enriched CD4 T cells were obtained as described in Example 8. The Treg-enriched CD4 T cells were activated as described in Example 8. Cells were then pulse incubated with an IL-2 FM (h9.4Fab-IL2 for 1 hr at 37 C, washed two times, and then plated at a density of 500,000 cells/mL in full medium (containing RPMI 1640 and 10% FBS). IL-2 surface levels following pulse incubation with the IL-2 FM were analyzed by flow cytometry. FIG. 20 shows IL-2 surface levels after titration of an FM comprising IL-2 pulse-loaded onto Treg-enriched CD4s. The IL-2 FM shows titratable surface loading at Day 0 and persistence on the cell surface for at least 3 days. In addition, the cells show dose-dependent proliferation, and all concentrations of the IL-2 FM evaluated (concentration range 1.5 -100 nM) were capable of maintaining cell viability above those of the negative control (FIG. 20).

[0526] To evaluate effects of FMs comprising IL-2 on Tregs we designed and produced multiple FMs comprising human IL-2 and a variety of human CD45-targeting moieties and linker configurations including Fab-IL2 (h9.4Fab-IL2), Fab-HSA-IL2 (h9.4Fab-HSA-IL2), scFv-HSA-IL2 (scFv-h9.4-HSA-IL2), scFv-FcγR3B-IL2 (scFv-h9.4-FcγR3B-IL2). In vitro tests of these molecules on human T cells demonstrated strong binding and surface persistence, as well as strong expansion of both Treg-enriched CD4s and total CD3s. Briefly, Treg-enriched cells were loaded by incubating with 100 nM tethered fusions (TF) or HBSS (mock), washed to removed unbound TF, plated, and then analyzed for cell expansion over time. After 18 hours, soluble recombinant IL2 (sIL2) was added to one of the mock wells at a concentration of 100 ng/mL to serve as a positive control for expansion by saturating levels of IL-2. As shown in FIG. 21, left panel, at 18 hours, strong surface staining was observed for all IL-2 TFs, with intensity of Fab-IL2 > Fab-HSA-IL2 > scFv-HSA-IL2. Cell expansion at day 3 was correlated with surface staining observed on day 1 (FIG. 21); cell viability, however, was consistent across all TFs and sIL2 (FIG. 21, right panel), and exhibited a 2-fold increase over mock. We conclude that multiple configurations of the targeting moiety are capable of facilitating IL-2 loading and persistence on the cell surface and subsequent cellular expansion and viability.

[0527] To examine the ability of IL-2-containing FMs to support cell proliferation and survival over longer time courses, CD4 cells were pulsed with vehicle or IL-2 FMs and cultured for 9 days. A separate mock-treated condition was supplemented with constant incubation with 100 ng/mL

recombinant IL-2 to serve as a positive control. The IL-2 TFs expanded Treg-enriched CD4 T cells and increased viability over time (FIG. 22). Cells loaded with each of the TF constructs showed equal or better expansion compared to constant stimulation with sIL2 through 6 days post-pulse incubation; Fab-IL2 had the highest surface stain and best growth and survival effects. Mock pulsed cells showed reduced viability and cell number by day 3, and retained only 5% viability by day 6. Conversely, the IL-2 TFs supported sustained cell expansion and had >75% viability 6 days post-BP. We conclude that multiple configurations of IL-2 TFs are capable of promoting expansion and survival of Treg-enriched CD4s for at least 9 days in vitro.

[0528] To evaluate the ability for IL-2 TFs to promote expansion and survival in vivo, tethered fusion-pulsed cells were injected into NOD scid gamma (NSG) mice. 5 million human CD4 T cells were loaded with Fab-HSA-IL2 as described above and injected into NSG mice (d0). 1 million activated human CD8s were injected on d3 in order to mimic the clinical application of HSCT and measure off-target effects. Blood was drawn on d4, 7, 10, 13, 20, 27, 30, etc. to measure Treg-enriched CD4s and CD8s. As shown in FIG. 23, adoptively transferred cells were detectable in the blood starting d7, and loading of Fab-HSA-IL2 significantly increased cell numbers at early and late time points. Peak increased expansion was detected at d10 with >5-fold more Fab-HSA-IL2-loaded cells in the blood stream. Furthermore, FIG. 24 shows that the CD4:CD8 ratio was higher in the mice treated with the FM-pulsed CD4 cells. This indicates that there were greater effects of the IL-2 on pulsed cells than on bystander CD8s, and that pulse loading of an IL-2 FM provides pro-survival and proliferative signals preferentially to the pulsed cells.

[0529] In summary, all four TFs show strong binding to unsorted CD3s or Treg-enriched CD4s. Binding is concentration-dependent and saturates between 25 and 100 nM for all constructs. All TFs promote survival and expansion similar to soluble IL-2 at saturating concentrations in vitro. In vivo, Fab-HSA-IL2 promotes early expansion and prolonged engraftment of Treg-enriched CD4s (5 fold at d10).

[0530] It should be noted that with antibody-tethered cytokine fusions (TFs), a certain amount of activity on non-pulsed cells has been observed. This can result from either TF dissociation from the loaded cell, or through trans or paracrine effects on neighboring cells. The next step is to reduce these off-target effects, giving maximal selectivity of IL-2 activity for the Tregs. This can be accomplished by engineering TFs for (a) reduced IL-2 affinity for its receptor and (b) enhanced strength, stability and selectivity of Treg binding. Because Tregs have extremely high expression of the IL-2 receptor and the cytokine will be held in close proximity to the receptor via antibody targeting, reduced affinity IL-2 will still drive signaling on loaded cells without off target effects on nearby cells or in the circulation. By increasing antibody specificity for Tregs, it can be ensured that any antibody that dissociates from loaded cells will not bind and initiate signaling on off-target cells. Together, these modifications can create a highly specific molecule with maximal Treg on-target signaling, without off-target toxicities.

[0531] Specifically, IL-2 affinity modulated mutants can be engineered to increase Treg-specific signaling. This can increase specificity of activation by reducing paracrine off-target IL-2 signaling on non-loaded cells. Reduced affinity

human and mouse IL-2 molecules can be made based on structure of receptor binding and analogous work with IL-15 (see Example 7). Optimal mutant can be selected in vitro using mixed loaded/non-loaded cell assay and maximizing ratio of on-target to off-target activity. Reduced off-target effects of affinity modulated IL-2 in vivo can be validated.

[0532] Potency and Treg selectivity of antibody binding motifs can also be optimized. This can increase specificity and strength of IL-2 tethering to Tregs to reduce off target effects, and enable selective loading of stabler and more active Treg subsets. Affinity matured, humanized CD45 antibody and humanized anti-CD4 (OKT4) can be produced. Antibody campaigns for CD25 (general Treg) and CD39 (active subset specific) can also be conducted. Optimal antibody binding motifs can be selected using the following criteria: (a) Stable and selective binding to Tregs; (b) Maximized stability of binding to Tregs. With highly stable binding, selectivity is less important since clinical flow-based sorting and ex vivo expansion results in a 90-95% pure Treg population; and (c) Treg selectivity is more important if tethered fusions come off the target cell and enter circulation. In that case, Treg-specificity will prevent re-binding and activation of off-target cell types.

[0533] Next, affinity-modulated IL-2 with optimized tethering domains can be combined to produce highly Treg-selective lead molecules. Optimized lead molecules can include bi-specific antibody tethering (e.g., in a design similar to FIG. 3E), which allows for increased persistence and cell type selectivity, and/or complex surface marker selectivity. Human leads and mouse surrogates can then be validated using in vitro binding, expansion and off-target mixed cell assays. Efficacy, off-target cell effects and toxicity can be validated using mouse surrogates in Treg-controllable disease models, e.g., Naive T cell model of colitis (IBD model). Expansion, survival, engraftment and homing of final human molecule can be validated in the NSG human intestinal graft model.

[0534] Without wishing to be bound by theory, it is believed that the mono-specific TFs bind through a single, non-specific targeting motif (FIG. 25, upper panel). IL-2

signaling occurs primarily on loaded cells (1). However, trans signaling (2), dissociation and soluble signaling (3) and rebinding and signaling (4) result in low levels of off-target cell activation. As shown in FIG. 25, lower panel, by increasing antibody targeting potency and specificity (e.g., by using a bi-valent or multi-specific binding moiety), as well as reducing IL-2 affinity for its receptor, loaded cells can continue to receive maximal IL-2 signal while eliminating off-target effects. This can result in specific survival, expansion and engraftment of transferred Tregs, leading to improved outcomes for patients.

[0535] Modifications and variations of the described methods and compositions of the present disclosure will be apparent to those skilled in the art without departing from the scope and spirit of the disclosure. Although the disclosure has been described in connection with specific embodiments, it should be understood that the disclosure as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the disclosure are intended and understood by those skilled in the relevant field in which this disclosure resides to be within the scope of the disclosure as represented by the following claims.

INCORPORATION BY REFERENCE

[0536] All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference. The PCT International Application having Attorney Docket No. 174285-010900/PCT titled "POLY-NUCLEOTIDES ENCODING IMMUNOSTIMULATORY FUSION MOLECULES AND USES THEREOF" filed Jul. 3, 2018 and the PCT International Application having Attorney Docket No. 174285-010800/PCT titled "IMMUNOSTIMULATORY FUSION MOLECULES AND USES THEREOF" filed Jul. 3, 2018 are both incorporated herein by reference in its entirety.

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Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys	180	185	190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro	195	200	205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Gly Gly Gly Gly Ser Gly	210	215	220
Gly Gly Gly Ser Gly Gly Gly Gly Ser Ile Thr Cys Pro Pro Pro Met	225	230	235
Ser Val Glu His Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser	245	250	255
Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr	260	265	270
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165					170					175				
Tyr	Ser	Leu	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys
		180					185					190		
His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Pro
		195					200					205		
Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys	Gly	Gly	Gly	Ser	Gly
		210					215					220		
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asn	Trp	Val	Asn	Val	Ile
		225					230					235		240
Asp	Leu	Lys	Lys	Ile	Glu	Asp	Leu	Ile	Gln	Ser	Met	His	Ile	Asp
				245					250					255
Thr	Leu	Tyr	Thr	Glu	Ser	Asp	Val	His	Pro	Ser	Cys	Lys	Val	Thr
				260					265					270
Met	Lys	Cys	Phe	Leu	Leu	Glu	Leu	Gln	Val	Ile	Ser	Leu	Glu	Ser
				275					280					285
Asp	Ala	Ser	Ile	His	Asp	Thr	Val	Glu	Asn	Leu	Ile	Ile	Leu	Ala
				290					295					300
Asn	Ser	Leu	Ser	Ser	Asn	Gly	Asn	Val	Thr	Glu	Ser	Gly	Cys	Lys
				305					310					315
Cys	Glu	Glu	Leu	Glu	Glu	Lys	Asn	Ile	Lys	Glu	Phe	Leu	Gln	Ser
				325					330					335
Val	His	Ile	Val	Gln	Met	Phe	Ile	Asn	Thr	Ser				
				340					345					

<210> SEQ ID NO 4
 <211> LENGTH: 347
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 4

Asn	Trp	Val	Asn	Val	Ile	Ser	Asp	Leu	Lys	Lys	Ile	Glu	Asp	Leu	Ile
1				5					10					15	
Gln	Ser	Met	His	Ile	Asp	Ala	Thr	Leu	Tyr	Thr	Glu	Ser	Asp	Val	His
			20					25					30		
Pro	Ser	Cys	Lys	Val	Thr	Ala	Met	Lys	Cys	Phe	Leu	Leu	Glu	Leu	Gln
			35				40						45		
Val	Ile	Ser	Leu	Glu	Ser	Gly	Asp	Ala	Ser	Ile	His	Asp	Thr	Val	Glu
			50			55					60				
Asn	Leu	Ile	Ile	Leu	Ala	Asn	Asn	Ser	Leu	Ser	Ser	Asn	Gly	Asn	Val
				65		70				75				80	
Thr	Glu	Ser	Gly	Cys	Lys	Glu	Cys	Glu	Glu	Leu	Glu	Glu	Lys	Asn	Ile
				85				90						95	
Lys	Glu	Phe	Leu	Gln	Ser	Phe	Val	His	Ile	Val	Gln	Met	Phe	Ile	Asn
			100					105					110		
Thr	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly
			115				120					125			
Ser	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala	Val	Ser	Leu
			130			135					140				
Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Lys	Ser	Val	Ser	Thr
			145		150				155					160	
Ser	Gly	Tyr	Ser	Tyr	Leu	His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Pro

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165					170					175					
Pro	Lys	Leu	Leu	Ile	Tyr	Leu	Ala	Ser	Asn	Leu	Glu	Ser	Gly	Val	Pro
		180						185					190		
Ala	Arg	Phe	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Asn	Ile		
		195				200					205				
His	Pro	Val	Glu	Glu	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	His	Ser
	210					215					220				
Arg	Glu	Leu	Pro	Phe	Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys
	225					230					235				240
Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu
			245						250					255	
Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe
		260						265						270	
Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln
		275					280					285			
Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser
	290					295					300				
Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu
	305					310					315				320
Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser
			325						330					335	
Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys					
			340					345							

<210> SEQ ID NO 5
 <211> LENGTH: 224
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 5

Gln	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1			5					10					15		
Ser	Leu	Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Asp	Phe	Ser	Arg	Tyr
		20						25					30		
Trp	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Ile
		35					40					45			
Gly	Glu	Ile	Asn	Pro	Thr	Ser	Ser	Thr	Ile	Asn	Phe	Thr	Pro	Ser	Leu
	50					55					60				
Lys	Asp	Lys	Val	Phe	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	Tyr
	65				70						75				80
Leu	Gln	Met	Ser	Lys	Val	Arg	Ser	Glu	Asp	Thr	Ala	Leu	Tyr	Tyr	Cys
			85					90						95	
Ala	Arg	Gly	Asn	Tyr	Tyr	Arg	Tyr	Gly	Asp	Ala	Met	Asp	Tyr	Trp	Gly
			100					105					110		
Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser
		115					120					125			
Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala
		130				135						140			
Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val
	145				150						155				160
Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala

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165					170					175					
Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val
		180						185					190		
Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His
		195					200					205			
Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys
	210					215					220				
<210> SEQ ID NO 6															
<211> LENGTH: 448															
<212> TYPE: PRT															
<213> ORGANISM: Unknown															
<220> FEATURE:															
<223> OTHER INFORMATION: Synthetic															
<400> SEQUENCE: 6															
Gln	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Asp	Phe	Ser	Arg	Tyr
		20						25					30		
Trp	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Ile
		35				40						45			
Gly	Glu	Ile	Asn	Pro	Thr	Ser	Ser	Thr	Ile	Asn	Phe	Thr	Pro	Ser	Leu
	50					55				60					
Lys	Asp	Lys	Val	Phe	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Thr	Leu	Tyr
	65				70					75					80
Leu	Gln	Met	Ser	Lys	Val	Arg	Ser	Glu	Asp	Thr	Ala	Leu	Tyr	Tyr	Cys
			85					90						95	
Ala	Arg	Gly	Asn	Tyr	Tyr	Arg	Tyr	Gly	Asp	Ala	Met	Asp	Tyr	Trp	Gly
		100						105					110		
Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser
		115					120					125			
Val	Phe	Pro	Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser	Glu	Ser	Thr	Ala
	130					135					140				
Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val
	145				150					155					160
Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala
			165						170					175	
Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val
		180						185					190		
Pro	Ser	Ser	Ser	Leu	Gly	Thr	Lys	Thr	Tyr	Thr	Cys	Asn	Val	Asp	His
		195					200					205			
Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Ser	Lys	Tyr	Gly
	210					215					220				
Pro	Pro	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Phe	Leu	Gly	Gly	Pro	Ser
	225				230					235					240
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg
			245						250					255	
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	Gln	Glu	Asp	Pro
		260						265					270		
Glu	Val	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala
		275					280					285			
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val

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290	295	300
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr		
305	310	315 320
Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr		
	325	330 335
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu		
	340	345 350
Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys		
	355	360 365
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser		
	370	375 380
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp		
385	390	395 400
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser		
	405	410 415
Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala		
	420	425 430
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys		
	435	440 445

<210> SEQ ID NO 7
 <211> LENGTH: 218
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 7

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser
20 25 30
Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45
Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80
Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ser Arg
85 90 95
Glu Leu Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg
100 105 110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro

