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# DESCRIPTION

## INTRODUCTION

**[0001]** Throughout this application various publications are referred to in square brackets. Full citations for these references may be found at the end of the specification.

**[0002]** The rapid progress over the past decade in the development of high throughput technologies for clinically relevant biomarker discovery has been paralleled by the stepwise development and application of biologics, drugs in which the active substance is produced by or extracted from a biological source (e.g., monoclonal antibodies, therapeutic proteins, and peptides), and has revolutionized the treatment of immune-borne conditions. However, current biologic therapies are prompting safety regulatory actions at double the rate of their synthetic counterparts (17% for biologics, 8.5% synthetics) [1]. This is thought to manifest from the mode of action of these immune-modulating biologics: *global* immunosuppression in the case of autoimmunity (e.g., Humira [2]) and *global* immunostimulation for the treatment of cancers (e.g., Yervoy [3]). These treatments do not adequately restrict immunomodulation to pathogenically relevant cells and as a result, predispose patients to potentially *deadly* infections and a host of troubling side effects [4-6]. Further, the moderate efficacy and safety profiles of these drugs [7] has elicited a recent trend toward targeted therapeutics. First generation "targeted" biologics direct their effects on more restricted T cell subsets (e.g., antibodies and protein therapeutics such as anti-4-1BB, anti-CD27, LAG-3, and TIM-3) [8-11]. However, like previous therapies, these "1<sup>st</sup>-gen" efforts remain unable to target only disease-relevant cells.

**[0003]** At the core of the molecular events comprising an adaptive immune response is the engagement of the T cell receptor (TCR) with a small peptide antigen non-covalently presented by a major histocompatibility complex (MHC) molecule. This represents the immune system's *targeting* mechanism and is a requisite molecular interaction for T cell activation and effector function. Following epitope-specific cell targeting, the recruited T cells are activated through general engagement of costimulatory molecules found on the antigen presenting cell. Both signals are required to drive T cell specificity and activation or inhibition. Importantly, during T cell development, a genomic editing process results in the expression of a *unique* TCR on every T cell [12], whereas the costimulatory molecule is generally expressed on all T cells (or large T cell 'subsets'). Current approaches rely almost exclusively on the general engagement of the costimulatory molecule, resulting in "global therapies". These global immunotherapies are incredibly potent but indiscriminately target T cells leading to significant toxicity. If costimulatory molecules could preferentially bind to T cells bearing disease-relevant TCRs, their potency would advance from a liability to a strength.

**[0004]** There exist a number of approaches for T cell modulation, which include the use of soluble costimulatory molecules generally expressed as Fc fusions or antibodies directed at

costimulatory molecules capable of blocking costimulatory function [13, 14], antibody-drug conjugates (ADCs) [15], bi-specific antibodies (BsAbs) [16, 17] and free peptide antigens [18]. Notably, ADCs (often referred to as magic bullets) promise the targeted delivery of toxins (or other drug payloads) directly to pathologic cells. However ADCs currently suffer from a lack of preferred biomarkers for antibody targeting and poor internalization rates as only ~1.5% of the administered dose is found inside tumor cells, with internalization often being required for cell killing. Bispecific antibodies provide an attractive opportunity to combine additive and synergistic effects of multiple mAbs, and can be used to bridge tumor cells with T cells [17], and therefore do not require internalization to illicit a response. Although bispecific antibodies have been developed to have bivalent interactions with two different antigens [19], these constructs still lack modularity and suffer reduced affinity compared to the parental mAb [20]. Adoptive T cell (CAR-T) therapy partially addresses these issues, and is an attractive alternative to the traditional therapies described above [21]. CAR-T uses genetically modified primary T cells bearing chimeric antigen receptors (CARs) on their surface: patient's T cells are extracted, purified and genetically modified to target tumor specific antigens through the use of CARs. The CAR generally has an external single chain variable domain (an antibody fragment) that targets pathologic cells but harbors traditional costimulatory molecule cytoplasmic domains. Once the engineered T cells bind to target antigen, the internal stimulatory domains provide the necessary signals for the T cell to become fully active. In this fully active state, the T cells can more effectively proliferate and attack cancer cells. Tempering this response so as to avoid cytokine release syndrome and associated side effects, along with scalability issues (e.g., the significant expense and difficulty associated with the T-cell extraction and modification) currently prevent this technology from entering mainstream use [22].

**[0005]** Biologics, also known as biopharmaceuticals, are drugs in which the active substance is produced by or extracted from a biological source (in contrast to "small-molecule" drugs). Biologics are relatively recent additions to the global therapeutic market, being for the most part recombinant proteins produced through genetic engineering; these include monoclonal antibodies, therapeutic proteins, and peptides. Most of the currently marketed biologic drugs are used to relieve patients suffering from chronic diseases, such as cancer, diabetes, cardiovascular diseases, infertility and cystic fibrosis. The global biologics market was valued at \$163 billion in 2012 and is expected to reach \$252 billion by 2017 supporting a five-year compound annual growth rate of 9%. Driving this growth is the need for a more extensive drug pipeline, identification of attractive targets against challenging diseases and a push to pursue follow-on biologics (biosimilars, generic biologics) exemplified by the recent introduction of an abbreviated FDA approval pathway.

**[0006]** The present invention addresses the need for precision therapeutics for immunotherapy and autoimmunity - tailored therapeutics that clonally target only the disease-related T cells for upregulation (e.g., in the case of cancer) or suppression (e.g., in the case of autoimmunity) as opposed to the global and "pseudo-targeted" modulators currently on the market or in development.

## SUMMARY

**[0007]** This invention provides a multimeric polypeptide comprising a heterodimeric polypeptide comprising

(a) a first polypeptide comprising, in order from N-terminus to C-terminus:

1. i) a peptide epitope; and
2. ii) a first major histocompatibility complex (MHC) polypeptide;

b) a second polypeptide comprising a second MHC polypeptide, and

c) at least one immunomodulatory polypeptide,

wherein the first and/or the second polypeptide comprises the at least one immunomodulatory polypeptide,

wherein the first MHC polypeptide is a  $\beta$ 2-microglobulin polypeptide, and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide,

wherein the peptide epitope is a cancer-associated epitope, and optionally, wherein the multimeric polypeptide comprises an immunoglobulin (Ig) Fc polypeptide or a non-Ig scaffold..

**[0008]** The invention further provides a method of selectively modulating the activity of a T cell that binds to a cancer-associated epitope, the method comprising contacting the T cell *in vitro* with the multimeric polypeptide of the invention, wherein said contacting selectively modulates the activity of the T cell.

**[0009]** The invention also provides a composition comprising the multimeric polypeptide of the invention and a pharmaceutically acceptable excipient.

**[0010]** This disclosure also provides recombinant polypeptide comprising a sequence of amino acids identical to a first B2M leader sequence contiguous with a candidate epitope peptide contiguous with a first amino acid linker sequence contiguous with a sequence of amino acids identical to a human native B2M peptide sequence contiguous with a second amino acid linker sequence contiguous with a second B2M leader sequence contiguous with a T cell modulatory domain peptide sequence contiguous with a third amino acid linker contiguous with a sequence of amino acids identical to a MHC heavy chain contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain.