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195	200	205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys		
210	215	
<210> SEQ ID NO 8		
<211> LENGTH: 577		
<212> TYPE: PRT		
<213> ORGANISM: Unknown		
<220> FEATURE:		
<223> OTHER INFORMATION: Synthetic		
<400> SEQUENCE: 8		
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly		
1	5	10
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr		
	20	25
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile		
	35	40
Gly Glu Ile Asn Pro Thr Ser Ser Thr Ile Asn Phe Thr Pro Ser Leu		
	50	55
Lys Asp Lys Val Phe Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr		
	65	70
Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys		
	85	90
Ala Arg Gly Asn Tyr Tyr Arg Tyr Gly Asp Ala Met Asp Tyr Trp Gly		
	100	105
Gln Gly Thr Ser Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser		
	115	120
Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala		
	130	135
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val		
	145	150
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala		
	165	170
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val		
	180	185
Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His		
	195	200
Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly		
	210	215
Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser		
	225	230
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg		
	245	250
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro		
	260	265
Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala		
	275	280
Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val		
	290	295
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr		
	305	310
Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr		
	315	320

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325					330					335					
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu
			340					345					350		
Pro	Pro	Ser	Gln	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys
		355					360					365			
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser
	370					375					380				
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp
385					390					395					400
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser
			405						410					415	
Arg	Trp	Gln	Glu	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala
		420						425					430		
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Leu	Gly	Lys
		435					440					445			
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asn
	450					455					460				
Trp	Val	Asn	Val	Ile	Ser	Asp	Leu	Lys	Lys	Ile	Glu	Asp	Leu	Ile	Gln
465					470					475					480
Ser	Met	His	Ile	Asp	Ala	Thr	Leu	Tyr	Thr	Glu	Ser	Asp	Val	His	Pro
				485					490					495	
Ser	Cys	Lys	Val	Thr	Ala	Met	Lys	Cys	Phe	Leu	Leu	Glu	Leu	Gln	Val
		500						505					510		
Ile	Ser	Leu	Glu	Ser	Gly	Asp	Ala	Ser	Ile	His	Asp	Thr	Val	Glu	Asn
		515					520					525			
Leu	Ile	Ile	Leu	Ala	Asn	Asn	Ser	Leu	Ser	Ser	Asn	Gly	Asn	Val	Thr
	530					535					540				
Glu	Ser	Gly	Cys	Lys	Glu	Cys	Glu	Glu	Leu	Glu	Glu	Lys	Asn	Ile	Lys
545					550					555					560
Glu	Phe	Leu	Gln	Ser	Phe	Val	His	Ile	Val	Gln	Met	Phe	Ile	Asn	Thr
			565						570					575	

Ser

<210> SEQ ID NO 9
 <211> LENGTH: 65
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Ile	Thr	Cys	Pro	Pro	Pro	Met	Ser	Val	Glu	His	Ala	Asp	Ile	Trp	Val
1				5					10					15	
Lys	Ser	Tyr	Ser	Leu	Tyr	Ser	Arg	Glu	Arg	Tyr	Ile	Cys	Asn	Ser	Gly
		20						25				30			
Phe	Lys	Arg	Lys	Ala	Gly	Thr	Ser	Ser	Leu	Thr	Glu	Cys	Val	Leu	Asn
	35					40					45				
Lys	Ala	Thr	Asn	Val	Ala	His	Trp	Thr	Thr	Pro	Ser	Leu	Lys	Cys	Ile
	50					55				60					

Arg
 65

<210> SEQ ID NO 10
 <211> LENGTH: 114
 <212> TYPE: PRT

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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile
1 5 10 15
Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His
20 25 30
Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln
35 40 45
Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu
50 55 60
Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val
65 70 75 80
Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile
85 90 95
Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn
100 105 110
Thr Ser

<210> SEQ ID NO 11

<211> LENGTH: 114

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 11

Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile
1 5 10 15
Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His
20 25 30
Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln
35 40 45
Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu
50 55 60
Asn Leu Ile Ile Leu Ala Asn Asp Ser Leu Ser Ser Asn Gly Asn Val
65 70 75 80
Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile
85 90 95
Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn
100 105 110
Thr Ser

<210> SEQ ID NO 12

<211> LENGTH: 297

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 12

Ile Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val
1 5 10 15
Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly
20 25 30

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Phe	Lys	Arg	Lys	Ala	Gly	Thr	Ser	Ser	Leu	Thr	Glu	Cys	Val	Ile	Asn	
		35					40					45				
Lys	Ala	Thr	Asn	Val	Ala	His	Trp	Thr	Thr	Pro	Ser	Leu	Lys	Cys	Ile	
	50					55					60					
Arg	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	
65					70					75					80	
Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	
			85						90					95		
Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	
			100					105					110			
Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	
		115					120					125				
Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	
	130					135					140					
Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	
145					150					155					160	
Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	
			165						170					175		
Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	
			180					185					190			
Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	
		195					200					205				
Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	
	210					215					220					
Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	
225					230					235					240	
Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	
			245						250					255		
Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	
			260					265					270			
Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	
		275					280					285				
Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys								
	290					295										

<210> SEQ ID NO 13

<211> LENGTH: 297

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 13

Ile	Thr	Cys	Pro	Pro	Pro	Met	Ser	Val	Glu	His	Ala	Asp	Ile	Trp	Val	
1				5					10				15			
Lys	Ser	Tyr	Ser	Leu	Tyr	Ser	Arg	Glu	Arg	Tyr	Ile	Cys	Asn	Ser	Gly	
		20						25				30				
Phe	Lys	Arg	Lys	Ala	Gly	Thr	Ser	Ser	Leu	Thr	Glu	Cys	Val	Leu	Asn	
		35					40					45				
Lys	Ala	Thr	Asn	Val	Ala	His	Trp	Thr	Thr	Pro	Ser	Leu	Lys	Cys	Ile	
	50					55					60					
Arg	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	
65					70					75					80	

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Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys
				85					90					95	
Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val
			100					105					110		
Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr
		115					120					125			
Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu
	130					135					140				
Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His
145					150					155					160
Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys
			165						170					175	
Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln
			180					185					190		
Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu
		195					200					205			
Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro
	210					215					220				
Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn
225					230					235					240
Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu
				245					250					255	
Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val
			260					265					270		
Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln
		275					280					285			
Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys							
	290					295									

<210> SEQ ID NO 14

<211> LENGTH: 220

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 14

Gln	Val	Gln	Leu	Gln	Gln	Leu	Gly	Ala	Glu	Leu	Ala	Arg	Pro	Gly	Ala
1				5					10					15	
Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr
			20					25					30		
Ser	Ile	Gln	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35				40						45			
Gly	Tyr	Ile	Asn	Pro	Ser	Ser	Gly	Tyr	Ile	Lys	Tyr	Asn	Gln	His	Phe
	50					55					60				
Arg	Asp	Arg	Ala	Thr	Leu	Thr	Ala	Asp	Arg	Ser	Ser	Ser	Thr	Ala	Tyr
65					70				75						80
Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys
			85						90					95	
Ala	Arg	Gly	Asn	Ser	Gly	Ser	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr
			100					105					110		
Leu	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu
			115				120						125		

-continued

Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys
130						135					140				
Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser
145					150					155					160
Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser
				165					170					175	
Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser
			180					185					190		
Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn
		195					200					205			
Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys				
	210					215					220				

<210> SEQ ID NO 15
 <211> LENGTH: 348
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 15

Asp	Ile	Val	Met	Thr	Gln	Ala	Ala	Pro	Ser	Val	Pro	Val	Thr	Pro	Gly
1				5					10					15	
Glu	Ser	Leu	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Lys	Ser	Leu	Leu	His	Ser
		20						25					30		
Ser	Gly	Ile	Thr	Tyr	Leu	Tyr	Trp	Phe	Leu	Gln	Arg	Pro	Gly	Gln	Ser
		35					40					45			
Pro	Gln	Leu	Leu	Ile	Tyr	Arg	Met	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro
	50					55					60				
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ala	Phe	Thr	Leu	Arg	Ile
65					70					75					80
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Met	Gln	His
			85					90						95	
Leu	Glu	Tyr	Pro	Phe	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys
		100						105					110		
Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu
		115					120					125			
Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe
	130					135					140				
Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln
145					150					155					160
Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser
			165						170					175	
Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu
			180					185					190		
Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser
		195					200					205			
Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys	Gly	Gly	Gly	Gly	Ser
	210					215					220				
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asn	Trp	Val	Asn	Val	Ile
225					230					235					240
Ser	Asp	Leu	Lys	Lys	Ile	Glu	Asp	Leu	Ile	Gln	Ser	Met	His	Ile	Asp
				245				250						255	

-continued

Ala	Thr	Leu	Tyr	Thr	Glu	Ser	Asp	Val	His	Pro	Ser	Cys	Lys	Val	Thr
		260						265					270		
Ala	Met	Lys	Cys	Phe	Leu	Leu	Glu	Leu	Gln	Val	Ile	Ser	Leu	Glu	Ser
		275					280					285			
Gly	Asp	Ala	Ser	Ile	His	Asp	Thr	Val	Glu	Asn	Leu	Ile	Ile	Leu	Ala
	290					295					300				
Asn	Asn	Ser	Leu	Ser	Ser	Asn	Gly	Asn	Val	Thr	Glu	Ser	Gly	Cys	Lys
305					310					315					320
Glu	Cys	Glu	Glu	Leu	Glu	Glu	Lys	Asn	Ile	Lys	Glu	Phe	Leu	Gln	Ser
				325					330					335	
Phe	Val	His	Ile	Val	Gln	Met	Phe	Ile	Asn	Thr	Ser				
		340						345							

<210> SEQ ID NO 16
 <211> LENGTH: 220
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 16

Gln	Val	Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Ala	Arg	Pro	Gly	Ala
1			5						10				15		
Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr
		20						25				30			
Thr	Met	Gln	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35				40						45			
Gly	Tyr	Ile	Asn	Pro	Ser	Ser	Gly	Tyr	Ile	Lys	Tyr	Asn	Gln	Lys	Phe
	50					55					60				
Lys	Asp	Lys	Val	Thr	Leu	Thr	Ala	Asp	Lys	Ser	Ser	Thr	Thr	Ala	Tyr
65					70					75					80
Met	Gln	Leu	Ser	Arg	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys
			85					90					95		
Ala	Arg	Arg	Gly	Ser	Tyr	Phe	Phe	Asp	Phe	Trp	Gly	Gln	Gly	Thr	Ser
			100					105					110		
Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu
		115					120					125			
Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys
	130					135					140				
Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser
145					150					155				160	
Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser
			165					170						175	
Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser
		180						185				190			
Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn
		195					200					205			
Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys				
	210					215					220				

<210> SEQ ID NO 17
 <211> LENGTH: 348
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:

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<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 17

Asp Ile Val Ile Thr Gln Asp Glu Leu Ser Asn Pro Val Thr Ser Gly
1 5 10 15
Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu Tyr Lys
20 25 30
Asp Gly Lys Thr Tyr Leu Asn Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Met Ser Thr Arg Ala Ser Gly Val Ser
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Glu Ile
65 70 75 80
Ser Arg Val Lys Ala Glu Asp Val Gly Val Tyr Tyr Cys Gln Gln Leu
85 90 95
Val Glu Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Val Lys
100 105 110
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125
Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140
Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145 150 155 160
Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175
Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
180 185 190
Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
195 200 205
Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Gly Gly Gly Gly Ser
210 215 220
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Trp Val Asn Val Ile
225 230 235 240
Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp
245 250 255
Ala Thr Leu Tyr Thr Glu Ser Asp Val His Pro Ser Cys Lys Val Thr
260 265 270
Ala Met Lys Cys Phe Leu Leu Glu Leu Gln Val Ile Ser Leu Glu Ser
275 280 285
Gly Asp Ala Ser Ile His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala
290 295 300
Asn Asn Ser Leu Ser Ser Asn Gly Asn Val Thr Glu Ser Gly Cys Lys
305 310 315 320
Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser
325 330 335
Phe Val His Ile Val Gln Met Phe Ile Asn Thr Ser
340 345

<210> SEQ ID NO 18

<211> LENGTH: 224

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

-continued

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 18

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn Pro Thr Ser Ser Thr Ile Asn Phe Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Val Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Asn Tyr Tyr Arg Tyr Gly Asp Ala Met Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

<210> SEQ ID NO 19

<211> LENGTH: 347

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 19

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Leu Gly
1 5 10 15
Glu Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser
20 25 30
Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
35 40 45
Lys Leu Leu Ile Tyr Leu Ala Ser Asn Arg Ala Thr Gly Val Pro Ala
50 55 60
Arg Phe Ser Gly Ser Gly Pro Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80
Ser Leu Glu Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His Ser Arg
85 90 95
Glu Leu Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105 110

-continued

Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln
	115						120					125			
Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr
	130					135					140				
Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser
145					150					155					160
Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr
			165						170					175	
Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys
		180					185						190		
His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro
	195						200					205			
Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys	Gly	Gly	Gly	Gly	Ser	Gly
	210					215					220				
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asn	Trp	Val	Asn	Val	Ile	Ser
225				230					235						240
Asp	Leu	Lys	Lys	Ile	Glu	Asp	Leu	Ile	Gln	Ser	Met	His	Ile	Asp	Ala
			245					250						255	
Thr	Leu	Tyr	Thr	Glu	Ser	Asp	Val	His	Pro	Ser	Cys	Lys	Val	Thr	Ala
		260						265					270		
Met	Lys	Cys	Phe	Leu	Leu	Glu	Leu	Gln	Val	Ile	Ser	Leu	Glu	Ser	Gly
	275						280					285			
Asp	Ala	Ser	Ile	His	Asp	Thr	Val	Glu	Asn	Leu	Ile	Ile	Leu	Ala	Asn
	290					295					300				
Asn	Ser	Leu	Ser	Ser	Asn	Gly	Asn	Val	Thr	Glu	Ser	Gly	Cys	Lys	Glu
305					310					315					320
Cys	Glu	Glu	Leu	Glu	Glu	Lys	Asn	Ile	Lys	Glu	Phe	Leu	Gln	Ser	Phe
			325					330						335	
Val	His	Ile	Val	Gln	Met	Phe	Ile	Asn	Thr	Ser					
		340					345								

<210> SEQ ID NO 20

<211> LENGTH: 224

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 20

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1			5					10					15		
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Asp	Phe	Ser	Arg	Tyr
	20						25						30		
Trp	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Ile
	35					40						45			
Gly	Glu	Ile	Asn	Pro	Thr	Ser	Ser	Thr	Ile	Asn	Phe	Ala	Asp	Ser	Val
	50					55				60					
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Ser	Leu	Tyr
65				70					75					80	
Leu	Gln	Met	Asn	Ser	Val	Arg	Asp	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
		85						90						95	
Ala	Arg	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Asp	Ala	Met	Asp	Tyr	Trp	Gly
		100						105						110	

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Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
 115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
 130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
 145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
 165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
 180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
 195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
 210 215 220

<210> SEQ ID NO 21
 <211> LENGTH: 340
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 21

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser
 20 25 30

Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
 35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
 65 70 75 80

Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ser Arg
 85 90 95

Glu Leu Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg
 100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
 195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Gly Gly Gly Ser Gly Gly
 210 215 220

Gly Ser Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp
 225 230 235 240

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<210> SEQ ID NO 22
<211> LENGTH: 344
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 22
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Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala	Val	Ser	Leu	Gly
1				5					10					15	
Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Lys	Ser	Val	Ser	Thr	Ser
			20					25					30		
Gly	Tyr	Ser	Tyr	Leu	His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Pro	Pro
		35					40					45			
Lys	Leu	Leu	Ile	Tyr	Leu	Ala	Ser	Asn	Leu	Glu	Ser	Gly	Val	Pro	Ala
	50					55					60				
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Asn	Ile	His
65					70					75					80
Pro	Val	Glu	Glu	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	His	Ser	Arg
				85					90					95	
Glu	Leu	Pro	Phe	Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg
			100					105					110		
Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln
			115				120					125			
Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr
	130					135				140					
Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser
145					150					155					160
Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr
				165				170						175	
Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys
			180					185					190		
His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro
		195					200					205			
Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys	Asp	Lys	Thr	His	Thr	Ser
					215						220				
Pro	Pro	Ser	Pro	Ala	Pro	Asn	Trp	Val	Asn	Val	Ile	Ser	Asp	Leu	Lys
225					230					235					240

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Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Asp	Phe	Ser	Arg	Tyr
			20					25					30		
Trp	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Ile
		35					40					45			
Gly	Glu	Ile	Asn	Pro	Thr	Ser	Ser	Thr	Ile	Asn	Phe	Ala	Asp	Ser	Val
	50					55					60				
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Ser	Leu	Tyr
65					70					75				80	
Leu	Gln	Met	Asn	Ser	Val	Arg	Asp	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
			85						90					95	
Ala	Arg	Gly	Asn	Tyr	Tyr	Arg	Tyr	Gly	Asp	Ala	Met	Asp	Tyr	Trp	Gly
			100					105					110		
Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser							
			115				120								