**[0011]** Also provided is a method of inhibiting a T cell clone which recognizes an epitope peptide comprising contacting a T cell of the clone with a recombinant peptide as described herein, wherein the recombinant peptide comprises the epitope peptide and comprises a T cell modulatory domain which is an inhibitory domain, in an amount effective to inhibit a T cell clone.

**[0012]** Also provided is a method of treating an autoimmune disorder by inhibiting a self-reactive T cell clone which recognizes an epitope peptide comprising contacting a T cell of the

clone with a recombinant peptide as described herein, wherein the recombinant peptide comprises the epitope peptide and comprises a T cell modulatory domain which is an inhibitory domain, in an amount effective to treat an autoimmune disorder.

**[0013]** Also provided is a method of stimulating a T cell clone which recognizes an epitope peptide comprising contacting a T cell of the clone with a recombinant peptide as described herein, wherein the recombinant peptide comprises the epitope peptide and comprises a T cell modulatory domain which is an stimulatory domain, in an amount effective to stimulate a T cell clone.

**[0014]** Also provided is a method of treating a cancer by stimulating a T cell clone which recognizes an epitope peptide on a cancer comprising contacting a T cell of the clone with a recombinant peptide as described herein, wherein the recombinant peptide comprises the epitope peptide and comprises a T cell modulatory domain which is an stimulatory domain, in an amount effective to treat the cancer.

**[0015]** Also provided is a recombinant polypeptide construct comprising (i) a candidate epitope peptide bound by a first amino acid linker sequence contiguous with a sequence of amino acids comprising a sequence identical to a human native B2M peptide sequence contiguous with a second amino acid linker sequence contiguous with a T cell modulatory domain peptide, wherein (i) is bound by one, or more than one, disulfide bond to (ii) a sequence of amino acids having the sequence of a MHC heavy chain contiguous with a third amino acid linker sequence contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain.

**[0016]** Also provided is recombinant polypeptide construct comprising (i) a candidate epitope peptide bound by a first amino acid linker sequence contiguous with a sequence of amino acids comprising a sequence identical to a human native B2M peptide sequence, wherein (i) is bound by one, or more than one, disulfide bond to (ii) a T cell modulatory domain peptide contiguous with a second amino acid linker sequence contiguous with a sequence of amino acids having the sequence of a MHC heavy chain contiguous a third amino acid linker sequence contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain.

**[0017]** Also provided is a protein comprising two of the recombinant polypeptide constructs described herein joined by one or more disulfide bonds between the respective immunoglobulin Fc domains thereof.

**[0018]** Also provided is a protein comprising two of the recombinant polypeptide constructs described herein joined by one or more disulfide bonds between the respective immunoglobulin Fc domains thereof.

**[0019]** This disclosure provides an isolated suspension-adapted cell transduced by or transfected with a heterologous nucleic acid comprising, in 5' to 3' order a sequence encoding a recombinant polypeptide as described herein.

**[0020]** The present disclosure provides a recombinant polypeptide comprising a sequence of amino acids identical to a first B2M leader sequence contiguous with a candidate epitope peptide contiguous with a first amino acid linker sequence contiguous with a sequence of amino acids identical to a human native B2M peptide sequence contiguous with a second amino acid linker sequence contiguous with a T cell modulatory domain peptide sequence contiguous with a third amino acid linker contiguous with a second B2M leader sequence contiguous with a sequence of amino acids identical to a MHC heavy chain contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain. In some cases, the candidate epitope comprises 7-20 amino acids. In some cases, the third amino acid linker is self-cleaving. In some cases, the second amino acid linker is self-cleaving. In some cases, the self-cleaving peptide is a viral 2A peptide or has the sequence thereof. In some cases, the first and/or second B2M leader sequence has the sequence of human B2M leader sequence. In some cases, the MHC heavy chain is a human MHC heavy chain. In some cases, the MHC heavy chain is an MHC I molecule. In some cases, the MHC heavy chain is an HLA-A02:01. In some cases, the MHC heavy chain is an MHC II molecule. In some cases, the immunoglobulin Fc domain is an IgG Fc domain. In some cases, the immunoglobulin Fc domain is an IgA Fc domain. In some cases, the immunoglobulin Fc domain is an IgM Fc domain. In some cases, the immunoglobulin Fc domain is a human immunoglobulin Fc domain. In some cases, the immunoglobulin Fc domain is an IgG1 Fc domain. In some cases, the recombinant polypeptide comprises a His-8 tag contiguous with the C-terminal thereof. In some cases, the T cell modulatory domain is an inhibitory domain. In some cases, the T cell modulatory domain is a stimulating domain. In some cases, the T cell modulatory domain is an antibody, and antibody fragment, a peptide ligand, a T cell costimulatory peptide, a cytokine or a toxin. In some cases, the T cell modulatory domain comprises a PD-L1 peptide, the Ig variable domain of a PD-L1 peptide, the T cell modulatory domain comprises 4-1BBL, the T cell modulatory domain comprises B7-1W88A, or the T cell modulatory domain comprises anti-CD28 single chain Fv. In some cases, the recombinant polypeptide comprises a mutation in a human native B2M peptide sequence thereof and in the Heavy Chain sequence thereof so as to effect a disulfide bond between the B2M peptide sequence and Heavy Chain sequence.

**[0021]** The present disclosure provides a recombinant polypeptide comprising a sequence of amino acids identical to a first B2M leader sequence contiguous with a candidate epitope peptide contiguous with a first amino acid linker sequence contiguous with a sequence of amino acids identical to a human native B2M peptide sequence contiguous with a second amino acid linker sequence contiguous with a second B2M leader sequence contiguous with a T cell modulatory domain peptide sequence contiguous with a third amino acid linker contiguous with a sequence of amino acids identical to a MHC heavy chain contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain. In some cases, the candidate epitope comprises 7-20 amino acids. In some cases, the third amino acid linker is self-cleaving. In some cases, the second amino acid linker is self-cleaving. In some cases, the self-cleaving peptide is a viral 2A peptide or has the sequence thereof. In some cases, the first and/or second B2M leader sequence has the sequence of human B2M leader sequence. In some cases, the MHC heavy chain is a human MHC heavy chain. In some cases, the MHC

heavy chain is an MHC I molecule. In some cases, the MHC heavy chain is an HLA-A02:01. In some cases, the MHC heavy chain is an MHC II molecule. In some cases, the immunoglobulin Fc domain is an IgG Fc domain. In some cases, the immunoglobulin Fc domain is an IgA Fc domain. In some cases, the immunoglobulin Fc domain is an IgM Fc domain. In some cases, the immunoglobulin Fc domain is a human immunoglobulin Fc domain. In some cases, the immunoglobulin Fc domain is an IgG1 Fc domain. In some cases, the recombinant polypeptide comprises a His-8 tag contiguous with the C-terminal thereof. In some cases, the T cell modulatory domain is an inhibitory domain. In some cases, the T cell modulatory domain is a stimulating domain. In some cases, the T cell modulatory domain is an antibody, and antibody fragment, a peptide ligand, a T cell costimulatory peptide, a cytokine or a toxin. In some cases, the T cell modulatory domain comprises a PD-L1 peptide, the Ig variable domain of a PD-L1 peptide, the T cell modulatory domain comprises 4-1BBL, the T cell modulatory domain comprises B7-1W88A, or the T cell modulatory domain comprises anti-CD28 single chain Fv. In some cases, the recombinant polypeptide comprises a mutation in a human native B2M peptide sequence thereof and in the Heavy Chain sequence thereof so as to effect a disulfide bond between the B2M peptide sequence and Heavy Chain sequence.