<400> SEQUENCE: 24

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser
20 25 30

Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Arg Ala Thr Gly Val Pro Ala

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50	55	60
Arg Phe Ser Gly Ser Gly	Pro Gly Thr Asp Phe	Thr Leu Thr Ile Ser
65	70	75 80
Ser Leu Glu Pro Glu Asp Phe	Ala Thr Tyr Tyr Cys	Gln His Ser Arg
	85	90 95
Glu Leu Pro Phe Thr Phe	Gly Gln Gly Thr Lys	Leu Glu Ile Lys
	100	105 110

<210> SEQ ID NO 25
 <211> LENGTH: 121
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 25

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly	
1	5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr	
	20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile	
	35 40 45
Gly Glu Ile Asn Pro Thr Ser Ser Thr Ile Asn Phe Ala Asp Ser Val	
	50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr	
	65 70 75 80
Leu Gln Met Asn Ser Val Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys	
	85 90 95
Ala Arg Gly Ser Gly Gly Gly Ser Gly Asp Ala Met Asp Tyr Trp Gly	
	100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser	
	115 120

<210> SEQ ID NO 26
 <211> LENGTH: 224
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 26

Glu Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Met Arg Pro Gly Ala	
1	5 10 15
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly His	
	20 25 30
Trp Met Asn Trp Val Arg Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile	
	35 40 45
Gly Met Ile His Pro Ser Asp Ser Glu Thr Arg Leu Asn Gln Lys Phe	
	50 55 60
Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Ser Ala Tyr	
	65 70 75 80
Met Gln Leu Ser Ser Pro Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys	
	85 90 95
Ala Arg Gly Ile Tyr Phe Tyr Gly Thr Thr Tyr Phe Asp Tyr Trp Gly	
	100 105 110

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Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser
		115					120					125			
Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala
	130					135					140				
Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val
145				150						155					160
Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala
		165						170						175	
Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val
	180						185					190			
Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His
	195					200						205			
Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys
	210					215					220				

<210> SEQ ID NO 27

<211> LENGTH: 343

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 27

Asp	Val	Gln	Ile	Thr	Gln	Ser	Pro	Ser	Tyr	Leu	Ala	Ala	Ser	Pro	Gly
1				5					10					15	
Glu	Thr	Ile	Ser	Ile	Asn	Cys	Arg	Ala	Ser	Lys	Thr	Ile	Ser	Lys	Tyr
		20					25						30		
Leu	Ala	Trp	Tyr	Gln	Glu	Lys	Pro	Gly	Lys	Thr	Asn	Lys	Leu	Leu	Ile
	35					40					45				
Tyr	Ser	Gly	Ser	Thr	Leu	Gln	Ser	Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly
	50				55					60					
Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Glu	Pro
65				70					75					80	
Glu	Asp	Phe	Ala	Met	Tyr	Tyr	Cys	Gln	Gln	His	Asn	Glu	Tyr	Pro	Leu
		85						90					95		
Thr	Phe	Gly	Thr	Gly	Thr	Lys	Leu	Glu	Leu	Lys	Arg	Thr	Val	Ala	Ala
		100					105						110		
Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly
	115					120					125				
Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala
	130				135						140				
Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln
145				150					155					160	
Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser
		165						170					175		
Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr
		180					185						190		
Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser
	195					200					205				
Phe	Asn	Arg	Gly	Glu	Cys	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser
210					215						220				
Gly	Gly	Gly	Gly	Ser	Asn	Trp	Val	Asn	Val	Ile	Ser	Asp	Leu	Lys	Lys
225				230					235					240	

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Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr
      245                      250                      255

Glu Ser Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe
      260                      265                      270

Leu Leu Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile
      275                      280                      285

His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser
      290                      295                      300

Ser Asn Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu
      305                      310                      315                      320

Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val
      325                      330                      335

Gln Met Phe Ile Asn Thr Ser
      340

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<210> SEQ ID NO 28
<211> LENGTH: 223
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

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<400> SEQUENCE: 28

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Asp Val Lys Leu Val Glu Ser Gly Gly Asp Leu Val Lys Leu Gly Gly
1      5      10      15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
      20      25      30

Tyr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Leu Val
      35      40      45

Ala Ala Ile Asp Asn Asp Gly Gly Ser Ile Ser Tyr Pro Asp Thr Val
      50      55      60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
      65      70      75      80

Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
      85      90      95

Ala Arg Gln Gly Arg Leu Arg Arg Asp Tyr Phe Asp Tyr Trp Gly Gln
      100     105     110

Gly Thr Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
      115     120     125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
      130     135     140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
      145     150     155     160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
      165     170     175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
      180     185     190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
      195     200     205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
      210     215     220

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<210> SEQ ID NO 29
<211> LENGTH: 347

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<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 29

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1           5           10          15
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr
          20           25          30
Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
          35           40          45
Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Ile Pro Ala
          50           55          60
Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Asn
65           70           75          80
Pro Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys Gln Gln Ser Asn
          85           90          95
Glu Asp Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
          100          105          110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
          115          120          125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
          130          135          140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145          150          155          160
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
          165          170          175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
          180          185          190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
          195          200          205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Gly Gly Gly Gly Ser Gly
210          215          220
Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Trp Val Asn Val Ile Ser
225          230          235          240
Asp Leu Lys Lys Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp Ala
          245          250          255
Thr Leu Tyr Thr Glu Ser Asp Val His Pro Ser Cys Lys Val Thr Ala
260          265          270
Met Lys Cys Phe Leu Leu Glu Leu Gln Val Ile Ser Leu Glu Ser Gly
          275          280          285
Asp Ala Ser Ile His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn
290          295          300
Asn Ser Leu Ser Ser Asn Gly Asn Val Thr Glu Ser Gly Cys Lys Glu
305          310          315          320
Cys Glu Glu Leu Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe
          325          330          335
Val His Ile Val Gln Met Phe Ile Asn Thr Ser
          340          345

<210> SEQ ID NO 30
<211> LENGTH: 221

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<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 30
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
1          5          10          15
Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr
20          25          30
Tyr Ile His Phe Val Arg Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
35          40          45
Gly Arg Ile Asp Pro Ala Asn Asp Asn Thr Leu Tyr Ala Ser Lys Phe
50          55          60
Gln Gly Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
65          70          75          80
Met His Leu Ser Ser Leu Thr Ser Gly Asp Thr Ala Val Tyr Tyr Cys
85          90          95
Gly Arg Gly Tyr Gly Tyr Tyr Val Phe Asp His Trp Gly Gln Gly Thr
100         105         110
Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115         120         125
Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
130         135         140
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145         150         155         160
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165         170         175
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180         185         190
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195         200         205
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210         215         220

<210> SEQ ID NO 31
<211> LENGTH: 343
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 31
Asp Val Gln Ile Asn Gln Ser Pro Ser Phe Leu Ala Ala Ser Pro Gly
1          5          10          15
Glu Thr Ile Thr Ile Asn Cys Arg Thr Ser Arg Ser Ile Ser Gln Tyr
20          25          30
Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys Leu Leu Ile
35          40          45
Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe Ser Gly
50          55          60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Gly Leu Glu Pro
65          70          75          80
Glu Asp Phe Ala Met Tyr Tyr Cys Gln Gln His Asn Glu Asn Pro Leu
85          90          95

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Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
 145 150 155 160
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190
 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205
 Phe Asn Arg Gly Glu Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 210 215 220
 Gly Gly Gly Gly Ser Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys
 225 230 235 240
 Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr
 245 250 255
 Glu Ser Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe
 260 265 270
 Leu Leu Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile
 275 280 285
 His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser
 290 295 300
 Ser Asn Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu
 305 310 315 320
 Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val
 325 330 335
 Gln Met Phe Ile Asn Thr Ser
 340

<210> SEQ ID NO 32
 <211> LENGTH: 380
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 32

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
 20 25 30
 Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45
 Gly Glu Ile Asn Pro Thr Ser Ser Thr Ile Asn Phe Thr Pro Ser Leu
 50 55 60
 Lys Asp Lys Val Phe Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95

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Ala Arg Gly Asn Tyr Tyr Arg Tyr Gly Asp Ala Met Asp Tyr Trp Gly
 100 105 110
 Gln Gly Thr Ser Val Thr Val Ser Gly Gly Gly Gly Ser Gly Gly Gly
 115 120 125
 Gly Ser Gly Gly Gly Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala
 130 135 140
 Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala
 145 150 155 160
 Ser Lys Ser Val Ser Thr Ser Gly Tyr Ser Tyr Leu His Trp Tyr Gln
 165 170 175
 Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn
 180 185 190
 Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr
 195 200 205
 Asp Phe Thr Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr
 210 215 220
 Tyr Tyr Cys Gln His Ser Arg Glu Leu Pro Phe Thr Phe Gly Ser Gly
 225 230 235 240
 Thr Lys Leu Glu Ile Lys Arg Ser Gly Ser Gly Gly Gly Gly Ser Leu
 245 250 255
 Gln Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu
 260 265 270
 Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val
 275 280 285
 His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu
 290 295 300
 Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val
 305 310 315 320
 Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn
 325 330 335
 Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn
 340 345 350
 Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile
 355 360 365
 Asn Thr Ser Ala Ala Ala His His His His His His
 370 375 380

<210> SEQ ID NO 33
 <211> LENGTH: 22
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 33

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
 1 5 10 15

Leu Pro Gly Ala Lys Cys
 20

<210> SEQ ID NO 34
 <211> LENGTH: 19
 <212> TYPE: PRT
 <213> ORGANISM: Unknown

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 34

Met Glu Leu Gly Leu Cys Trp Val Phe Leu Val Ala Ile Leu Glu Gly
1 5 10 15

Val Gln Cys

<210> SEQ ID NO 35
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 35

Met Ala Pro Arg Arg Ala Arg Gly Cys Arg Thr Leu Gly Leu Pro Ala
1 5 10 15

Leu Leu Leu Leu Leu Leu Leu Arg Pro Pro Ala Thr Arg Gly
20 25 30

<210> SEQ ID NO 36
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 36

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 37
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 37

Gly Gly Gly Ser Gly Gly Gly Ser
1 5

<210> SEQ ID NO 38
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 38

Asp Lys Thr His Thr Ser Pro Pro Ser Pro Ala Pro
1 5 10

<210> SEQ ID NO 39
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 39

Arg Ser Gly Ser Gly Gly Gly Ser Leu Gln

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1	5	10
<210> SEQ ID NO 40		
<211> LENGTH: 162		
<212> TYPE: PRT		
<213> ORGANISM: Homo sapiens		
<400> SEQUENCE: 40		
Met Arg Ile Ser Lys Pro His Leu Arg Ser Ile Ser Ile Gln Cys Tyr		
1	5	10 15
Leu Cys Leu Leu Leu Asn Ser His Phe Leu Thr Glu Ala Gly Ile His		
	20	25 30
Val Phe Ile Leu Gly Cys Phe Ser Ala Gly Leu Pro Lys Thr Glu Ala		
	35	40 45
Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile		
	50	55 60
Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His		
	65	70 75 80
Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln		
	85	90 95
Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu		
	100	105 110
Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val		
	115	120 125
Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile		
	130	135 140
Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn		
	145	150 155 160
Thr Ser		

<210> SEQ ID NO 41		
<211> LENGTH: 267		
<212> TYPE: PRT		
<213> ORGANISM: Homo sapiens		
<400> SEQUENCE: 41		
Met Ala Pro Arg Arg Ala Arg Gly Cys Arg Thr Leu Gly Leu Pro Ala		
1	5	10 15
Leu Leu Leu Leu Leu Leu Arg Pro Ala Thr Arg Gly Ile Thr		
	20	25 30
Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser		
	35	40 45
Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys		
	50	55 60
Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala		
	65	70 75 80
Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg Asp		
	85	90 95
Pro Ala Leu Val His Gln Arg Pro Ala Pro Pro Ser Thr Val Thr Thr		
	100	105 110
Ala Gly Val Thr Pro Gln Pro Glu Ser Leu Ser Pro Ser Gly Lys Glu		
	115	120 125
Pro Ala Ala Ser Ser Pro Ser Ser Asn Asn Thr Ala Ala Thr Thr Ala		
	130	135 140

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Ala Ile Val Pro Gly Ser Gln Leu Met Pro Ser Lys Ser Pro Ser Thr
 145 150 155 160

Gly Thr Thr Glu Ile Ser Ser His Glu Ser Ser His Gly Thr Pro Ser
 165 170 175

Gln Thr Thr Ala Lys Asn Trp Glu Leu Thr Ala Ser Ala Ser His Gln
 180 185 190

Pro Pro Gly Val Tyr Pro Gln Gly His Ser Asp Thr Thr Val Ala Ile
 195 200 205

Ser Thr Ser Thr Val Leu Leu Cys Gly Leu Ser Ala Val Ser Leu Leu
 210 215 220

Ala Cys Tyr Leu Lys Ser Arg Gln Thr Pro Pro Leu Ala Ser Val Glu
 225 230 235 240

Met Glu Ala Met Glu Ala Leu Pro Val Thr Trp Gly Thr Ser Ser Arg
 245 250 255

Asp Glu Asp Leu Glu Asn Cys Ser His His Leu
 260 265

<210> SEQ ID NO 42
 <211> LENGTH: 177
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 42

Met Phe His Val Ser Phe Arg Tyr Ile Phe Gly Leu Pro Pro Leu Ile
 1 5 10 15

Leu Val Leu Leu Pro Val Ala Ser Ser Asp Cys Asp Ile Glu Gly Lys
 20 25 30

Asp Gly Lys Gln Tyr Glu Ser Val Leu Met Val Ser Ile Asp Gln Leu
 35 40 45

Leu Asp Ser Met Lys Glu Ile Gly Ser Asn Cys Leu Asn Asn Glu Phe
 50 55 60

Asn Phe Phe Lys Arg His Ile Cys Asp Ala Asn Lys Glu Gly Met Phe
 65 70 75 80

Leu Phe Arg Ala Ala Arg Lys Leu Arg Gln Phe Leu Lys Met Asn Ser
 85 90 95

Thr Gly Asp Phe Asp Leu His Leu Leu Lys Val Ser Glu Gly Thr Thr
 100 105 110

Ile Leu Leu Asn Cys Thr Gly Gln Val Lys Gly Arg Lys Pro Ala Ala
 115 120 125

Leu Gly Glu Ala Gln Pro Thr Lys Ser Leu Glu Glu Asn Lys Ser Leu
 130 135 140

Lys Glu Gln Lys Lys Leu Asn Asp Leu Cys Phe Leu Lys Arg Leu Leu
 145 150 155 160

Gln Glu Ile Lys Thr Cys Trp Asn Lys Ile Leu Met Gly Thr Lys Glu
 165 170 175

His

<210> SEQ ID NO 43
 <211> LENGTH: 152
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 43

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Asp	Cys	Asp	Ile	Glu	Gly	Lys	Asp	Gly	Lys	Gln	Tyr	Glu	Ser	Val	Leu
1				5					10					15	
Met	Val	Ser	Ile	Asp	Gln	Leu	Leu	Asp	Ser	Met	Lys	Glu	Ile	Gly	Ser
			20					25					30		
Asn	Cys	Leu	Asn	Asn	Glu	Phe	Asn	Phe	Phe	Lys	Arg	His	Ile	Cys	Asp
		35					40					45			
Ala	Asn	Lys	Glu	Gly	Met	Phe	Leu	Phe	Arg	Ala	Ala	Arg	Lys	Leu	Arg
	50					55					60				
Gln	Phe	Leu	Lys	Met	Asn	Ser	Thr	Gly	Asp	Phe	Asp	Leu	His	Leu	Leu
65					70				75					80	
Lys	Val	Ser	Glu	Gly	Thr	Thr	Ile	Leu	Leu	Asn	Cys	Thr	Gly	Gln	Val
			85					90						95	
Lys	Gly	Arg	Lys	Pro	Ala	Ala	Leu	Gly	Glu	Ala	Gln	Pro	Thr	Lys	Ser
			100					105					110		
Leu	Glu	Glu	Asn	Lys	Ser	Leu	Lys	Glu	Gln	Lys	Lys	Leu	Asn	Asp	Leu
		115					120					125			
Cys	Phe	Leu	Lys	Arg	Leu	Leu	Gln	Glu	Ile	Lys	Thr	Cys	Trp	Asn	Lys
	130					135					140				
Ile	Leu	Met	Gly	Thr	Lys	Glu	His								
145					150										

<210> SEQ ID NO 44

<211> LENGTH: 162

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 44

Met	Arg	Ser	Ser	Pro	Gly	Asn	Met	Glu	Arg	Ile	Val	Ile	Cys	Leu	Met
1				5					10					15	
Val	Ile	Phe	Leu	Gly	Thr	Leu	Val	His	Lys	Ser	Ser	Ser	Gln	Gly	Gln
			20					25					30		
Asp	Arg	His	Met	Ile	Arg	Met	Arg	Gln	Leu	Ile	Asp	Ile	Val	Asp	Gln
		35				40						45			
Leu	Lys	Asn	Tyr	Val	Asn	Asp	Leu	Val	Pro	Glu	Phe	Leu	Pro	Ala	Pro
	50				55					60					
Glu	Asp	Val	Glu	Thr	Asn	Cys	Glu	Trp	Ser	Ala	Phe	Ser	Cys	Phe	Gln
65					70				75					80	
Lys	Ala	Gln	Leu	Lys	Ser	Ala	Asn	Thr	Gly	Asn	Asn	Glu	Arg	Ile	Ile
			85					90					95		
Asn	Val	Ser	Ile	Lys	Lys	Leu	Lys	Arg	Lys	Pro	Pro	Ser	Thr	Asn	Ala
		100					105						110		
Gly	Arg	Arg	Gln	Lys	His	Arg	Leu	Thr	Cys	Pro	Ser	Cys	Asp	Ser	Tyr
	115					120						125			
Glu	Lys	Lys	Pro	Pro	Lys	Glu	Phe	Leu	Glu	Arg	Phe	Lys	Ser	Leu	Leu
	130				135					140					
Gln	Lys	Met	Ile	His	Gln	His	Leu	Ser	Ser	Arg	Thr	His	Gly	Ser	Glu
145				150						155				160	

Asp Ser

<210> SEQ ID NO 45

<211> LENGTH: 132

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

-continued

<400> SEQUENCE: 45

Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile Asp Ile Val
 1 5 10 15
 Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu Phe Leu Pro
 20 25 30
 Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala Phe Ser Cys
 35 40 45
 Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn Asn Glu Arg
 50 55 60
 Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Ser Thr
 65 70 75 80
 Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro Ser Cys Asp
 85 90 95
 Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe Lys Ser
 100 105 110
 Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr His Gly
 115 120 125
 Ser Glu Asp Ser
 130

<210> SEQ ID NO 46

<211> LENGTH: 219

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 46

Met Cys Pro Ala Arg Ser Leu Leu Leu Val Ala Thr Leu Val Leu Leu
 1 5 10 15
 Asp His Leu Ser Leu Ala Arg Asn Leu Pro Val Ala Thr Pro Asp Pro
 20 25 30
 Gly Met Phe Pro Cys Leu His His Ser Gln Asn Leu Leu Arg Ala Val
 35 40 45
 Ser Asn Met Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys
 50 55 60
 Thr Ser Glu Glu Ile Asp His Glu Asp Ile Thr Lys Asp Lys Thr Ser
 65 70 75 80
 Thr Val Glu Ala Cys Leu Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys
 85 90 95
 Leu Asn Ser Arg Glu Thr Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala
 100 105 110
 Ser Arg Lys Thr Ser Phe Met Met Ala Leu Cys Leu Ser Ser Ile Tyr
 115 120 125
 Glu Asp Leu Lys Met Tyr Gln Val Glu Phe Lys Thr Met Asn Ala Lys
 130 135 140
 Leu Leu Met Asp Pro Lys Arg Gln Ile Phe Leu Asp Gln Asn Met Leu
 145 150 155 160
 Ala Val Ile Asp Glu Leu Met Gln Ala Leu Asn Phe Asn Ser Glu Thr
 165 170 175
 Val Pro Gln Lys Ser Ser Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys
 180 185 190
 Ile Lys Leu Cys Ile Leu Leu His Ala Phe Arg Ile Arg Ala Val Thr
 195 200 205

-continued

Ile Asp Arg Val Met Ser Tyr Leu Asn Ala Ser
210 215

<210> SEQ ID NO 47
 <211> LENGTH: 197
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 47

Arg Asn Leu Pro Val Ala Thr Pro Asp Pro Gly Met Phe Pro Cys Leu
1 5 10 15
 His His Ser Gln Asn Leu Leu Arg Ala Val Ser Asn Met Leu Gln Lys
20 25 30
 Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys Thr Ser Glu Glu Ile Asp
35 40 45
 His Glu Asp Ile Thr Lys Asp Lys Thr Ser Thr Val Glu Ala Cys Leu
50 55 60
 Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys Leu Asn Ser Arg Glu Thr
65 70 75 80
 Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala Ser Arg Lys Thr Ser Phe
85 90 95
 Met Met Ala Leu Cys Leu Ser Ser Ile Tyr Glu Asp Leu Lys Met Tyr
100 105 110
 Gln Val Glu Phe Lys Thr Met Asn Ala Lys Leu Leu Met Asp Pro Lys
115 120 125
 Arg Gln Ile Phe Leu Asp Gln Asn Met Leu Ala Val Ile Asp Glu Leu
130 135 140
 Met Gln Ala Leu Asn Phe Asn Ser Glu Thr Val Pro Gln Lys Ser Ser
145 150 155 160
 Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys Ile Lys Leu Cys Ile Leu
165 170 175
 Leu His Ala Phe Arg Ile Arg Ala Val Thr Ile Asp Arg Val Met Ser
180 185 190
 Tyr Leu Asn Ala Ser
195

<210> SEQ ID NO 48
 <211> LENGTH: 328
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 48

Met Cys His Gln Gln Leu Val Ile Ser Trp Phe Ser Leu Val Phe Leu
1 5 10 15
 Ala Ser Pro Leu Val Ala Ile Trp Glu Leu Lys Lys Asp Val Tyr Val
20 25 30
 Val Glu Leu Asp Trp Tyr Pro Asp Ala Pro Gly Glu Met Val Val Leu
35 40 45
 Thr Cys Asp Thr Pro Glu Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln
50 55 60
 Ser Ser Glu Val Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys
65 70 75 80
 Glu Phe Gly Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val
85 90 95

-continued

Leu	Ser	His	Ser	Leu	Leu	Leu	His	Lys	Lys	Glu	Asp	Gly	Ile	Trp
			100				105					110		
Ser	Thr	Asp	Ile	Leu	Lys	Asp	Gln	Lys	Glu	Pro	Lys	Asn	Lys	Thr
		115					120					125		Phe
Leu	Arg	Cys	Glu	Ala	Lys	Asn	Tyr	Ser	Gly	Arg	Phe	Thr	Cys	Trp
	130					135					140			Trp
Leu	Thr	Thr	Ile	Ser	Thr	Asp	Leu	Thr	Phe	Ser	Val	Lys	Ser	Arg
145					150					155				160
Gly	Ser	Ser	Asp	Pro	Gln	Gly	Val	Thr	Cys	Gly	Ala	Ala	Thr	Leu
				165					170					175
Ala	Glu	Arg	Val	Arg	Gly	Asp	Asn	Lys	Glu	Tyr	Glu	Tyr	Ser	Val
			180					185					190	Glu
Cys	Gln	Glu	Asp	Ser	Ala	Cys	Pro	Ala	Ala	Glu	Glu	Ser	Leu	Pro
		195					200					205		Ile
Glu	Val	Met	Val	Asp	Ala	Val	His	Lys	Leu	Lys	Tyr	Glu	Asn	Tyr
	210					215					220			Thr
Ser	Ser	Phe	Phe	Ile	Arg	Asp	Ile	Ile	Lys	Pro	Asp	Pro	Pro	Lys
225					230					235				240
Leu	Gln	Leu	Lys	Pro	Leu	Lys	Asn	Ser	Arg	Gln	Val	Glu	Val	Ser
				245					250					255
Glu	Tyr	Pro	Asp	Thr	Trp	Ser	Thr	Pro	His	Ser	Tyr	Phe	Ser	Leu
			260					265					270	Thr
Phe	Cys	Val	Gln	Val	Gln	Gly	Lys	Ser	Lys	Arg	Glu	Lys	Lys	Asp
		275					280					285		Arg
Val	Phe	Thr	Asp	Lys	Thr	Ser	Ala	Thr	Val	Ile	Cys	Arg	Lys	Asn
	290					295					300			Ala
Ser	Ile	Ser	Val	Arg	Ala	Gln	Asp	Arg	Tyr	Tyr	Ser	Ser	Ser	Trp
305					310					315				320
Glu	Trp	Ala	Ser	Val	Pro	Cys	Ser							
				325										

<210> SEQ ID NO 49

<211> LENGTH: 306

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 49

Ile	Trp	Glu	Leu	Lys	Lys	Asp	Val	Tyr	Val	Val	Glu	Leu	Asp	Trp	Tyr
1			5					10					15		
Pro	Asp	Ala	Pro	Gly	Glu	Met	Val	Val	Leu	Thr	Cys	Asp	Thr	Pro	Glu
		20					25						30		
Glu	Asp	Gly	Ile	Thr	Trp	Thr	Leu	Asp	Gln	Ser	Ser	Glu	Val	Leu	Gly
	35						40					45			
Ser	Gly	Lys	Thr	Leu	Thr	Ile	Gln	Val	Lys	Glu	Phe	Gly	Asp	Ala	Gly
	50					55					60				
Gln	Tyr	Thr	Cys	His	Lys	Gly	Gly	Glu	Val	Leu	Ser	His	Ser	Leu	Leu
65					70					75				80	
Leu	Leu	His	Lys	Lys	Glu	Asp	Gly	Ile	Trp	Ser	Thr	Asp	Ile	Leu	Lys
			85					90						95	
Asp	Gln	Lys	Glu	Pro	Lys	Asn	Lys	Thr	Phe	Leu	Arg	Cys	Glu	Ala	Lys
			100					105					110		
Asn	Tyr	Ser	Gly	Arg	Phe	Thr	Cys	Trp	Trp	Leu	Thr	Thr	Ile	Ser	Thr
		115						120					125		

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Asp Leu Thr Phe Ser Val Lys Ser Ser Arg Gly Ser Ser Asp Pro Gln
 130 135 140
 Gly Val Thr Cys Gly Ala Ala Thr Leu Ser Ala Glu Arg Val Arg Gly
 145 150 155 160
 Asp Asn Lys Glu Tyr Glu Tyr Ser Val Glu Cys Gln Glu Asp Ser Ala
 165 170 175
 Cys Pro Ala Ala Glu Glu Ser Leu Pro Ile Glu Val Met Val Asp Ala
 180 185 190
 Val His Lys Leu Lys Tyr Glu Asn Tyr Thr Ser Ser Phe Phe Ile Arg
 195 200 205
 Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu Gln Leu Lys Pro Leu
 210 215 220
 Lys Asn Ser Arg Gln Val Glu Val Ser Trp Glu Tyr Pro Asp Thr Trp
 225 230 235 240
 Ser Thr Pro His Ser Tyr Phe Ser Leu Thr Phe Cys Val Gln Val Gln
 245 250 255
 Gly Lys Ser Lys Arg Glu Lys Lys Asp Arg Val Phe Thr Asp Lys Thr
 260 265 270
 Ser Ala Thr Val Ile Cys Arg Lys Asn Ala Ser Ile Ser Val Arg Ala
 275 280 285
 Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser Glu Trp Ala Ser Val Pro
 290 295 300
 Cys Ser
 305

<210> SEQ ID NO 50
 <211> LENGTH: 519
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 50

Ile Trp Glu Leu Lys Lys Asp Val Tyr Val Val Glu Leu Asp Trp Tyr
 1 5 10 15
 Pro Asp Ala Pro Gly Glu Met Val Val Leu Thr Cys Asp Thr Pro Glu
 20 25 30
 Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln Ser Ser Glu Val Leu Gly
 35 40 45
 Ser Gly Lys Thr Leu Thr Ile Gln Val Lys Glu Phe Gly Asp Ala Gly
 50 55 60
 Gln Tyr Thr Cys His Lys Gly Gly Glu Val Leu Ser His Ser Leu Leu
 65 70 75 80
 Leu Leu His Lys Lys Glu Asp Gly Ile Trp Ser Thr Asp Ile Leu Lys
 85 90 95
 Asp Gln Lys Glu Pro Lys Asn Lys Thr Phe Leu Arg Cys Glu Ala Lys
 100 105 110
 Asn Tyr Ser Gly Arg Phe Thr Cys Trp Trp Leu Thr Thr Ile Ser Thr
 115 120 125
 Asp Leu Thr Phe Ser Val Lys Ser Ser Arg Gly Ser Ser Asp Pro Gln
 130 135 140
 Gly Val Thr Cys Gly Ala Ala Thr Leu Ser Ala Glu Arg Val Arg Gly
 145 150 155 160

-continued

Asp	Asn	Lys	Glu	Tyr	Glu	Tyr	Ser	Val	Glu	Cys	Gln	Glu	Asp	Ser	Ala	
			165						170					175		
Cys	Pro	Ala	Ala	Glu	Glu	Ser	Leu	Pro	Ile	Glu	Val	Met	Val	Asp	Ala	
		180						185					190			
Val	His	Lys	Leu	Lys	Tyr	Glu	Asn	Tyr	Thr	Ser	Ser	Phe	Phe	Ile	Arg	
	195						200					205				
Asp	Ile	Ile	Lys	Pro	Asp	Pro	Pro	Lys	Asn	Leu	Gln	Leu	Lys	Pro	Leu	
	210				215						220					
Lys	Asn	Ser	Arg	Gln	Val	Glu	Val	Ser	Trp	Glu	Tyr	Pro	Asp	Thr	Trp	
225				230						235					240	
Ser	Thr	Pro	His	Ser	Tyr	Phe	Ser	Leu	Thr	Phe	Cys	Val	Gln	Val	Gln	
			245					250						255		
Gly	Lys	Ser	Lys	Arg	Glu	Lys	Lys	Asp	Arg	Val	Phe	Thr	Asp	Lys	Thr	
			260					265					270			
Ser	Ala	Thr	Val	Ile	Cys	Arg	Lys	Asn	Ala	Ser	Ile	Ser	Val	Arg	Ala	
	275						280					285				
Gln	Asp	Arg	Tyr	Tyr	Ser	Ser	Ser	Trp	Ser	Glu	Trp	Ala	Ser	Val	Pro	
	290				295						300					
Cys	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	
305				310						315				320		
Gly	Ser	Arg	Asn	Leu	Pro	Val	Ala	Thr	Pro	Asp	Pro	Gly	Met	Phe	Pro	
			325					330					335			
Cys	Leu	His	His	Ser	Gln	Asn	Leu	Leu	Arg	Ala	Val	Ser	Asn	Met	Leu	
		340					345						350			
Gln	Lys	Ala	Arg	Gln	Thr	Leu	Glu	Phe	Tyr	Pro	Cys	Thr	Ser	Glu	Glu	
	355					360					365					
Ile	Asp	His	Glu	Asp	Ile	Thr	Lys	Asp	Lys	Thr	Ser	Thr	Val	Glu	Ala	
	370				375					380						
Cys	Leu	Pro	Leu	Glu	Leu	Thr	Lys	Asn	Glu	Ser	Cys	Leu	Asn	Ser	Arg	
385				390					395						400	
Glu	Thr	Ser	Phe	Ile	Thr	Asn	Gly	Ser	Cys	Leu	Ala	Ser	Arg	Lys	Thr	
			405				410						415			
Ser	Phe	Met	Met	Ala	Leu	Cys	Leu	Ser	Ser	Ile	Tyr	Glu	Asp	Leu	Lys	
		420					425					430				
Met	Tyr	Gln	Val	Glu	Phe	Lys	Thr	Met	Asn	Ala	Lys	Leu	Leu	Met	Asp	
	435						440					445				
Pro	Lys	Arg	Gln	Ile	Phe	Leu	Asp	Gln	Asn	Met	Leu	Ala	Val	Ile	Asp	
	450				455						460					
Glu	Leu	Met	Gln	Ala	Leu	Asn	Phe	Asn	Ser	Glu	Thr	Val	Pro	Gln	Lys	
465				470					475					480		
Ser	Ser	Leu	Glu	Glu	Pro	Asp	Phe	Tyr	Lys	Thr	Lys	Ile	Lys	Leu	Cys	
			485				490					495				
Ile	Leu	Leu	His	Ala	Phe	Arg	Ile	Arg	Ala	Val	Thr	Ile	Asp	Arg	Val	
		500					505					510				
Met	Ser	Tyr	Leu	Asn	Ala	Ser										
		515														

<210> SEQ ID NO 51
 <211> LENGTH: 519
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:

-continued

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 51

Arg	Asn	Leu	Pro	Val	Ala	Thr	Pro	Asp	Pro	Gly	Met	Phe	Pro	Cys	Leu	1	5	10	15
His	His	Ser	Gln	Asn	Leu	Leu	Arg	Ala	Val	Ser	Asn	Met	Leu	Gln	Lys	20	25	30	
Ala	Arg	Gln	Thr	Leu	Glu	Phe	Tyr	Pro	Cys	Thr	Ser	Glu	Glu	Ile	Asp	35	40	45	
His	Glu	Asp	Ile	Thr	Lys	Asp	Lys	Thr	Ser	Thr	Val	Glu	Ala	Cys	Leu	50	55	60	
Pro	Leu	Glu	Leu	Thr	Lys	Asn	Glu	Ser	Cys	Leu	Asn	Ser	Arg	Glu	Thr	65	70	75	80
Ser	Phe	Ile	Thr	Asn	Gly	Ser	Cys	Leu	Ala	Ser	Arg	Lys	Thr	Ser	Phe	85	90	95	
Met	Met	Ala	Leu	Cys	Leu	Ser	Ser	Ile	Tyr	Glu	Asp	Leu	Lys	Met	Tyr	100	105	110	
Gln	Val	Glu	Phe	Lys	Thr	Met	Asn	Ala	Lys	Leu	Leu	Met	Asp	Pro	Lys	115	120	125	
Arg	Gln	Ile	Phe	Leu	Asp	Gln	Asn	Met	Leu	Ala	Val	Ile	Asp	Glu	Leu	130	135	140	
Met	Gln	Ala	Leu	Asn	Phe	Asn	Ser	Glu	Thr	Val	Pro	Gln	Lys	Ser	Ser	145	150	155	160
Leu	Glu	Glu	Pro	Asp	Phe	Tyr	Lys	Thr	Lys	Ile	Lys	Leu	Cys	Ile	Leu	165	170	175	
Leu	His	Ala	Phe	Arg	Ile	Arg	Ala	Val	Thr	Ile	Asp	Arg	Val	Met	Ser	180	185	190	
Tyr	Leu	Asn	Ala	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	195	200	205	
Ser	Gly	Gly	Gly	Ser	Ile	Trp	Glu	Leu	Lys	Lys	Asp	Val	Tyr	Val	Val	210	215	220	
Glu	Leu	Asp	Trp	Tyr	Pro	Asp	Ala	Pro	Gly	Glu	Met	Val	Val	Leu	Thr	225	230	235	240
Cys	Asp	Thr	Pro	Glu	Glu	Asp	Gly	Ile	Thr	Trp	Thr	Leu	Asp	Gln	Ser	245	250	255	
Ser	Glu	Val	Leu	Gly	Ser	Gly	Lys	Thr	Leu	Thr	Ile	Gln	Val	Lys	Glu	260	265	270	
Phe	Gly	Asp	Ala	Gly	Gln	Tyr	Thr	Cys	His	Lys	Gly	Gly	Glu	Val	Leu	275	280	285	
Ser	His	Ser	Leu	Leu	Leu	Leu	His	Lys	Lys	Glu	Asp	Gly	Ile	Trp	Ser	290	295	300	
Thr	Asp	Ile	Leu	Lys	Asp	Gln	Lys	Glu	Pro	Lys	Asn	Lys	Thr	Phe	Leu	305	310	315	320
Arg	Cys	Glu	Ala	Lys	Asn	Tyr	Ser	Gly	Arg	Phe	Thr	Cys	Trp	Trp	Leu	325	330	335	
Thr	Thr	Ile	Ser	Thr	Asp	Leu	Thr	Phe	Ser	Val	Lys	Ser	Ser	Arg	Gly	340	345	350	
Ser	Ser	Asp	Pro	Gln	Gly	Val	Thr	Cys	Gly	Ala	Ala	Thr	Leu	Ser	Ala	355	360	365	
Glu	Arg	Val	Arg	Gly	Asp	Asn	Lys	Glu	Tyr	Glu	Tyr	Ser	Val	Glu	Cys	370	375	380	

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Gln Glu Asp Ser Ala Cys Pro Ala Ala Glu Glu Ser Leu Pro Ile Glu
385 390 395 400

Val Met Val Asp Ala Val His Lys Leu Lys Tyr Glu Asn Tyr Thr Ser
405 410 415

Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu
420 425 430

Gln Leu Lys Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser Trp Glu
435 440 445

Tyr Pro Asp Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Thr Phe
450 455 460

Cys Val Gln Val Gln Gly Lys Ser Lys Arg Glu Lys Lys Asp Arg Val
465 470 475 480

Phe Thr Asp Lys Thr Ser Ala Thr Val Ile Cys Arg Lys Asn Ala Ser
485 490 495

Ile Ser Val Arg Ala Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser Glu
500 505 510

Trp Ala Ser Val Pro Cys Ser
515

<210> SEQ ID NO 52
 <211> LENGTH: 61
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 52

Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser
1 5 10 15

Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys
20 25 30

Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala
35 40 45

Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys
50 55 60

<210> SEQ ID NO 53
 <211> LENGTH: 232
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 53

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1 5 10 15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

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Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
   115                               120               125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
   130                               135               140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
   145                               150               155               160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
           165                               170               175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
           180                               185               190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
           195                               200               205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
   210                               215               220

Ser Leu Ser Leu Ser Pro Gly Lys
   225                               230

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<210> SEQ ID NO 54

<211> LENGTH: 228

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 54

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Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val
1      5      10      15

Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
   20      25      30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
   35      40      45

His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
   50      55      60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
   65      70      75      80

Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn
   85      90      95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro
   100     105     110

Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln
   115     120     125

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
   130     135     140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
   145     150     155     160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
   165     170     175

Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
   180     185     190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
   195     200     205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
   210     215     220

Ser Pro Gly Lys
   225

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-continued

<210> SEQ ID NO 55
 <211> LENGTH: 228
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 55

Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val
 1 5 10 15
 Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 20 25 30
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 35 40 45
 His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
 50 55 60
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
 65 70 75 80
 Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn
 85 90 95
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser
 100 105 110
 Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln
 115 120 125
 Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
 130 135 140
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 145 150 155 160
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 165 170 175
 Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 180 185 190
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
 195 200 205
 Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
 210 215 220
 Ser Pro Gly Lys
 225

<210> SEQ ID NO 56
 <211> LENGTH: 293
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 56

Ile Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val
 1 5 10 15
 Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly
 20 25 30
 Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn
 35 40 45
 Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile

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50	55	60
Arg Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro		
65	70	75 80
Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr		
	85	90 95
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val		
	100	105 110
Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val		
	115	120 125
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser		
	130	135 140
Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu		
	145	150 155 160
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser		
	165	170 175
Ser Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro		
	180	185 190
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln		
	195	200 205
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala		
	210	215 220
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr		
	225	230 235 240
Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu		
	245	250 255
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser		
	260	265 270
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser		
	275	280 285
Leu Ser Pro Gly Lys		
290		

<210> SEQ ID NO 57
 <211> LENGTH: 121
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 57

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn Pro Thr Ser Ser Thr Ile Asn Phe Thr Pro Ser Leu
50 55 60
Lys Asp Lys Val Phe Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95
Ala Arg Gly Asn Tyr Tyr Arg Tyr Gly Asp Ala Met Asp Tyr Trp Gly

-continued

100	105	110
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Gln Gly Thr Ser Val Thr Val Ser Ser
 115 120

<210> SEQ ID NO 58
 <211> LENGTH: 111
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 58

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser
 20 25 30

Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
 35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
 65 70 75 80

Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ser Arg
 85 90 95

Glu Leu Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
 100 105 110

<210> SEQ ID NO 59
 <211> LENGTH: 117
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 59

Gln Val Gln Leu Gln Gln Leu Gly Ala Glu Leu Ala Arg Pro Gly Ala
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

Ser Ile Gln Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Tyr Ile Asn Pro Ser Ser Gly Tyr Ile Lys Tyr Asn Gln His Phe
 50 55 60

Arg Asp Arg Ala Thr Leu Thr Ala Asp Arg Ser Ser Ser Thr Ala Tyr
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Gly Asn Ser Gly Ser Phe Asp Tyr Trp Gly Gln Gly Thr Thr
 100 105 110

Leu Thr Val Ser Ser
 115

<210> SEQ ID NO 60
 <211> LENGTH: 112
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:

-continued

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 60

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
 1 5 10 15

Glu Ser Leu Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
 20 25 30

Ser Gly Ile Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
 35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
 85 90 95

Leu Glu Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110

<210> SEQ ID NO 61

<211> LENGTH: 117

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 61

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

Thr Met Gln Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Tyr Ile Asn Pro Ser Ser Gly Tyr Ile Lys Tyr Asn Gln Lys Phe
 50 55 60

Lys Asp Lys Val Thr Leu Thr Ala Asp Lys Ser Ser Thr Thr Ala Tyr
 65 70 75 80

Met Gln Leu Ser Arg Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Arg Gly Ser Tyr Phe Phe Asp Phe Trp Gly Gln Gly Thr Ser
 100 105 110

Val Thr Val Ser Ser
 115

<210> SEQ ID NO 62

<211> LENGTH: 112

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 62

Asp Ile Val Ile Thr Gln Asp Glu Leu Ser Asn Pro Val Thr Ser Gly
 1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu Tyr Lys
 20 25 30

Asp Gly Lys Thr Tyr Leu Asn Trp Phe Leu Gln Arg Pro Gly Gln Ser

-continued

35	40	45
Pro Gln Leu Leu Ile Tyr	Leu Met Ser Thr Arg	Ala Ser Gly Val Ser
50	55	60
Asp Arg Phe Ser Gly Ser	Gly Ser Gly Thr Asp	Phe Thr Leu Glu Ile
65	70	75
Ser Arg Val Lys Ala Glu	Asp Val Gly Val Tyr	Tyr Cys Gln Gln Leu
85	90	95
Val Glu Tyr Pro Phe Thr	Phe Gly Gly Gly Thr	Lys Leu Glu Val Lys
100	105	110

<210> SEQ ID NO 63
 <211> LENGTH: 205
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 63

Met Ala Pro Arg Arg Ala	Arg Gly Cys Arg Thr	Leu Gly Leu Pro Ala
1	5	10
Leu Leu Leu Leu Leu Leu	Arg Pro Pro Ala Thr	Arg Gly Ile Thr
20	25	30
Cys Pro Pro Pro Met Ser	Val Glu His Ala Asp	Ile Trp Val Lys Ser
35	40	45
Tyr Ser Leu Tyr Ser Arg	Glu Arg Tyr Ile Cys	Asn Ser Gly Phe Lys
50	55	60
Arg Lys Ala Gly Thr Ser	Ser Leu Thr Glu Cys	Val Leu Asn Lys Ala
65	70	75
Thr Asn Val Ala His Trp	Thr Thr Pro Ser Leu	Lys Cys Ile Arg Asp
85	90	95
Pro Ala Leu Val His Gln	Arg Pro Ala Pro Pro	Ser Thr Val Thr Thr
100	105	110
Ala Gly Val Thr Pro Gln	Pro Glu Ser Leu Ser	Pro Ser Gly Lys Glu
115	120	125
Pro Ala Ala Ser Ser Pro	Ser Ser Asn Asn Thr	Ala Ala Thr Thr Ala
130	135	140
Ala Ile Val Pro Gly Ser	Gln Leu Met Pro Ser	Lys Ser Pro Ser Thr
145	150	155
Gly Thr Thr Glu Ile Ser	Ser Ser His Glu Ser	Ser His Gly Thr Pro
165	170	175
Gln Thr Thr Ala Lys Asn	Trp Glu Leu Thr Ala	Ser Ala Ser His Gln
180	185	190
Pro Pro Gly Val Tyr Pro	Gln Gly His Ser Asp	Thr Thr
195	200	205

<210> SEQ ID NO 64
 <211> LENGTH: 172
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 64

Met Ala Pro Arg Arg Ala	Arg Gly Cys Arg Thr	Leu Gly Leu Pro Ala
1	5	10
Leu Leu Leu Leu Leu Leu	Arg Pro Pro Ala Thr	Arg Gly Ile Thr
20	25	30
Cys Pro Pro Pro Met Ser	Val Glu His Ala Asp	Ile Trp Val Lys Ser

-continued

35	40	45
Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys		
50	55	60
Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala		
65	70	75
Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Lys Pro		
85	90	95
Ala Ala Ser Ser Pro Ser Ser Asn Asn Thr Ala Ala Thr Thr Ala Ala		
100	105	110
Ile Val Pro Gly Ser Gln Leu Met Pro Ser Lys Ser Pro Ser Thr Gly		
115	120	125
Thr Thr Glu Ile Ser Ser His Glu Ser Ser His Gly Thr Pro Ser Gln		
130	135	140
Thr Thr Ala Lys Asn Trp Glu Leu Thr Ala Ser Ala Ser His Gln Pro		
145	150	155
Pro Gly Val Tyr Pro Gln Gly His Ser Asp Thr Thr		
165	170	

<210> SEQ ID NO 65
 <211> LENGTH: 62
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 65

Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser		
1	5	10
Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys		
20	25	30
Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Ile Asn Lys Ala		
35	40	45
Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile		
50	55	60

<210> SEQ ID NO 66
 <211> LENGTH: 65
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 66

Ile Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val		
1	5	10
Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly		
20	25	30
Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Ile Asn		
35	40	45
Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile		
50	55	60

Arg
65

<210> SEQ ID NO 67
 <211> LENGTH: 232

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<212> TYPE: PRT
<213> ORGANISM: unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 67

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1          5          10          15
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
          20          25          30
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
          35          40          45
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
          50          55          60
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65          70          75          80
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
          85          90          95
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
          100          105          110
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
          115          120          125
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
          130          135          140
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145          150          155          160
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
          165          170          175
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
          180          185          190
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
          195          200          205
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210          215          220
Ser Leu Ser Leu Ser Pro Gly Lys
225          230

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<210> SEQ ID NO 68
<211> LENGTH: 326
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 68

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1          5          10          15
Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
          20          25          30
Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
          35          40          45
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
          50          55          60
Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65          70          75          80
Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys

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85					90					95					
Thr	Val	Glu	Arg	Lys	Cys	Cys	Val	Glu	Cys	Pro	Pro	Cys	Pro	Ala	Pro
			100					105					110		
Pro	Val	Ala	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp
			115					120					125		
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp
			130					135					140		
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly
			145					150					155		160
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Phe	Asn
				165					170					175	
Ser	Thr	Phe	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Val	His	Gln	Asp	Trp
			180					185					190		
Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro
			195					200					205		
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Thr	Lys	Gly	Gln	Pro	Arg	Glu
			210					215					220		
Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn
			225					230					235		240
Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile
			245						250					255	
Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr
			260					265					270		
Thr	Pro	Pro	Met	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys
			275					280					285		
Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys
			290					295					300		
Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu
			305					310					315		320
Ser	Leu	Ser	Pro	Gly	Lys										
				325											

<210> SEQ ID NO 69

<211> LENGTH: 224

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 69

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1			5						10				15		
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Tyr	Ser	Phe	Thr	Gly	His
			20					25					30		
Trp	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
			35				40						45		
Gly	Met	Ile	His	Pro	Ser	Asp	Ser	Glu	Thr	Arg	Tyr	Asn	Gln	Lys	Phe
			50			55						60			
Lys	Asp	Arg	Phe	Thr	Ile	Ser	Val	Asp	Lys	Ser	Lys	Asn	Thr	Leu	Tyr
			65			70						75		80	
Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85				90						95	
Ala	Arg	Gly	Ile	Tyr	Phe	Tyr	Gly	Thr	Thr	Tyr	Phe	Asp	Tyr	Trp	Gly

-continued

100	105	110
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser		
115	120	125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala		
130	135	140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val		
145	150	155
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala		
	165	170
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val		
	180	185
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His		
	195	200
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys		
	210	215
		220

<210> SEQ ID NO 70
 <211> LENGTH: 16
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 70

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 71
 <211> LENGTH: 20
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 71

Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser

20

<210> SEQ ID NO 72
 <211> LENGTH: 523
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 72

Ile Trp Glu Leu Lys Lys Asp Val Tyr Val Val Glu Leu Asp Trp Tyr
1 5 10 15
Pro Asp Ala Pro Gly Glu Met Val Val Leu Thr Cys Asp Thr Pro Glu
20 25 30
Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln Ser Ser Glu Val Leu Gly
35 40 45
Ser Gly Lys Thr Leu Thr Ile Gln Val Lys Glu Phe Gly Asp Ala Gly
50 55 60
Gln Tyr Thr Cys His Lys Gly Gly Glu Val Leu Ser His Ser Leu Leu
65 70 75 80