**[0022]** In some cases, the recombinant polypeptide comprises a mutation in a human native B2M peptide sequence thereof and in the Heavy Chain sequence thereof so as to effect a disulfide bond between the B2M peptide sequence and Heavy Chain sequence. In some cases, the Heavy Chain sequence is an HLA and wherein the disulfide bond links one of the following pairs of residues: B2M residue 12, HLA residue 236; B2M residue 12, HLA residue 237; B2M residue 8, HLA residue 234; B2M residue 10, HLA residue 235; B2M residue 24, HLA residue 236; B2M residue 28, HLA residue 232; B2M residue 98, HLA residue 192; B2M residue 99, HLA residue 234; B2M residue 3, HLA residue 120; B2M residue 31, HLA residue 96; B2M residue 53, HLA residue 35; B2M residue 60, HLA residue 96; B2M residue 60, HLA residue 122; B2M residue 63, HLA residue 27; B2M residue Arg3, HLA residue Gly120; B2M residue His31, HLA residue Gln96; B2M residue Asp53, HLA residue Arg35; B2M residue Trp60, HLA residue Gln96; B2M residue Trp60, HLA residue Asp122; B2M residue Tyr63, HLA residue Tyr27; B2M residue Lys6, HLA residue Glu232; B2M residue Gln8, HLA residue Arg234; B2M residue Tyr10, HLA residue Pro235; B2M residue Ser11, HLA residue Gln242; B2M residue Asn24, HLA residue Ala236; B2M residue Ser28, HLA residue Glu232; B2M residue Asp98, HLA residue His192; and B2M residue Met99, HLA residue Arg234.

**[0023]** In some cases, the recombinant polypeptide comprises a mutation in a human native B2M peptide sequence thereof and in the Heavy Chain sequence thereof so as to effect a disulfide bond between the B2M peptide sequence and Heavy Chain sequence. In some cases, the Heavy Chain sequence is an HLA and wherein the disulfide bond links one of the following pairs of residues: first linker position Gly 2, Heavy Chain (HLA) position Tyr 84; Light Chain (B2M) position Arg 12, HLA Ala236; and/or B2M residue Arg12, HLA residue Gly237.

**[0024]** In some cases, the T cell modulatory domain is an inhibitory domain. In some cases, the T cell modulatory domain is a stimulating domain. In some cases, the T cell modulatory domain is an antibody, and antibody fragment, a peptide ligand, a T cell costimulatory peptide,

a cytokine or a toxin. In some cases, the T cell modulatory domain comprises a PD-L1 peptide, the Ig variable domain of a PD-L1 peptide, the T Cell modulatory domain comprises 4-1BBL, the T Cell modulatory domain comprises B7- 1W88A, or the T cell modulatory domain comprises anti-CD28 single chain Fv.

**[0025]** The present disclosure provides a nucleic acid encoding any of the recombinant polypeptides described above, or elsewhere herein. The present disclosure provides a cell transformed with a nucleic acid encoding any of the recombinant polypeptides described above, or elsewhere herein.

**[0026]** The present disclosure provides a method of inhibiting a T cell clone which recognizes an epitope peptide comprising contacting a T cell of the clone with a recombinant peptide of any of described above, or elsewhere herein, wherein the recombinant peptide comprises the epitope peptide and comprises a T cell modulatory domain which is an inhibitory domain, in an amount effective to inhibit a T cell clone.

**[0027]** The present disclosure provides a method of treating an autoimmune disorder by inhibiting a self-reactive T cell clone which recognizes an epitope peptide comprising contacting a T cell of the clone with a recombinant peptide described above, or elsewhere herein, wherein the recombinant peptide comprises the epitope peptide and comprises a T cell modulatory domain which is an inhibitory domain, in an amount effective to treat an autoimmune disorder.

**[0028]** The present disclosure provides a method of stimulating a T cell clone which recognizes an epitope peptide comprising contacting a T cell of the clone with a recombinant peptide described above, or elsewhere herein, wherein the recombinant peptide comprises the epitope peptide and comprises a T cell modulatory domain which is an stimulatory domain, in an amount effective to stimulate a T cell clone.

**[0029]** The present disclosure provides a method of treating a cancer by stimulating a T cell clone which recognizes an epitope peptide on a cancer comprising contacting a T cell of the clone with a recombinant peptide described above, or elsewhere herein, wherein the recombinant peptide comprises the epitope peptide and comprises a T cell modulatory domain which is an stimulatory domain, in an amount effective to treat the cancer.

**[0030]** The present disclosure provides a recombinant polypeptide construct comprising (i) a candidate epitope peptide bound by a first amino acid linker sequence contiguous with a sequence of amino acids comprising a sequence identical to a human native B2M peptide sequence contiguous with a second amino acid linker sequence contiguous with a T cell modulatory domain peptide, wherein (i) is bound by one, or more than one, disulfide bond to (ii) a sequence of amino acids having the sequence of a MHC heavy chain contiguous with a third amino acid linker sequence contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain. The present disclosure provides a protein comprising two of the recombinant polypeptide constructs joined by one or more disulfide bonds between the

respective immunoglobulin Fc domains thereof.

**[0031]** The present disclosure provides a recombinant polypeptide construct comprising (i) a candidate epitope peptide bound by a first amino acid linker sequence contiguous with a sequence of amino acids comprising a sequence identical to a human native B2M peptide sequence, wherein (i) is bound by one, or more than one, disulfide bond to (ii) a T cell modulatory domain peptide contiguous with a second amino acid linker sequence contiguous with a sequence of amino acids having the sequence of a MHC heavy chain contiguous a third amino acid linker sequence contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain. The present disclosure provides a protein comprising two of the recombinant polypeptide constructs joined by one or more disulfide bonds between the respective immunoglobulin Fc domains thereof.

**[0032]** The present disclosure provides multimeric polypeptides comprising at least a first polypeptide and a second polypeptide, where the first polypeptide comprises, in order from N-terminus to C-terminus: i) an epitope; and ii) a first major histocompatibility complex (MHC) polypeptide; and where the second polypeptide comprises, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an immunoglobulin (Ig) Fc polypeptide, where the multimeric polypeptide comprises an immunomodulatory domain at the C-terminus of the first polypeptide or at the N-terminus of the second polypeptide. The present disclosure provides nucleic acids comprising nucleotide sequences encoding the multimeric polypeptide. The present disclosure provides recombinant expression vectors comprising the nucleic acids. The present disclosure provides genetically modified host cells, where the genetically modified host cells are genetically modified with a nucleic acid of the present disclosure or a recombinant expression vector of the present disclosure. The present disclosure provides compositions, including pharmaceutical compositions, comprising the multimeric polypeptides. The present disclosure provides methods of modulating an activity of a T cell, the methods involving contacting the T cell with a multimeric polypeptide of the present disclosure. The present disclosure provides methods of treatment involving administering to an individual in need thereof an effective amount of a multimeric polypeptide of the present disclosure. The present disclosure provides a container comprising a multimeric polypeptide of the present disclosure, or a composition (e.g., a pharmaceutical composition) comprising a multimeric polypeptide of the present disclosure.