-continued

Leu	Leu	His	Lys	Lys	Glu	Asp	Gly	Ile	Trp	Ser	Thr	Asp	Ile	Leu	Lys	85	90	95	
Asp	Gln	Lys	Glu	Pro	Lys	Asn	Lys	Thr	Phe	Leu	Arg	Cys	Glu	Ala	Lys	100	105	110	
Asn	Tyr	Ser	Gly	Arg	Phe	Thr	Cys	Trp	Trp	Leu	Thr	Thr	Ile	Ser	Thr	115	120	125	
Asp	Leu	Thr	Phe	Ser	Val	Lys	Ser	Ser	Arg	Gly	Ser	Ser	Asp	Pro	Gln	130	135	140	
Gly	Val	Thr	Cys	Gly	Ala	Ala	Thr	Leu	Ser	Ala	Glu	Arg	Val	Arg	Gly	145	150	155	160
Asp	Asn	Lys	Glu	Tyr	Glu	Tyr	Ser	Val	Glu	Cys	Gln	Glu	Asp	Ser	Ala	165	170	175	
Cys	Pro	Ala	Ala	Glu	Glu	Ser	Leu	Pro	Ile	Glu	Val	Met	Val	Asp	Ala	180	185	190	
Val	His	Lys	Leu	Lys	Tyr	Glu	Asn	Tyr	Thr	Ser	Ser	Phe	Phe	Ile	Arg	195	200	205	
Asp	Ile	Ile	Lys	Pro	Asp	Pro	Pro	Lys	Asn	Leu	Gln	Leu	Lys	Pro	Leu	210	215	220	
Lys	Asn	Ser	Arg	Gln	Val	Glu	Val	Ser	Trp	Glu	Tyr	Pro	Asp	Thr	Trp	225	230	235	240
Ser	Thr	Pro	His	Ser	Tyr	Phe	Ser	Leu	Thr	Phe	Cys	Val	Gln	Val	Gln	245	250	255	
Gly	Lys	Ser	Lys	Arg	Glu	Lys	Lys	Asp	Arg	Val	Phe	Thr	Asp	Lys	Thr	260	265	270	
Ser	Ala	Thr	Val	Ile	Cys	Arg	Lys	Asn	Ala	Ser	Ile	Ser	Val	Arg	Ala	275	280	285	
Gln	Asp	Arg	Tyr	Tyr	Ser	Ser	Ser	Trp	Ser	Glu	Trp	Ala	Ser	Val	Pro	290	295	300	
Cys	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	305	310	315	320
Ser	Gly	Gly	Gly	Gly	Ser	Arg	Asn	Leu	Pro	Val	Ala	Thr	Pro	Asp	Pro	325	330	335	
Gly	Met	Phe	Pro	Cys	Leu	His	His	Ser	Gln	Asn	Leu	Leu	Arg	Ala	Val	340	345	350	
Ser	Asn	Met	Leu	Gln	Lys	Ala	Arg	Gln	Thr	Leu	Glu	Phe	Tyr	Pro	Cys	355	360	365	
Thr	Ser	Glu	Glu	Ile	Asp	His	Glu	Asp	Ile	Thr	Lys	Asp	Lys	Thr	Ser	370	375	380	
Thr	Val	Glu	Ala	Cys	Leu	Pro	Leu	Glu	Leu	Thr	Lys	Asn	Glu	Ser	Cys	385	390	395	400
Leu	Asn	Ser	Arg	Glu	Thr	Ser	Phe	Ile	Thr	Asn	Gly	Ser	Cys	Leu	Ala	405	410	415	
Ser	Arg	Lys	Thr	Ser	Phe	Met	Met	Ala	Leu	Cys	Leu	Ser	Ser	Ile	Tyr	420	425	430	
Glu	Asp	Leu	Lys	Met	Tyr	Gln	Val	Glu	Phe	Lys	Thr	Met	Asn	Ala	Lys	435	440	445	
Leu	Leu	Met	Asp	Pro	Lys	Arg	Gln	Ile	Phe	Leu	Asp	Gln	Asn	Met	Leu	450	455	460	
Ala	Val	Ile	Asp	Glu	Leu	Met	Gln	Ala	Leu	Asn	Phe	Asn	Ser	Glu	Thr	465	470	475	480

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Val	Pro	Gln	Lys	Ser	Ser	Leu	Glu	Glu	Pro	Asp	Phe	Tyr	Lys	Thr	Lys	485	490	495
Ile	Lys	Leu	Cys	Ile	Leu	Leu	His	Ala	Phe	Arg	Ile	Arg	Ala	Val	Thr	500	505	510
Ile	Asp	Arg	Val	Met	Ser	Tyr	Leu	Asn	Ala	Ser						515	520	

<210> SEQ ID NO 73
 <211> LENGTH: 523
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 73

Arg	Asn	Leu	Pro	Val	Ala	Thr	Pro	Asp	Pro	Gly	Met	Phe	Pro	Cys	Leu	1	5	10	15
His	His	Ser	Gln	Asn	Leu	Leu	Arg	Ala	Val	Ser	Asn	Met	Leu	Gln	Lys	20	25	30	
Ala	Arg	Gln	Thr	Leu	Glu	Phe	Tyr	Pro	Cys	Thr	Ser	Glu	Glu	Ile	Asp	35	40	45	
His	Glu	Asp	Ile	Thr	Lys	Asp	Lys	Thr	Ser	Thr	Val	Glu	Ala	Cys	Leu	50	55	60	
Pro	Leu	Glu	Leu	Thr	Lys	Asn	Glu	Ser	Cys	Leu	Asn	Ser	Arg	Glu	Thr	65	70	75	80
Ser	Phe	Ile	Thr	Asn	Gly	Ser	Cys	Leu	Ala	Ser	Arg	Lys	Thr	Ser	Phe	85	90	95	
Met	Met	Ala	Leu	Cys	Leu	Ser	Ser	Ile	Tyr	Glu	Asp	Leu	Lys	Met	Tyr	100	105	110	
Gln	Val	Glu	Phe	Lys	Thr	Met	Asn	Ala	Lys	Leu	Leu	Met	Asp	Pro	Lys	115	120	125	
Arg	Gln	Ile	Phe	Leu	Asp	Gln	Asn	Met	Leu	Ala	Val	Ile	Asp	Glu	Leu	130	135	140	
Met	Gln	Ala	Leu	Asn	Phe	Asn	Ser	Glu	Thr	Val	Pro	Gln	Lys	Ser	Ser	145	150	155	160
Leu	Glu	Glu	Pro	Asp	Phe	Tyr	Lys	Thr	Lys	Ile	Lys	Leu	Cys	Ile	Leu	165	170	175	
Leu	His	Ala	Phe	Arg	Ile	Arg	Ala	Val	Thr	Ile	Asp	Arg	Val	Met	Ser	180	185	190	
Tyr	Leu	Asn	Ala	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	195	200	205	
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ile	Trp	Glu	Leu	Lys	Lys	Asp	210	215	220	
Val	Tyr	Val	Val	Glu	Leu	Asp	Trp	Tyr	Pro	Asp	Ala	Pro	Gly	Glu	Met	225	230	235	240
Val	Val	Leu	Thr	Cys	Asp	Thr	Pro	Glu	Glu	Asp	Gly	Ile	Thr	Trp	Thr	245	250	255	
Leu	Asp	Gln	Ser	Ser	Glu	Val	Leu	Gly	Ser	Gly	Lys	Thr	Leu	Thr	Ile	260	265	270	
Gln	Val	Lys	Glu	Phe	Gly	Asp	Ala	Gly	Gln	Tyr	Thr	Cys	His	Lys	Gly	275	280	285	
Gly	Glu	Val	Leu	Ser	His	Ser	Leu	Leu	Leu	Leu	His	Lys	Lys	Glu	Asp	290	295	300	

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Gly	Ile	Trp	Ser	Thr	Asp	Ile	Leu	Lys	Asp	Gln	Lys	Glu	Pro	Lys	Asn	
305					310					315					320	
Lys	Thr	Phe	Leu	Arg	Cys	Glu	Ala	Lys	Asn	Tyr	Ser	Gly	Arg	Phe	Thr	
			325						330					335		
Cys	Trp	Trp	Leu	Thr	Thr	Ile	Ser	Thr	Asp	Leu	Thr	Phe	Ser	Val	Lys	
			340					345					350			
Ser	Ser	Arg	Gly	Ser	Ser	Asp	Pro	Gln	Gly	Val	Thr	Cys	Gly	Ala	Ala	
		355					360					365				
Thr	Leu	Ser	Ala	Glu	Arg	Val	Arg	Gly	Asp	Asn	Lys	Glu	Tyr	Glu	Tyr	
	370					375					380					
Ser	Val	Glu	Cys	Gln	Glu	Asp	Ser	Ala	Cys	Pro	Ala	Ala	Glu	Glu	Ser	
385					390					395					400	
Leu	Pro	Ile	Glu	Val	Met	Val	Asp	Ala	Val	His	Lys	Leu	Lys	Tyr	Glu	
			405					410						415		
Asn	Tyr	Thr	Ser	Ser	Phe	Phe	Ile	Arg	Asp	Ile	Ile	Lys	Pro	Asp	Pro	
			420					425					430			
Pro	Lys	Asn	Leu	Gln	Leu	Lys	Pro	Leu	Lys	Asn	Ser	Arg	Gln	Val	Glu	
		435					440					445				
Val	Ser	Trp	Glu	Tyr	Pro	Asp	Thr	Trp	Ser	Thr	Pro	His	Ser	Tyr	Phe	
	450					455					460					
Ser	Leu	Thr	Phe	Cys	Val	Gln	Val	Gln	Gly	Lys	Ser	Lys	Arg	Glu	Lys	
465					470					475					480	
Lys	Asp	Arg	Val	Phe	Thr	Asp	Lys	Thr	Ser	Ala	Thr	Val	Ile	Cys	Arg	
			485					490						495		
Lys	Asn	Ala	Ser	Ile	Ser	Val	Arg	Ala	Gln	Asp	Arg	Tyr	Tyr	Ser	Ser	
		500						505					510			
Ser	Trp	Ser	Glu	Trp	Ala	Ser	Val	Pro	Cys	Ser						
	515						520									

<210> SEQ ID NO 74

<211> LENGTH: 107

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 74

Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	
1				5					10					15		
Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Phe		
		20					25						30			
Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	
	35					40						45				
Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	
	50				55						60					
Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	
65				70						75				80		
Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	
			85						90					95		
Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys						
		100						105								

<210> SEQ ID NO 75

<211> LENGTH: 106

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

-continued

<400> SEQUENCE: 75

```

Gly Gln Pro Lys Ala Asn Pro Thr Val Thr Leu Phe Pro Pro Ser Ser
1           5           10           15
Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
20           25           30
Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro
35           40           45
Val Lys Ala Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn
50           55           60
Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
65           70           75           80
Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val
85           90           95
Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
100           105

```

<210> SEQ ID NO 76

<211> LENGTH: 106

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 76

```

Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser
1           5           10           15
Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
20           25           30
Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro
35           40           45
Val Lys Ala Gly Val Glu Thr Thr Pro Ser Lys Gln Ser Asn Asn
50           55           60
Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
65           70           75           80
Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val
85           90           95
Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
100           105

```

<210> SEQ ID NO 77

<211> LENGTH: 106

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 77

```

Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser
1           5           10           15
Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
20           25           30
Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro
35           40           45
Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn
50           55           60
Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
65           70           75           80

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Ser	His	Lys	Ser	Tyr	Ser	Cys	Gln	Val	Thr	His	Glu	Gly	Ser	Thr	Val
			85						90					95	

Glu	Lys	Thr	Val	Ala	Pro	Thr	Glu	Cys	Ser
			100					105	

<210> SEQ ID NO 78
 <211> LENGTH: 106
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 78

Ser	Gln	Pro	Lys	Ala	Ala	Pro	Ser	Val	Thr	Leu	Phe	Pro	Pro	Ser	Ser
1			5					10						15	

Glu	Glu	Leu	Gln	Ala	Asn	Lys	Ala	Thr	Leu	Val	Cys	Leu	Val	Ser	Asp
		20					25					30			

Phe	Tyr	Pro	Gly	Ala	Val	Thr	Val	Ala	Trp	Lys	Ala	Asp	Gly	Ser	Pro
	35					40					45				

Val	Lys	Val	Gly	Val	Glu	Thr	Thr	Lys	Pro	Ser	Lys	Gln	Ser	Asn	Asn
	50					55					60				

Lys	Tyr	Ala	Ala	Ser	Ser	Tyr	Leu	Ser	Leu	Thr	Pro	Glu	Gln	Trp	Lys
65				70					75					80	

Ser	His	Arg	Ser	Tyr	Ser	Cys	Arg	Val	Thr	His	Glu	Gly	Ser	Thr	Val
			85					90						95	

Glu	Lys	Thr	Val	Ala	Pro	Ala	Glu	Cys	Ser
			100					105	

<210> SEQ ID NO 79
 <211> LENGTH: 219
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 79

Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly
1			5					10						15	

Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Leu	His	Ser
		20					25					30			

Ser	Gly	Ile	Thr	Tyr	Leu	Tyr	Trp	Phe	Leu	Gln	Lys	Pro	Gly	Gln	Ser
	35					40					45				

Pro	Gln	Leu	Leu	Ile	Tyr	Arg	Met	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro
	50				55					60					

Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65				70					75					80	

Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Met	Gln	His
		85					90							95	

Leu	Glu	Tyr	Pro	Phe	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys
		100					105						110		

Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu
		115				120						125			

Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe
	130				135						140				

Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln
145					150					155				160	

Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

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<210> SEQ ID NO 80
<211> LENGTH: 133
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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[illegible]

<400> SEQUENCE: 81

Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ala
1				5					10					15	
Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr
			20					25						30	
Ser	Ile	Gln	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Arg	Leu	Glu	Trp	Ile
			35				40						45		
Gly	Tyr	Ile	Asn	Pro	Ser	Ser	Gly	Tyr	Ile	Lys	Tyr	Asn	Gln	His	Phe
	50					55					60				
Arg	Gly	Arg	Ala	Thr	Leu	Thr	Ala	Asp	Arg	Ser	Ala	Ser	Thr	Ala	Tyr
65					70					75					80
Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	
Ala	Arg	Gly	Asn	Ser	Gly	Ser	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu
			100					105					110		

```

<210> SEQ ID NO 82
<211> LENGTH: 712
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 82

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1          5          10          15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
          20          25          30
Ser Gly Ile Thr Tyr Leu Tyr Trp Phe Leu Gln Lys Pro Gly Gln Ser
          35          40          45
Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
          50          55          60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65          70          75          80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
          85          90          95
Leu Glu Tyr Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys

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100					105					110				
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Asp
	115						120				125			
Ala	His	Lys	Ser	Glu	Val	Ala	His	Arg	Phe	Lys	Asp	Leu	Gly	Glu
	130						135				140			
Asn	Phe	Lys	Ala	Leu	Val	Leu	Ile	Ala	Phe	Ala	Gln	Tyr	Leu	Gln
	145				150					155				160
Cys	Pro	Phe	Glu	Asp	His	Val	Lys	Leu	Val	Asn	Glu	Val	Thr	Glu
				165					170					175
Ala	Lys	Thr	Cys	Val	Ala	Asp	Glu	Ser	Ala	Glu	Asn	Cys	Asp	Lys
			180						185				190	Ser
Leu	His	Thr	Leu	Phe	Gly	Asp	Lys	Leu	Cys	Thr	Val	Ala	Thr	Leu
		195					200					205		Arg
Glu	Thr	Tyr	Gly	Glu	Met	Ala	Asp	Cys	Cys	Ala	Lys	Gln	Glu	Pro
	210					215					220			Glu
Arg	Asn	Glu	Cys	Phe	Leu	Gln	His	Lys	Asp	Asp	Asn	Pro	Asn	Leu
	225					230				235				240
Arg	Leu	Val	Arg	Pro	Glu	Val	Asp	Val	Met	Cys	Thr	Ala	Phe	His
				245					250					255
Asn	Glu	Glu	Thr	Phe	Leu	Lys	Lys	Tyr	Leu	Tyr	Glu	Ile	Ala	Arg
			260					265					270	Arg
His	Pro	Tyr	Phe	Tyr	Ala	Pro	Glu	Leu	Leu	Phe	Phe	Ala	Lys	Arg
		275					280					285		Tyr
Lys	Ala	Ala	Phe	Thr	Glu	Cys	Cys	Gln	Ala	Ala	Asp	Lys	Ala	Ala
	290					295					300			Cys
Leu	Leu	Pro	Lys	Leu	Asp	Glu	Leu	Arg	Asp	Glu	Gly	Lys	Ala	Ser
	305				310					315				320
Ala	Lys	Gln	Arg	Leu	Lys	Cys	Ala	Ser	Leu	Gln	Lys	Phe	Gly	Glu
				325					330					335
Ala	Phe	Lys	Ala	Trp	Ala	Val	Ala	Arg	Leu	Ser	Gln	Arg	Phe	Pro
			340					345					350	Lys
Ala	Glu	Phe	Ala	Glu	Val	Ser	Lys	Leu	Val	Thr	Asp	Leu	Thr	Lys
		355					360					365		Val
His	Thr	Glu	Cys	Cys	His	Gly	Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp
	370					375					380			Arg
Ala	Asp	Leu	Ala	Lys	Tyr	Ile	Cys	Glu	Asn	Gln	Asp	Ser	Ile	Ser
	385				390					395				400
Lys	Leu	Lys	Glu	Cys	Cys	Glu	Lys	Pro	Leu	Leu	Glu	Lys	Ser	His
				405					410					415
Ile	Ala	Glu	Val	Glu	Asn	Asp	Glu	Met	Pro	Ala	Asp	Leu	Pro	Ser
		420						425					430	Leu
Ala	Ala	Asp	Phe	Val	Glu	Ser	Lys	Asp	Val	Cys	Lys	Asn	Tyr	Ala
		435					440					445		Glu
Ala	Lys	Asp	Val	Phe	Leu	Gly	Met	Phe	Leu	Tyr	Glu	Tyr	Ala	Arg
	450					455					460			Arg
His	Pro	Asp	Tyr	Ser	Val	Val	Leu	Leu	Leu	Arg	Leu	Ala	Lys	Thr
	465				470					475				480
Glu	Thr	Thr	Leu	Glu	Lys	Cys	Cys	Ala	Ala	Ala	Asp	Pro	His	Glu
				485					490					495
Tyr	Ala	Lys	Val	Phe	Asp	Glu	Phe	Lys	Pro	Leu	Val	Glu	Glu	Pro
			500					505						Gln

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Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr
  515                      520                      525

Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln
  530                      535                      540

Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val
  545                      550                      555                      560

Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala
                      565                      570                      575

Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu
  580                      585                      590

Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu
  595                      600                      605

Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr
  610                      615                      620

Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile
  625                      630                      635                      640

Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu
  645                      650                      655

Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys
  660                      665                      670

Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala
  675                      680                      685

Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala
  690                      695                      700

Ala Ser Gln Ala Ala Leu Gly Leu
  705                      710

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<210> SEQ ID NO 83
<211> LENGTH: 987
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

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<400> SEQUENCE: 83

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Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
  1           5           10           15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
  20           25           30

Ser Ile Gln Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile
  35           40           45

Gly Tyr Ile Asn Pro Ser Ser Gly Tyr Ile Lys Tyr Asn Gln His Phe
  50           55           60

Arg Gly Arg Ala Thr Leu Thr Ala Asp Arg Ser Ala Ser Thr Ala Tyr
  65           70           75           80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
  85           90           95

Ala Arg Gly Asn Ser Gly Ser Phe Asp Tyr Trp Gly Gln Gly Thr Leu
  100          105          110

Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
  115          120          125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser
  130          135          140

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Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly	Glu	Pro	Ala	Ser	Ile	Ser	Cys
145					150					155					160
Arg	Ser	Ser	Gln	Ser	Leu	Leu	His	Ser	Ser	Gly	Ile	Thr	Tyr	Leu	Tyr
			165						170					175	
Trp	Phe	Leu	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Gln	Leu	Leu	Ile	Tyr	Arg
		180						185					190		
Met	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly
	195						200				205				
Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile	Ser	Arg	Val	Glu	Ala	Glu	Asp
210						215					220				
Val	Gly	Val	Tyr	Tyr	Cys	Met	Gln	His	Leu	Glu	Tyr	Pro	Phe	Thr	Phe
225					230					235					240
Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Gly	Gly	Gly	Gly	Ser	Gly	Gly
			245						250					255	
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ala	His	Lys	Ser	Glu	Val	Ala
		260						265					270		
His	Arg	Phe	Lys	Asp	Leu	Gly	Glu	Glu	Asn	Phe	Lys	Ala	Leu	Val	Leu
	275						280					285			
Ile	Ala	Phe	Ala	Gln	Tyr	Leu	Gln	Gln	Cys	Pro	Phe	Glu	Asp	His	Val
290						295					300				
Lys	Leu	Val	Asn	Glu	Val	Thr	Glu	Phe	Ala	Lys	Thr	Cys	Val	Ala	Asp
305					310					315					320
Glu	Ser	Ala	Glu	Asn	Cys	Asp	Lys	Ser	Leu	His	Thr	Leu	Phe	Gly	Asp
			325						330					335	
Lys	Leu	Cys	Thr	Val	Ala	Thr	Leu	Arg	Glu	Thr	Tyr	Gly	Glu	Met	Ala
			340					345					350		
Asp	Cys	Cys	Ala	Lys	Gln	Glu	Pro	Glu	Arg	Asn	Glu	Cys	Phe	Leu	Gln
	355						360					365			
His	Lys	Asp	Asp	Asn	Pro	Asn	Leu	Pro	Arg	Leu	Val	Arg	Pro	Glu	Val
370						375					380				
Asp	Val	Met	Cys	Thr	Ala	Phe	His	Asp	Asn	Glu	Glu	Thr	Phe	Leu	Lys
385					390					395					400
Lys	Tyr	Leu	Tyr	Glu	Ile	Ala	Arg	Arg	His	Pro	Tyr	Phe	Tyr	Ala	Pro
			405						410					415	
Glu	Leu	Leu	Phe	Phe	Ala	Lys	Arg	Tyr	Lys	Ala	Ala	Phe	Thr	Glu	Cys
			420					425					430		
Cys	Gln	Ala	Ala	Asp	Lys	Ala	Ala	Cys	Leu	Leu	Pro	Lys	Leu	Asp	Glu
	435						440					445			
Leu	Arg	Asp	Glu	Gly	Lys	Ala	Ser	Ser	Ala	Lys	Gln	Arg	Leu	Lys	Cys
450						455					460				
Ala	Ser	Leu	Gln	Lys	Phe	Gly	Glu	Arg	Ala	Phe	Lys	Ala	Trp	Ala	Val
465					470					475					480
Ala	Arg	Leu	Ser	Gln	Arg	Phe	Pro	Lys	Ala	Glu	Phe	Ala	Glu	Val	Ser
			485						490					495	
Lys	Leu	Val	Thr	Asp	Leu	Thr	Lys	Val	His	Thr	Glu	Cys	Cys	His	Gly
		500						505					510		
Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp	Arg	Ala	Asp	Leu	Ala	Lys	Tyr	Ile
	515						520					525			
Cys	Glu	Asn	Gln	Asp	Ser	Ile	Ser	Ser	Lys	Leu	Lys	Glu	Cys	Cys	Glu
530						535					540				

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Lys	Pro	Leu	Leu	Glu	Lys	Ser	His	Cys	Ile	Ala	Glu	Val	Glu	Asn	Asp
545					550					555					560
Glu	Met	Pro	Ala	Asp	Leu	Pro	Ser	Leu	Ala	Ala	Asp	Phe	Val	Glu	Ser
				565					570					575	
Lys	Asp	Val	Cys	Lys	Asn	Tyr	Ala	Glu	Ala	Lys	Asp	Val	Phe	Leu	Gly
			580					585					590		
Met	Phe	Leu	Tyr	Glu	Tyr	Ala	Arg	Arg	His	Pro	Asp	Tyr	Ser	Val	Val
		595					600					605			
Leu	Leu	Leu	Arg	Leu	Ala	Lys	Thr	Tyr	Glu	Thr	Thr	Leu	Glu	Lys	Cys
	610					615					620				
Cys	Ala	Ala	Ala	Asp	Pro	His	Glu	Cys	Tyr	Ala	Lys	Val	Phe	Asp	Glu
625					630					635					640
Phe	Lys	Pro	Leu	Val	Glu	Glu	Pro	Gln	Asn	Leu	Ile	Lys	Gln	Asn	Cys
			645					650						655	
Glu	Leu	Phe	Glu	Gln	Leu	Gly	Glu	Tyr	Lys	Phe	Gln	Asn	Ala	Leu	Leu
			660					665					670		
Val	Arg	Tyr	Thr	Lys	Lys	Val	Pro	Gln	Val	Ser	Thr	Pro	Thr	Leu	Val
		675					680					685			
Glu	Val	Ser	Arg	Asn	Leu	Gly	Lys	Val	Gly	Ser	Lys	Cys	Cys	Lys	His
	690					695					700				
Pro	Glu	Ala	Lys	Arg	Met	Pro	Cys	Ala	Glu	Asp	Tyr	Leu	Ser	Val	Val
705					710					715					720
Leu	Asn	Gln	Leu	Cys	Val	Leu	His	Glu	Lys	Thr	Pro	Val	Ser	Asp	Arg
			725						730					735	
Val	Thr	Lys	Cys	Cys	Thr	Glu	Ser	Leu	Val	Asn	Arg	Arg	Pro	Cys	Phe
			740					745					750		
Ser	Ala	Leu	Glu	Val	Asp	Glu	Thr	Tyr	Val	Pro	Lys	Glu	Phe	Asn	Ala
		755					760					765			
Glu	Thr	Phe	Thr	Phe	His	Ala	Asp	Ile	Cys	Thr	Leu	Ser	Glu	Lys	Glu
	770					775					780				
Arg	Gln	Ile	Lys	Lys	Gln	Thr	Ala	Leu	Val	Glu	Leu	Val	Lys	His	Lys
785					790					795					800
Pro	Lys	Ala	Thr	Lys	Glu	Gln	Leu	Lys	Ala	Val	Met	Asp	Asp	Phe	Ala
				805					810					815	
Ala	Phe	Val	Glu	Lys	Cys	Cys	Lys	Ala	Asp	Asp	Lys	Glu	Thr	Cys	Phe
			820					825					830		
Ala	Glu	Glu	Gly	Lys	Lys	Leu	Val	Ala	Ala	Ser	Gln	Ala	Ala	Leu	Gly
		835					840					845			
Leu	Gly	Gly	Gly	Gly	Ser	Ala	Pro	Thr	Ser	Ser	Ser	Thr	Lys	Lys	Thr
	850					855					860				
Gln	Leu	Gln	Leu	Glu	His	Leu	Leu	Leu	Asp	Leu	Gln	Met	Ile	Leu	Asn
865					870					875					880
Gly	Ile	Asn	Asn	Tyr	Lys	Asn	Pro	Lys	Leu	Thr	Arg	Met	Leu	Thr	Phe
				885					890					895	
Lys	Phe	Tyr	Met	Pro	Lys	Lys	Ala	Thr	Glu	Leu	Lys	His	Leu	Gln	Cys
			900					905					910		
Leu	Glu	Glu	Glu	Leu	Lys	Pro	Leu	Glu	Glu	Val	Leu	Asn	Leu	Ala	Gln
		915					920					925			
Ser	Lys	Asn	Phe	His	Leu	Arg	Pro	Arg	Asp	Leu	Ile	Ser	Asn	Ile	Asn
	930					935					940				
Val	Ile	Val	Leu	Glu	Leu	Lys	Gly	Ser	Glu	Thr	Thr	Phe	Met	Cys	Glu

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945	950	955	960
Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile			
	965	970	975
Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Thr			
	980	985	

<210> SEQ ID NO 84
 <211> LENGTH: 190
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 84

Gly Met Arg Thr Glu Asp Leu Pro Lys Ala Val Val Phe Leu Glu Pro			
1	5	10	15
Gln Trp Tyr Ser Val Leu Glu Lys Asp Ser Val Thr Leu Lys Cys Gln			
	20	25	30
Gly Ala Tyr Ser Pro Glu Asp Asn Ser Thr Gln Trp Phe His Asn Glu			
	35	40	45
Ser Leu Ile Ser Ser Gln Ala Ser Ser Tyr Phe Ile Asp Ala Ala Thr			
	50	55	60
Val Asn Asp Ser Gly Glu Tyr Arg Cys Gln Thr Asn Leu Ser Thr Leu			
	65	70	75
Ser Asp Pro Val Gln Leu Glu Val His Ile Gly Trp Leu Leu Leu Gln			
	85	90	95
Ala Pro Arg Trp Val Phe Lys Glu Glu Asp Pro Ile His Leu Arg Cys			
	100	105	110
His Ser Trp Lys Asn Thr Ala Leu His Lys Val Thr Tyr Leu Gln Asn			
	115	120	125
Gly Lys Asp Arg Lys Tyr Phe His His Asn Ser Asp Phe His Ile Pro			
	130	135	140
Lys Ala Thr Leu Lys Asp Ser Gly Ser Tyr Phe Cys Arg Gly Leu Val			
	145	150	155
Gly Ser Lys Asn Val Ser Ser Glu Thr Val Asn Ile Thr Ile Thr Gln			
	165	170	175
Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Ser Pro Pro Gly			
	180	185	190

<210> SEQ ID NO 85
 <211> LENGTH: 602
 <212> TYPE: PRT
 <213> ORGANISM: Unknown
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 85

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala			
1	5	10	15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr			
	20	25	30
Ser Ile Gln Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile			
	35	40	45
Gly Tyr Ile Asn Pro Ser Ser Gly Tyr Ile Lys Tyr Asn Gln His Phe			
	50	55	60
Arg Gly Arg Ala Thr Leu Thr Ala Asp Arg Ser Ala Ser Thr Ala Tyr			
	65	70	75
			80

Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
85				100				90				95			
Ala	Arg	Gly	Asn	Ser	Gly	Ser	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu
100				105				110				115			
Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly
115				120				125				130			
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Val	Met	Thr	Gln	Ser
130				135				140				145			
Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly	Glu	Pro	Ala	Ser	Ile	Ser	Cys
145				150				155				160			
Arg	Ser	Ser	Gln	Ser	Leu	Leu	His	Ser	Ser	Gly	Ile	Thr	Tyr	Leu	Tyr
165				170				175				180			
Trp	Phe	Leu	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Gln	Leu	Leu	Ile	Tyr	Arg
180				185				190				195			
Met	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly
195				200				205				210			
Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile	Ser	Arg	Val	Glu	Ala	Glu	Asp
210				215				220				225			
Val	Gly	Val	Tyr	Tyr	Cys	Met	Gln	His	Leu	Glu	Tyr	Pro	Phe	Thr	Phe
225				230				235				240			
Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Gly	Gly	Gly	Gly	Ser	Gly	Gly
245				250				255				260			
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Met	Arg	Thr	Glu	Asp	Leu	Pro
260				265				270				275			
Lys	Ala	Val	Val	Phe	Leu	Glu	Pro	Gln	Trp	Tyr	Ser	Val	Leu	Glu	Lys
275				280				285				290			
Asp	Ser	Val	Thr	Leu	Lys	Cys	Gln	Gly	Ala	Tyr	Ser	Pro	Glu	Asp	Asn
290				295				300				305			
Ser	Thr	Gln	Trp	Phe	His	Asn	Glu	Ser	Leu	Ile	Ser	Ser	Gln	Ala	Ser
310				315				320				325			
Ser	Tyr	Phe	Ile	Asp	Ala	Ala	Thr	Val	Asn	Asp	Ser	Gly	Glu	Tyr	Arg
325				330				335				340			
Cys	Gln	Thr	Asn	Leu	Ser	Thr	Leu	Ser	Asp	Pro	Val	Gln	Leu	Glu	Val
340				345				350				355			
His	Ile	Gly	Trp	Leu	Leu	Leu	Gln	Ala	Pro	Arg	Trp	Val	Phe	Lys	Glu
355				360				365				370			
Glu	Asp	Pro	Ile	His	Leu	Arg	Cys	His	Ser	Trp	Lys	Asn	Thr	Ala	Leu
370				375				380				385			
His	Lys	Val	Thr	Tyr	Leu	Gln	Asn	Gly	Lys	Asp	Arg	Lys	Tyr	Phe	His
385				390				395				400			
His	Asn	Ser	Asp	Phe	His	Ile	Pro	Lys	Ala	Thr	Leu	Lys	Asp	Ser	Gly
405				410				415				420			
Ser	Tyr	Phe	Cys	Arg	Gly	Leu	Val	Gly	Ser	Lys	Asn	Val	Ser	Ser	Glu
420				425				430				435			
Thr	Val	Asn	Ile	Thr	Ile	Thr	Gln	Gly	Leu	Ala	Val	Ser	Thr	Ile	Ser
435				440				445				450			
Ser	Phe	Ser	Pro	Pro	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala
450				455				460				465			
Pro	Thr	Ser	Ser	Ser	Thr	Lys	Lys	Thr	Gln	Leu	Gln	Leu	Glu	His	Leu
465				470				475				480			

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Leu	Leu	Asp	Leu	Gln	Met	Ile	Leu	Asn	Gly	Ile	Asn	Asn	Tyr	Lys	Asn
				485					490					495	
Pro	Lys	Leu	Thr	Arg	Met	Leu	Thr	Phe	Lys	Phe	Tyr	Met	Pro	Lys	Lys
			500					505					510		
Ala	Thr	Glu	Leu	Lys	His	Leu	Gln	Cys	Leu	Glu	Glu	Glu	Leu	Lys	Pro
		515					520					525			
Leu	Glu	Glu	Val	Leu	Asn	Leu	Ala	Gln	Ser	Lys	Asn	Phe	His	Leu	Arg
	530					535					540				
Pro	Arg	Asp	Leu	Ile	Ser	Asn	Ile	Asn	Val	Ile	Val	Leu	Glu	Leu	Lys
545					550					555					560
Gly	Ser	Glu	Thr	Thr	Phe	Met	Cys	Glu	Tyr	Ala	Asp	Glu	Thr	Ala	Thr
				565					570					575	
Ile	Val	Glu	Phe	Leu	Asn	Arg	Trp	Ile	Thr	Phe	Cys	Gln	Ser	Ile	Ile
			580					585					590		
Ser	Thr	Leu	Thr	His	His	His	His	His	His						
		595					600								

<210> SEQ ID NO 86

<211> LENGTH: 958

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 86

Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ala
1				5					10					15	
Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr
		20						25					30		
Ser	Ile	Gln	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Arg	Leu	Glu	Trp	Ile
		35					40					45			
Gly	Tyr	Ile	Asn	Pro	Ser	Ser	Gly	Tyr	Ile	Lys	Tyr	Asn	Gln	His	Phe
	50					55					60				
Arg	Gly	Arg	Ala	Thr	Leu	Thr	Ala	Asp	Arg	Ser	Ala	Ser	Thr	Ala	Tyr
65					70				75					80	
Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
			85					90						95	
Ala	Arg	Gly	Asn	Ser	Gly	Ser	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu
		100						105					110		
Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu
		115					120					125			
Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys
	130					135					140				
Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser
145					150					155				160	
Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser
			165					170						175	
Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser
		180						185					190		
Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn
	195						200					205			
Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Gly	Gly	Gly	Gly
	210					215					220				

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Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ala	His	Lys	Ser
225					230					235					240
Glu	Val	Ala	His	Arg	Phe	Lys	Asp	Leu	Gly	Glu	Glu	Asn	Phe	Lys	Ala
				245					250					255	
Leu	Val	Leu	Ile	Ala	Phe	Ala	Gln	Tyr	Leu	Gln	Gln	Cys	Pro	Phe	Glu
			260					265					270		
Asp	His	Val	Lys	Leu	Val	Asn	Glu	Val	Thr	Glu	Phe	Ala	Lys	Thr	Cys
		275					280					285			
Val	Ala	Asp	Glu	Ser	Ala	Glu	Asn	Cys	Asp	Lys	Ser	Leu	His	Thr	Leu
	290					295					300				
Phe	Gly	Asp	Lys	Leu	Cys	Thr	Val	Ala	Thr	Leu	Arg	Glu	Thr	Tyr	Gly
305					310					315					320
Glu	Met	Ala	Asp	Cys	Cys	Ala	Lys	Gln	Glu	Pro	Glu	Arg	Asn	Glu	Cys
			325						330					335	
Phe	Leu	Gln	His	Lys	Asp	Asp	Asn	Pro	Asn	Leu	Pro	Arg	Leu	Val	Arg
			340					345					350		
Pro	Glu	Val	Asp	Val	Met	Cys	Thr	Ala	Phe	His	Asp	Asn	Glu	Glu	Thr
		355					360					365			
Phe	Leu	Lys	Lys	Tyr	Leu	Tyr	Glu	Ile	Ala	Arg	Arg	His	Pro	Tyr	Phe
	370					375					380				
Tyr	Ala	Pro	Glu	Leu	Leu	Phe	Phe	Ala	Lys	Arg	Tyr	Lys	Ala	Ala	Phe
385					390					395					400
Thr	Glu	Cys	Cys	Gln	Ala	Ala	Asp	Lys	Ala	Ala	Cys	Leu	Leu	Pro	Lys
				405					410					415	
Leu	Asp	Glu	Leu	Arg	Asp	Glu	Gly	Lys	Ala	Ser	Ser	Ala	Lys	Gln	Arg
			420					425					430		
Leu	Lys	Cys	Ala	Ser	Leu	Gln	Lys	Phe	Gly	Glu	Arg	Ala	Phe	Lys	Ala
		435					440					445			
Trp	Ala	Val	Ala	Arg	Leu	Ser	Gln	Arg	Phe	Pro	Lys	Ala	Glu	Phe	Ala
	450					455					460				
Glu	Val	Ser	Lys	Leu	Val	Thr	Asp	Leu	Thr	Lys	Val	His	Thr	Glu	Cys
465					470					475					480
Cys	His	Gly	Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp	Arg	Ala	Asp	Leu	Ala
				485					490					495	
Lys	Tyr	Ile	Cys	Glu	Asn	Gln	Asp	Ser	Ile	Ser	Ser	Lys	Leu	Lys	Glu
			500					505					510		
Cys	Cys	Glu	Lys	Pro	Leu	Leu	Glu	Lys	Ser	His	Cys	Ile	Ala	Glu	Val
		515					520					525			
Glu	Asn	Asp	Glu	Met	Pro	Ala	Asp	Leu	Pro	Ser	Leu	Ala	Ala	Asp	Phe
	530					535					540				
Val	Glu	Ser	Lys	Asp	Val	Cys	Lys	Asn	Tyr	Ala	Glu	Ala	Lys	Asp	Val
545					550					555					560
Phe	Leu	Gly	Met	Phe	Leu	Tyr	Glu	Tyr	Ala	Arg	Arg	His	Pro	Asp	Tyr
				565					570					575	
Ser	Val	Val	Leu	Leu	Leu	Arg	Leu	Ala	Lys	Thr	Tyr	Glu	Thr	Thr	Leu
			580					585					590		
Glu	Lys	Cys	Cys	Ala	Ala	Ala	Asp	Pro	His	Glu	Cys	Tyr	Ala	Lys	Val
		595					600					605			
Phe	Asp	Glu	Phe	Lys	Pro	Leu	Val	Glu	Glu	Pro	Gln	Asn	Leu	Ile	Lys
	610					615					620				
Gln	Asn	Cys	Glu	Leu	Phe	Glu	Gln	Leu	Gly	Glu	Tyr	Lys	Phe	Gln	Asn

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625	630	635	640
Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro			
	645	650	655
Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys			
	660	665	670
Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu			
	675	680	685
Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val			
	690	695	700
Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg			
	705	710	715
Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu			
	725	730	735
Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser			
	740	745	750
Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val			
	755	760	765
Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp			
	770	775	780
Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu			
	785	790	795
Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala			
	805	810	815
Ala Leu Gly Leu Gly Gly Gly Gly Ser Ala Pro Thr Ser Ser Ser Thr			
	820	825	830
Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met			
	835	840	845
Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met			
	850	855	860
Leu Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His			
	865	870	875
Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn			
	885	890	895
Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser			
	900	905	910
Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe			
	915	920	925
Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn			
	930	935	940
Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Thr			
	945	950	955

<210> SEQ ID NO 87

<211> LENGTH: 4

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 87

Gly Gly Gly Ser

1

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<210> SEQ ID NO 88
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 88

Gly Gly Gly Gly Ser
1 5

<210> SEQ ID NO 89
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 89

Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5

<210> SEQ ID NO 90
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 90

Ser Gly Gly Gly Ser
1 5

<210> SEQ ID NO 91
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 91

Asp Lys Thr His Thr Cys Pro Pro Ser Cys Ala Pro Glu
1 5 10

<210> SEQ ID NO 92
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 92

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro Pro Ser Pro Ala
1 5 10 15

Pro Glu

<210> SEQ ID NO 93
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 93

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Ser Val Glu Ser Pro Pro Ser Pro
1 5

<210> SEQ ID NO 94
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 94

Glu Arg Lys Ser Ser Val Glu Ser Pro Pro Ser Pro
1 5 10

<210> SEQ ID NO 95
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 95

Pro Pro Ser Pro Ser Ser Pro
1 5

<210> SEQ ID NO 96
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 96

Glu Ser Lys Tyr Gly Pro Pro Ser Pro Ser Ser Pro
1 5 10

<210> SEQ ID NO 97
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 97

Gly Phe Ser Gly
1

<210> SEQ ID NO 98
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 98

Gly Lys Val Ser
1

<210> SEQ ID NO 99
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

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<400> SEQUENCE: 99

Gly Trp Ile Gly

1

<210> SEQ ID NO 100

<211> LENGTH: 4

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 100

Gly Lys Lys Trp

1

<210> SEQ ID NO 101

<211> LENGTH: 4

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 101

Gly Ala Tyr Met

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<210> SEQ ID NO 102

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 102

Val Pro Leu Ser Leu Tyr Ser Gly

1

5

<210> SEQ ID NO 103

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 103

Gly Pro Gln Gly Ile Ala Gly Gln

1

5

<210> SEQ ID NO 104

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 104

Val Pro Met Ser Met Arg Gly Gly

1

5

<210> SEQ ID NO 105

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 105

Ile Pro Val Ser Leu Arg Ser Gly
1 5

<210> SEQ ID NO 106

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 106

Arg Pro Phe Ser Met Ile Met Gly
1 5

<210> SEQ ID NO 107

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 107

Val Pro Leu Ser Leu Thr Met Gly
1 5

<210> SEQ ID NO 108

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 108

Ile Pro Glu Ser Leu Arg Ala Gly
1 5

<210> SEQ ID NO 109

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<220> FEATURE:

<221> NAME/KEY: MISC_FEATURE

<222> LOCATION: (7) .. (7)

<223> OTHER INFORMATION: Nva

<400> SEQUENCE: 109

Arg Pro Lys Pro Val Glu Xaa Trp Arg Lys
1 5 10

<210> SEQ ID NO 110

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Unknown

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

<220> FEATURE:

<221> NAME/KEY: MISC_FEATURE

<222> LOCATION: (7) .. (7)

<223> OTHER INFORMATION: Nva

<400> SEQUENCE: 110

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Arg Pro Lys Pro Tyr Ala Xaa Trp Met Lys
1 5 10

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<210> SEQ ID NO 111
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
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<400> SEQUENCE: 111

Pro Gln Gly Ile Ala Gly Gln Arg
1 5

```
<210> SEQ ID NO 112
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
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<400> SEQUENCE: 112

Pro Leu Gly Ile Ala Gly Arg
1 5

```
<210> SEQ ID NO 113
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
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<400> SEQUENCE: 113

Gly Pro Leu Gly Pro
1 5

```
<210> SEQ ID NO 114
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
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<400> SEQUENCE: 114

Gly Pro Ile Gly Pro
1 5

1. A fusion molecule, comprising:
 - (a) a cytokine or growth factor molecule; and
 - (b) a regulatory T cell (Treg) targeting moiety comprising an antigen-binding fragment of an antibody having an affinity to an antigen on the surface of a Treg;wherein the cytokine or growth factor molecule is operatively linked to the antigen-binding fragment.
2. (canceled)
3. The fusion molecule of claim 1, wherein the targeting moiety comprises a bispecific molecule having an antigen-binding fragment specific for CD4 and an antigen-binding fragment specific for CD25.
4. The fusion molecule of claim 1, wherein the antigen is one or more of CD4, CD45, CD3, CD2, CD25, CD127, CD197 (CCR7), CXCR3, CXCR4, CXCR5, CD38, CD27.

CCR4, CCR5, CD137, CD39, CCR4, CCR5, CCR6 (CD196), CCR8, CCR10, OX40, GITR, CTLA4, LAG3, CD73, CD103, CD62L, CCR2, CCR9, Neuropilin 1 (NRP1), CD8, CD11a, CD18, or a variant of any of the foregoing.

5-6. (canceled)

7. The fusion molecule of claim 1, wherein the cytokine or growth factor molecule is one or more of IL-15, IL-2, IL-18, IL-27, IL-10, IL-35, Amphiregulin, IL-33, TGF- β , IL-7, IL-21, IL-6, IL-12, or IL-23, or a variant of any of the foregoing.

8. The fusion molecule of claim 1, wherein the Treg is a healthy and/or non-malignant Treg.

9. The fusion molecule of claim 1, further comprising a linker for operably linking the cytokine or growth factor molecule and the targeting moiety.

10. The fusion molecule of claim **9**, wherein the linker is selected from one or more of a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, or a non-helical linker.

11. The fusion molecule of claim **10**, wherein the peptide linker is a human serum albumin or a variant thereof.

12. The fusion molecule of claim **9**, wherein the linker, at either end, is fused to the cytokine or growth factor molecule and the targeting moiety via a peptide comprising Gly and Ser.

13. The fusion molecule of claim **12**, wherein the peptide is a (GGGS)_N (SEQ ID NO: 87) or (GGGS)_N (SEQ ID NO: 88) linker, where N indicates the number of repeats of the motif and is an integer selected from 1-10.

14. The fusion molecule of claim **1**, wherein the antigen-binding fragment is a Fab fragment comprising a light chain and a heavy chain fragment linked by a disulfide bond, and wherein the cytokine or growth factor molecule is operably linked to the Fab fragment at a C-terminus of the light chain, an N-terminus of the light chain, a C-terminus of the heavy chain fragment, or an N-terminus of the heavy chain fragment.

15-19. (canceled)

20. A pharmaceutical composition comprising the fusion molecule of claim **1** and a pharmaceutically acceptable carrier, excipient, or stabilizer.

21. A modified Treg, comprising a healthy and/or non-malignant Treg and the fusion molecule of claim **1** bound or targeted thereto.

22. A method for ex vivo expansion of Tregs, comprising providing a population of PBMCs from a subject, and selectively expanding Tregs therein in the presence of a plurality of fusion molecules of claim **1**, thereby producing a plurality of expanded Tregs.

23. A method for providing immunosuppressive therapy, comprising administering to a subject in need thereof a composition comprising a plurality of fusion molecules of claim **1**.

24. The method of claim **23**, wherein the immunosuppressive therapy is used to treat diseases selected from allo-immune diseases, auto-immune diseases, allergy, and inflammatory diseases.

25. The method of claim **24**, wherein the allo-immune diseases include organ transplant rejection, graft versus host

disease (GVHD) (e.g., post allogeneic hematopoietic stem cell transplant, HSCT) and GVHD post allogeneic stem cell transplantation (SCT) the auto-immune diseases include Type 1 diabetes, Multiple Sclerosis and Alopecia, and the inflammatory diseases include Inflammatory Bowel Disease, Rheumatoid Arthritis and Lupus.

26-35. (canceled)

36. A method of preparing modified Tregs, comprising:

- (a) providing a population of Tregs; and
- (b) incubating the fusion molecule of claim **1** with the population of Tregs so as to permit targeted binding of the fusion molecule thereto, thereby producing a population of Tregs having fusion molecules bound on the cell surface.

37-38. (canceled)

39. A method for the suppressing or preventing an immune response in a human subject, the method comprising administering to the human subject a cell therapeutic composition, the composition comprising:

- (a) a plurality of fusion molecules, each fusion molecule comprising
 - (i) a cytokine or growth factor molecule; and
 - (ii) a Treg targeting moiety having an affinity to a cell surface antigen of a Treg; and

(b) a population of Tregs,

wherein the plurality of fusion molecules are bound to the surface of the Tregs, and wherein the cytokine or growth factor molecule acts in vivo upon the population of Tregs in the human subject to suppress or prevent an immune response in the human subject.

40-46. (canceled)

47. A composition comprising:

- (a) a fusion molecule comprising
 - (i) a cytokine or growth factor molecule; and
 - (ii) an immune cell targeting moiety having an affinity to a Treg cell surface antigen;
- (b) a Treg expressing or otherwise displaying the cell surface antigen, wherein the fusion molecule is bound to the surface of the Treg through interaction with the cell surface antigen; and
- (c) a nanoparticle, nanogel, or liposome.

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