**[0033]** The present disclosure provides a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an immunomodulatory domain; iii) a second MHC polypeptide; and ii) an Ig Fc polypeptide. The present disclosure provides a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; ii) a first MHC polypeptide; and iii) an immunomodulatory domain; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an immunoglobulin (Ig) Fc polypeptide. In some cases, the first MHC polypeptide is a  $\beta$ 2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain

polypeptide. In some cases, the  $\beta$ 2-microglobulin polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4. In some cases, the MHC class I heavy chain polypeptide is an HLA-A, an HLA-B, or an HLA-C, heavy chain. In some cases, the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, or 100%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:5. In some cases, the first MHC polypeptide is an MHC Class II alpha chain polypeptide; and wherein the second MHC polypeptide is an MHC class II beta chain polypeptide. In some cases, the epitope is a T-cell epitope. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide, an IgG2 Fc polypeptide, an IgG3 Fc polypeptide, an IgG4 Fc polypeptide, an IgA Fc polypeptide, or an IgM Fc polypeptide. In some cases, the Ig Fc polypeptide comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, or 100%, amino acid sequence identity to an amino acid sequence depicted in FIG. 24A-24C. In some cases, the first polypeptide and the second polypeptide are non-covalently associated. In some cases, the first polypeptide and the second polypeptide are covalently linked. In some cases, the covalent linkage is via a disulfide bond. In some cases, the first MHC polypeptide or a linker between the epitope and the first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, and wherein the disulfide linkage is between the first and the second Cys residues. In some cases, the multimeric polypeptide comprises a first linker interposed between the epitope and the first MHC polypeptide. In some cases, the immunomodulatory polypeptide is selected from a 4-1BBL polypeptide, a B7-1 polypeptide; a B7-2 polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a CD86 polypeptide, a PD-L1 polypeptide, a FasL polypeptide, and a PD-L2 polypeptide. In some cases, the first polypeptide or the second polypeptide comprises 2 or more immunomodulatory polypeptides. In some cases, the 2 or more immunomodulatory polypeptides are in tandem. In some cases, the multimeric polypeptide comprises a third polypeptide, wherein the third polypeptide comprises an immunomodulatory polypeptide comprising an amino acid sequence having at least 90% amino acid sequence identity to the immunomodulatory polypeptide of the first polypeptide. In some cases, the third polypeptide is covalently linked to the first polypeptide. In some cases, wherein the second polypeptide comprises, in order from N-terminus to C-terminus: i) the second MHC polypeptide; ii) the immunoglobulin (Ig) Fc polypeptide; and iii) an affinity tag.

**[0034]** The present disclosure provides a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) optionally an Ig Fc polypeptide or a non-Ig scaffold, wherein the multimeric polypeptide comprises optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig scaffold, wherein the multimeric polypeptide comprises one or more immunomodulatory domains, wherein the one or more immunomodulatory domain is: A) at the C-terminus of the first polypeptide; B) at the N-terminus of the second polypeptide; C) at the C-terminus of the second polypeptide; or D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide. In some cases, a multimeric polypeptide comprises a

single immunomodulatory polypeptide. In some cases, a multimeric polypeptide comprises two immunomodulatory polypeptides (e.g., two copies of the same immunomodulatory polypeptide). In some cases, a multimeric polypeptide comprises three immunomodulatory polypeptides (e.g., three copies of the same immunomodulatory polypeptide). In some cases, a multimeric polypeptide comprises four immunomodulatory polypeptides (e.g., four copies of the same immunomodulatory polypeptide). In some cases, a multimeric polypeptide comprises a single immunomodulatory polypeptide. In some cases, a multimeric polypeptide comprises two immunomodulatory polypeptides (e.g., two copies of the same immunomodulatory polypeptide). In some cases, a multimeric polypeptide comprises three immunomodulatory polypeptides (e.g., three copies of the same immunomodulatory polypeptide). In some cases, a multimeric polypeptide comprises four immunomodulatory polypeptides (e.g., four copies of the same immunomodulatory polypeptide). In some cases, the multimeric polypeptide comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; ii) a first MHC polypeptide; and iii) an immunomodulatory domain; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an Ig Fc polypeptide. In some cases, the multimeric polypeptide comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an immunomodulatory domain; iii) a second MHC polypeptide; and ii) an immunoglobulin (Ig) Fc polypeptide. In some cases, the multimeric polypeptide comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an Ig Fc polypeptide; and iii) an immunomodulatory domain. In some cases, the multimeric polypeptide comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an immunomodulatory domain. In some cases, the multimeric polypeptide comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an immunomodulatory domain; and ii) a second MHC polypeptide. In some cases, the multimeric polypeptide comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; ii) a first MHC polypeptide; and iii) an immunomodulatory domain; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide. In some cases, the non-Ig scaffold is an XTEN polypeptide, a transferrin polypeptide, an Fc receptor polypeptide, an elastin-like polypeptide, a silk-like polypeptide, or a silk-elastin-like polypeptide. In some cases, the first MHC polypeptide is a  $\beta$ 2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide. In some cases, the  $\beta$ 2-microglobulin polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4. In some cases, the MHC class I heavy chain polypeptide is an HLA-A, an HLA-B, or an HLA-C heavy chain. In some cases, the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:5. In some cases, the first MHC polypeptide is an MHC Class II alpha

chain polypeptide; and wherein the second MHC polypeptide is an MHC class II beta chain polypeptide. In some cases, the epitope is a T-cell epitope. In some cases, the multimeric polypeptide comprises an Fc polypeptide, and wherein the Ig Fc polypeptide is an IgG1 Fc polypeptide, an IgG2 Fc polypeptide, an IgG3 Fc polypeptide, an IgG4 Fc polypeptide, an IgA Fc polypeptide, or an IgM Fc polypeptide. In some cases, the Ig Fc polypeptide comprises an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, or at least 100%, amino acid sequence identity to an amino acid sequence depicted in FIG. 24A-24C. In some cases, the first polypeptide and the second polypeptide are non-covalently associated. In some cases, the first polypeptide and the second polypeptide are covalently linked. In some cases, the covalent linkage is via a disulfide bond. In some cases, the first MHC polypeptide or a linker between the epitope and the first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, and wherein the disulfide linkage is between the first and the second Cys residues. In some cases, the multimeric polypeptide comprises a first linker interposed between the epitope and the first MHC polypeptide. In some cases, the immunomodulatory polypeptide is selected from a 4-1BBL polypeptide, a B7-1 polypeptide; a B7-2 polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a CD86 polypeptide, a PD-L1 polypeptide, a FasL polypeptide, and a PD-L2 polypeptide. In some cases, the multimeric polypeptide comprises 2 or more immunomodulatory polypeptides. In some cases, the 2 or more immunomodulatory polypeptides are in tandem. In some cases, the multimeric polypeptide comprises a third polypeptide, wherein the third polypeptide comprises an immunomodulatory polypeptide comprising an amino acid sequence having at least 90% amino acid sequence identity to the immunomodulatory polypeptide of the first polypeptide or the second polypeptide. In some cases, the third polypeptide is covalently linked to the first polypeptide. In some cases, the second polypeptide comprises, in order from N-terminus to C-terminus: i) the second MHC polypeptide; ii) the Ig Fc polypeptide; and iii) an affinity tag.

**[0035]** The present disclosure provides a nucleic acid comprising nucleotide sequences encoding the polypeptide chains of a multimeric polypeptide of the present disclosure; in some cases, the nucleic acid is present in a recombinant expression vector. The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a recombinant polypeptide, i) wherein the recombinant polypeptide comprises, in order from N-terminus to C-terminus: a) an epitope; b) a first MHC polypeptide; c) an immunomodulatory polypeptide; d) a proteolytically cleavable linker or a ribosome skipping signal; e) a second MHC polypeptide; and f) an immunoglobulin (Ig) Fc polypeptide or a non-Ig-based scaffold; or ii) wherein the recombinant polypeptide comprises, in order from N-terminus to C-terminus: a) an epitope; b) a first MHC polypeptide; c) a proteolytically cleavable linker or a ribosome skipping signal; d) an immunomodulatory polypeptide; e) a second MHC polypeptide; and f) an Ig Fc polypeptide or a non-Ig-based scaffold. In some cases, the first MHC polypeptide is a  $\beta$ 2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide. In some cases, the  $\beta$ 2-microglobulin polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4. In some cases, the MHC class I heavy chain polypeptide is an HLA-A,

HLA-B, or HLA-C heavy chain. In some cases, the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:5. In some cases, the first MHC polypeptide is an MHC Class II alpha chain polypeptide; and wherein the second MHC polypeptide is an MHC class II beta chain polypeptide. In some cases, the epitope is a T-cell epitope. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide, an IgG2 Fc polypeptide, an IgG3 Fc polypeptide, an IgG4 Fc polypeptide, an IgA Fc polypeptide, or an IgM Fc polypeptide. In some cases, the Ig Fc polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to an amino acid sequence depicted in Figures 24A-24C. In some cases, the immunomodulatory polypeptide is selected from a 4-1BBL polypeptide, a B7-1 polypeptide; a B7-2 polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a CD86 polypeptide, a PD-L1 polypeptide, a FasL polypeptide, and a PD-L2 polypeptide. In some cases, the immunomodulatory polypeptide is selected from a CD7, CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, and HVEM. In some cases, the proteolytically cleavable linker or ribosome skipping signal comprises an amino acid sequence selected from: a) LEVLFQGP (SEQ ID NO:37); b) ENLYTQS (SEQ ID NO:34); c) a furin cleavage site; d) LVPR (SEQ ID NO:36); e) GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:64); f) GSGEGRGSLLTCGDVEENPGP (SEQ ID NO:65); g) GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO:66); and h) GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:67). In some cases, the recombinant polypeptide comprises, in order from N-terminus to C-terminus: a) a first leader peptide; b) the epitope; c) the first MHC polypeptide; d) the immunomodulatory polypeptide; e) the proteolytically cleavable linker or ribosome skipping signal; f) a second leader peptide; g) the second MHC polypeptide; and h) the immunoglobulin (Ig) Fc polypeptide. In some cases, the first leader peptide and the second leader peptide is a  $\beta$ 2-M leader peptide. In some cases, the nucleotide sequence is operably linked to a transcriptional control element. In some cases, the transcriptional control element is a promoter that is functional in a eukaryotic cell. In some cases, the first MHC polypeptide or a linker between the epitope and the first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, and wherein the first and the second Cys residues provide for a disulfide linkage between the first MHC polypeptide and the second MHC polypeptide. The present disclosure provides a recombinant expression vector comprising any one of the nucleic acids described above or elsewhere herein. In some cases, the recombinant expression vector is a viral vector. In some cases, the recombinant expression vector is a non-viral vector. The present disclosure provides a host cell genetically modified with a recombinant expression vector as described above and elsewhere herein. In some cases, the host cell is *in vitro*. In some cases, the host cell is genetically modified such that the cell does not produce an endogenous MHC  $\beta$ 2-microglobulin polypeptide. In some cases, the host cell is a T lymphocyte.

**[0036]** The present disclosure provides a composition comprising: a) a first nucleic acid comprising a nucleotide sequence encoding a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; ii) a first MHC polypeptide; and iii) an immunomodulatory domain; and b) a first nucleic acid comprising a nucleotide sequence encoding a second

polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an Ig Fc polypeptide or a non-Ig-based scaffold. The present disclosure provides a composition comprising: a) a first nucleic acid comprising a nucleotide sequence encoding a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a first nucleic acid comprising a nucleotide sequence encoding a second polypeptide comprising, in order from N-terminus to C-terminus: i) an immunomodulatory domain ii) a second MHC polypeptide; and iii) an Ig Fc polypeptide. In some cases, the first and/or the second nucleic acid is present in a recombinant expression vector. The present disclosure provides a host cell genetically modified with a nucleic acid composition described above or elsewhere herein.

**[0037]** The present disclosure provides a method of producing a multimeric polypeptide as described above or elsewhere herein, the method comprising: a) culturing a host cell as described above or elsewhere herein *in vitro* in a culture medium under conditions such that the host cell synthesizes the multimeric polypeptide; and b) isolating the multimeric polypeptide from the host cell and/or from the culture medium. In some cases, the second polypeptide comprises an affinity tag, and wherein said isolating comprises contacting the multimeric polypeptide produced by the cell with a binding partner for the affinity tag, wherein the binding partner is immobilized, thereby immobilizing the multimeric polypeptide. In some cases, the method comprises eluting the immobilized multimeric polypeptide.

**[0038]** The present disclosure provides a method of selectively modulating the activity of an epitope-specific T cell, the method comprising contacting the T cell with a multimeric polypeptide as described above or elsewhere herein, wherein said contacting selectively modulates the activity of the epitope-specific T cell. In some cases, the immunomodulatory polypeptide is an activating polypeptide, and wherein the multimeric polypeptide activates the epitope-specific T cell. In some cases, the immunomodulatory polypeptide is an inhibiting polypeptide, and wherein the multimeric polypeptide inhibits the epitope-specific T cell. In some cases, the contacting is carried out *in vitro*. In some cases, the contacting is carried out *in vivo*.

**[0039]** The present disclosure provides a method of selectively modulating the activity of an epitope-specific T cell in an individual, the method comprising administering to the individual an effective amount of a multimeric polypeptide as described above or elsewhere herein effective to selectively modulate the activity of an epitope-specific T cell in an individual. In some cases, the immunomodulatory polypeptide is an activating polypeptide, and wherein the multimeric polypeptide activates the epitope-specific T cell. In some cases, the epitope is a cancer-associated epitope, and wherein said administering selectively increases the activity of a T cell specific for the cancer-associate epitope. In some cases, the immunomodulatory polypeptide is an inhibitory polypeptide, and wherein the multimeric polypeptide inhibits activity of the epitope-specific T cell. In some cases, the epitope is a self-epitope, and wherein said administering selectively inhibits the activity of a T cell specific for the self-epitope.

**[0040]** The present disclosure provides a method of treating an infection in an individual, the method comprising administering to the individual an effective amount of a) a multimeric

polypeptide as described above or elsewhere herein; or b) one or more recombinant expression vectors comprising nucleotide sequences encoding the multimeric polypeptide; or c) one or more mRNAs comprising nucleotide sequences encoding the multimeric polypeptide, wherein the epitope is a pathogen-associated epitope, wherein the immunomodulatory polypeptide is an activating polypeptide, and wherein said administering effective to selectively modulate the activity of a pathogen-associated epitope-specific T cell in an individual. In some cases, the pathogen is a virus, a bacterium, or a protozoan. In some cases, the administering is subcutaneous (i.e., the administering is carried out via subcutaneous administration). In some cases, the administering is intravenous (i.e., the administering is carried out via intravenous administration). In some cases, the administering is intramuscular (i.e., the administering is carried out via intramuscular administration). In some cases, the administering is systemic. In some cases, the administering is distal to a treatment site. (i.e., the administering is carried out via subcutaneous administration) the administering is local. (i.e., the administering is carried out via subcutaneous administration) the administering is at or near a treatment site.

**[0041]** The present disclosure provides a composition comprising: a) a multimeric polypeptide as described above or elsewhere herein; and b) a pharmaceutically acceptable excipient.

**[0042]** The present disclosure provides a composition comprising: a) a nucleic acid as described above or elsewhere herein, or a recombinant expression vector as described above or elsewhere herein; and b) a pharmaceutically acceptable excipient.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0043]**

FIG. 1: SynTac: an artificial immunological synapse for T cell activation. The panel on the left depicts the traditional two-signal hypothesis for T cell activation. Namely, targeted T cell engagement through unique TCR:MHC-epitope interactions between the T cell and Antigen Presenting Cell (APC), followed by stimulation or inhibition through costimulatory molecule engagement. The middle panel is a schematic representation of the synTac molecule followed by a mode of action for synTac (Right). Analogous to the natural response (Left), the synTac fusion protein allows for highly specific cell targeting through the MHC-epitope. Following this is a T cell modulatory domain ("MOD") which acts via costimulatory molecule engagement, and can provide for either activation or inhibition. This elicits a clonal, not global, T cell response. Notably, the MOD can be any known or approved antibody, antibody fragment, costimulatory molecule, or other literature validated payload (cytokines, toxins, etc.) as well as new entrants.

FIG. 2A-2C: The synTac Fc-fusion construction. One strategy exploits an Fc-fusion construction to increase the valency, stability and therapeutic window of the associated products. Briefly, the Fc region is a native covalent homo-dimer, formed through interaction of two identical immunoglobulin CH2-CH3 domains (termed Fc) and stabilized through two

disulfide bonds between the CH2 domains, illustrated as two thin lines. FIG. 2A shows a single chain peptide MHC protein linked at its carboxy terminus to an IgG Fc region. To introduce alternative protein linkages (such as an MOD), the construct was split into respective heavy and light chains and fuse both peptides and proteins to various ends. One construction, FIG. 2B, results in an amino-terminal association of the peptide to the light chain (beta 2 microglobulin, B2M) followed by a carboxy terminal extension of the light chain to the MOD effector molecule. In this scenario the heavy chain (HLA-molecule, HC) is fused to the Fc region. Constructs are held together covalently through disulfide bridges (labeled as S-S). An alternative orientation, FIG. 2C, places the MOD amino-terminal of the Fc fused heavy chain with the peptide still linked to the B2M light chain.

FIG. 3A-3B: The overall design for the two base synTac molecules. This construct utilizes a native human B2M leader sequence (LEADER) to allow for efficient secretion and ER processing immediately followed by a candidate epitope (labeled as PEPTIDE). For the light chain linkage (LC, FIG. 3A), this is coupled to the native B2M molecule through linker L1 and the MOD through linker L2. This entire cassette is linked to another B2M leader sequence, the MHC heavy chain and an IgG1 Fc domain by a viral porcine teschovirus-1 (P2A) "self-cleaving" peptide to allow for stoichiometric expression of each chain. The Heavy Chain (HC, FIG. 3B) linkage is similar however the viral P2A peptide now follows the B2M and the MOD follows the second leader peptide. Both constructs terminate in an 8x His tag.

FIG. 4: CRISPR/CAS mediated Knock-out of endogenous Beta-2-Microglobulin. Guide RNA was designed against endogenous B2M, transfected along with a plasmid encoding CRISPR/CAS and allowed to culture for three days. The cultured cells were surface stained for B2M and counter selected (sorted on loss of fluorescence) by fluorescence activated cell sorting (FACS). The sorted cells were allowed to recover and subjected to two more rounds of staining, counter-sorting and recovery (3 rounds in total) to ensure efficient knock-out. The final pool was quality checked by monitoring B2M surface expression via FACS, shown above.

FIG. 5A-5B: Production and activity testing of synTac constructs with engineered disulfide bonds. High-level expression was demonstrated for one construct (H236-L12, labeled as synTac 18) with modest expression for a second (H237-L12, synTac 17). The dt-SCT disulfide schema is used a positive control (labeled as synTac 2). Non-reducing PAGE suggests that the high molecular weight, disulfide linked, moiety was formed as expected (FIG. 5A). All expressing constructs were scaled up to the 100 ml scale, purified and activity tested through binding of cognate TCR expressed on the surface of HEK cells (termed A6), as monitored by FACS fluorescence, suggesting proper folding and activity (FIG. 5B). Cells expressing non-cognate TCR (termed AS01) were used as a negative control.

FIG. 6A-6B: Expression of various synTac protein fusions. Successful expression of (FIG. 6A) light chain linked synTac fusions with various targeting peptides and HLA isotypes with a PD-L1 MOD domain, specifically 1) HTLV-human-HLA-A02, 2) IGRP-murine H2-Kd and 3) TUM-murine H2-Kd, (FIG. 6B) IGRP based synTac fusion bearing various MOD domains, 4) the Ig variable domain of PD-L1, 5) 4-1BBL, 6) anti-CD28 single chain Fv, and 7) B7-1W88A, (FIG. 6C) IGRP based synTac fusions expressed as a heavy chain linkage, bearing various MODS,

8) PD-L1 and 9) anti-CD28 single chain Fv.

FIG. 7A-7B: TCR-synTac-PD1 Bridging: validating the integrity of the synTac protein components. HEK cells expressing a cognate TCR (A6) were used as a positive control and cells expressing a non-cognate TCR (AS01) were generated and used as a negative control along with untransduced parental cells. Cells were challenged with non-fluorescent purified HTLV-PD-L1 synTac variants and incubated with its cognate receptor PD1 fused to murine IgG2a. The PD1-Fc fusion was detected using a FITC labeled anti-mouse secondary antibody. A schematic of the reaction is illustrated in FIG. 7A, FACs results shown in FIG. 7B. As expected, co-localized fluorescence was only observed when HTLV presented synTac WITH a PD-L1 MOD was challenged against cognate (A6) HEK cell lines. Of note, this was not observed when challenged against non-cognate TCR bearing HEK cells or parental cells, when challenged against FITC-PD1-Fc only or when the MOD was absent.

FIG. 8A-8D: SynTac in action: *in vitro* T cell assays. CD8+ T cells from 8.3 transgenic NOD mice were cultures in the presence of immobilized anti-CD3 antibody to stimulate polyclonal T cell activation. Stimulated cultures were treated with soluble versions of either synTac TUM-PD-L1 (FIG. 8A) or synTac IGRP-PD-L1 (FIG. 8C) to examine the antigen specificity of any suppressive effect. A version of synTac IGRP without PD-L1 (FIG. 8B) served as an effector control for the MOD domain. Before seeding, cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) in order to monitor the extent of T cell activation-induced cellular proliferation. Cells were harvested at 5 days and examined using flow cytometry for viability and proliferation. Supernatants were also examined for the expression of the CD8+ T cell effector cytokines IFNy and TNF $\alpha$  using a multiplexed flow cytometric bead assay. All CD8+ T cell activation parameters examined were suppressed in an antigen-specific and effector (i.e. MOD) domain-dependent manner (FIG. 8D).

FIG. 9A-9F provide amino acid sequences and domain structure of synTac polypeptides.

FIG. 10A-10C depict constructs for 4-1BBL *trimeric expression*. Cartoon representation of (FIG. 10A) monomeric form of the native 4-1BBL ectodomain (residues 50-254), showing membrane proximal (Memb Prox, MP) and the TNF homology (TNF-H) domains, (FIG. 10B) 4-1BBL dimeric synTac, and (FIG. 10C) fully active dual trimeric form of 4-1BBL synTac generated through coexpression of traditional synTac constructs with a "free" from of 4-1BBL ecto-domain (residues 50-254, FIG. 10A) having no affinity tag. All constructs assemble when expressed together in mammalian cells. Purification proceeds through the Fc region (protein A/G) followed by size exclusion, allowing for separation of 4-1BBL trimeric synTac from free BBL.

FIG. 11A-11B. Multiangle light scattering (MALS) analysis of trimers 4-1BBL bearing synTac proteins. (FIG. 11A) Molecular weight of major species identified through MALS, showing examples of multiple independent measurements. (FIG. 11B) Representative traces from MALS of synTac 40+51, with relatively high levels of light scattering and low UV absorption, reflecting the presence of a small amount of protein with a high molecular weight. Low molecular weight buffer components result in large changes in refractive index (either positive or negative) without associated change in UV absorbance.

FIG. 12. SynTac 4-1BBL receptor binding. Protein A microbeads were coated to saturation with recombinant human or mouse 4-1BB-Fc fusion protein and used to bind synTac constructs bearing 4-1BB ligand (dimer and trimer) as the co-modulatory domain, followed by a fluorescent detection antibody specific for the synTac heavy chain isotype. The extent of specific binding of synTac 4-1BBL to bead-borne 4-1BB was then measured by high throughput flow cytometry. Using this system, the degree of cross reactivity and relative affinities of 4-1BBL for both human and murine 4-1BB was explored in the context of the synTac scaffold. 4-1BBL bearing synTacs were shown to bind cognate receptor, but not "receptor-less" (termed no MOD) Fc bound microbeads, suggesting a well-folded and active protein reagent. Notably, the trimer bound in an affinity range expected for dual trimeric engagement with the original dimer showing a 10 fold reduction in binding affinity and all constructs cross react between murine and human receptors.

FIG. 13. CD8<sup>+</sup> T cells from 8.3 transgenic NOD mice were cultured in the presence of immobilized anti-CD3 antibody to stimulate polyclonal T cell activation. Stimulated cultures were treated with soluble versions of either synTac TUM-41BBL (A) or synTac IGRP-41BBL (B and C) to examine the antigen specificity of any stimulatory effect. Control treatments were media alone (- CNTRL) or immobilized anti-CD3 (+ CNTRL) to benchmark response magnitude. Cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) in order to monitor the extent of T cell activation-induced cellular proliferation. After 4 days, the cells were harvested and examined using flow cytometry for viability and proliferation. Supernatants were also examined for the expression of the CD8<sup>+</sup> T cell effector cytokines IFNy and TNF $\alpha$  using a multiplexed flow cytometric bead assay. All CD8<sup>+</sup> T cell activation parameters examined were activated in an antigen-specific and effector (i.e. MOD) domain-dependent manner.

FIG. 14. Single Dose *in vivo* T cell stimulation assays. NOD mice were injected intraperitoneally with synTac IGRP-41BBL, synTac TUM-41BBL or PBS. Six days post injection, the mice were sacrificed and splenocytes were examined via flow cytometry for relative frequencies of IGRP-specific CD8 T cells using an appropriate peptide-MHC pentamer stain. IGRP-41BBL treatment was associated with a much higher frequency of IGRP-specific CD8 T cells versus controls, supporting a significant *in vivo* expansion from a single dose.

FIG. 15. Multi Dose *in vivo* T cell stimulation assays. NOD mice were injected intraperitoneally with synTac IGRP-41BBL, synTac TUM-41BBL or PBS for three doses over two weeks. Seven days post injection, the mice were sacrificed and PBMC's (from blood) were examined via flow cytometry for relative frequencies of IGRP-specific CD8 T cells using an appropriate peptide-MHC pentamer stain. IGRP-41BBL treatment was associated with a higher frequency of IGRP-specific CD8 T cells versus controls, supporting a significant *in vivo* expansion from a multiple doses, including rare-tumor specific T cells (TUM).

FIG. 16A-16B. Schematics of optimized constructs for 4-1BBL trimeric expression. Disulfide locking (FIG. 16A, DL) and single chain trimers (FIG. 16B, SCT).

FIG. 17. SynTac 4-1BBL receptor binding. Protein A microbeads were coated to saturation with

recombinant human or mouse 4-1BB-Fc fusion protein and used to bind synTac constructs bearing 4-1BB ligand (Disulfide Locked trimers (69, 70 and 71) and Single Chain trimer (SCT) as the co-modulatory domain, followed by a fluorescent detection antibody specific for the synTac heavy chain isotype. The Native trimer shown is a binding control (Trimer). The extent of specific binding of synTac 4-1BBL to bead-borne 4-1BB was then measured by high throughput flow cytometry. 4-1BBL bearing synTacs were shown to bind cognate receptor, but not "receptor-less" (termed "no MOD") Fc bound microbeads, suggesting a well-folded and active protein reagent. All constructs cross react between murine and human receptors.

FIG. 18. Expression Validation of optimized 4-1BBL constructs. SynTacs produced by co-expression, with the original 4-1BBL modulator (synTac 40/51, with no disulfide lock, labeled as "O" (for original)) and three optimized constructs containing engineered disulfide locks restraining the trimer conformation. Two native residues in each construct were replaced for cysteine residues (Q94C:P245C (labeled as "DL1" in gel), Q94C:P242C "DL2", and Q89C:L115C "DL3", termed synTac 69, 70 and 71 respectively), co-expressed in human cells with a "free" non tagged version harboring the same mutations (termed 98,99,100 respectively) to allow for covalent locking in the cell. The degree of disulfide bonding was observed by amount of released (non-covalently bound) "free" 4-1BBL in non-reduced SDS PAGE analysis. Free-BBL would migrate at ~20 kDa (BOX), confirming disulfide locking of engineered constructs. SynTac carrying a single-chain-trimer version (SCT) of 4-1BBL is also shown following affinity and gel filtration purification (labeled as "SCT"). Accurate mass confirmed by multi angle light scattering (MALS).

FIG. 19A-19I. Schematic depictions of embodiments of synTac constructs of the present disclosure. FIG. 19A-19C depict constructs described in relation to FIG. 2A-2C respectively; in FIG. 19B and 19C the P2A uncleaved polypeptide is depicted (top) and the cleaved polypeptide (through P2A-mediated self-cleavage) is depicted (bottom) with disulfide bonding (SS), mediated by cysteine substitution (\*), illustrated. FIG. 19D-19F depict constructs described above in relation to FIG. 8A-8C respectively; in each of FIG. 19D-19F the P2A uncleaved form is depicted above (top) the P2A-mediated self-cleaved polypeptide (bottom) with disulfide bonding (SS), mediated by cysteine substitution (\*), illustrated. FIG. 19G depicts a generalized version of the synTac40 construct in relationship to FIG. 9B with the uncleaved (top) and self-cleaved/disulfide bonded (bottom) polypeptides illustrated. FIG 19H depicts a generalized version of synTac69, synTac70 and synTac71 in relationship to FIG. 9C-9E, the uncleaved (top) and self-cleaved/disulfide bonded (bottom) polypeptides are illustrated and additional cysteine substitutions in the 4-1BBL domain are also indicated (\*). FIG. 19I depicts a generalized version of the synTac 4-1BBL single chain trimer (SCT) in relationship to FIG. 9F, the uncleaved (top) and self-cleaved/disulfide bonded (bottom) polypeptides are illustrated.

FIG. 20 provides an multiple amino acid sequence alignment of beta-2 microglobulin (B2M) precursors (i.e., including the leader sequence) from *Homo sapiens* (NP\_004039.1; SEQ ID NO:78), *Pan troglodytes* (NP\_001009066.1; SEQ ID NO:79), *Macaca mulatta* (NP\_001040602.1; SEQ ID NO:80), *Bos Taurus* (NP\_776318.1; SEQ ID NO:81) and *Mus musculus* (NP\_033865.2; SEQ ID NO:82).

FIG. 21 provides the domain structure of the construct of SEQ ID NO:6.

FIG. 22 provides the domain structure of the construct of SEQ ID NO:7.

FIG. 23 depicts the effect of *in vivo* administration of synTac IGRP-PDL1, synTac TUM-PDL1, or phosphate-buffered saline (PBS) on the frequency of IGRP-specific CD8<sup>+</sup> T cells.

FIG. 24A-24C provide amino acid sequences of immunoglobulin Fc polypeptides.

FIG. 25A-25C provide amino acid sequences of human leukocyte antigen (HLA) Class I heavy chain polypeptides.

FIG. 26A-26B provide amino acid sequences of PD-L1 polypeptides.

FIG. 27 provides an amino acid sequence of a 4-1BBL polypeptide.

FIG. 28 provides an amino acid sequence of an ICOS-L polypeptide.

FIG. 29 provides an amino acid sequence of an OX40L polypeptide.

FIG. 30 provides an amino acid sequence of a PD-L2 polypeptide.

FIG. 31 provides an amino acid sequence of a CD80 (B7-1) polypeptide.

FIG. 32 provides an amino acid sequence of a CD86 (B7-2) polypeptide.

FIG. 33 provides an amino acid sequence of a Fas ligand (FAS-L) polypeptide.

FIG. 34A-34H provide schematic depictions of embodiments of synTac constructs of the present disclosure, where disulfide bonding (SS), mediated by cysteine substitution (\*), is illustrated. In these embodiments, disulfide bonds are formed between MHC (e.g., HLA) polypeptides present in separate polypeptides.

## DEFINITIONS

**[0044]** A "leader sequence" as used herein includes any signal peptide that can be processed by a mammalian cell, including the human B2M leader. Such sequences are well-known in the art.

**[0045]** As used herein, "contiguous with" with regard to, for example, element A and element B, means element A is adjacent to element B and bonded to element B, preferably, unless otherwise specified, via a covalent bond. For example, for a first sequence of amino acids contiguous with a second sequence of amino acids, the C-terminal of the first sequence of amino acids can be joined by a peptide bond to the N-terminal of the second sequence of amino acids.

**[0046]** The terms "peptide," "polypeptide," and "protein" are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The terms also include polypeptides that have co-translational (e.g., signal peptide cleavage) and post-translational modifications of the polypeptide, such as, for example, disulfide-bond formation, glycosylation, acetylation, phosphorylation, proteolytic cleavage, and the like. Furthermore, as used herein, a "polypeptide" refers to a protein that includes modifications, such as deletions, additions, and substitutions (generally conservative in nature as would be known to a person in the art) to the native sequence, as long as the protein maintains the desired activity. These modifications can be deliberate, as through site-directed mutagenesis, or can be accidental, such as through mutations of hosts that produce the proteins, or errors due to PCR amplification or other recombinant DNA methods.

**[0047]** The term "recombinant", as used herein to describe a nucleic acid molecule, means a polynucleotide of genomic, cDNA, viral, semisynthetic, and/or synthetic origin, which, by virtue of its origin or manipulation, is not associated with all or a portion of the polynucleotide sequences with which it is associated in nature. The term "recombinant," as used with respect to a protein or polypeptide, refers to a polypeptide produced by expression from a recombinant polynucleotide. The term "recombinant," as used with respect to a host cell or a virus, refers to a host cell or virus into which a recombinant polynucleotide has been introduced. Recombinant is also used herein to refer to, with reference to material (e.g., a cell, a nucleic acid, a protein, or a vector) that the material has been modified by the introduction of a heterologous material (e.g., a cell, a nucleic acid, a protein, or a vector).

**[0048]** The terms "polynucleotide," "oligonucleotide," "nucleic acid" and "nucleic acid molecule" are used interchangeably herein to include a polymeric form of nucleotides, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, the terms include triple-, double- and single-stranded DNA, as well as triple-, double- and single-stranded RNA. The terms also include such molecules with modifications, such as by methylation and/or by capping, and unmodified forms of a polynucleotide. More particularly, the terms "polynucleotide," "oligonucleotide," "nucleic acid" and "nucleic acid molecule" include polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), any other type of polynucleotide which is an N- or C-glycoside of a purine or pyrimidine base, and other polymers containing non-nucleotidic backbones, polymers, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA.

**[0049]** The term "vector" as used herein refers a vehicle capable of transferring nucleic acid sequences to target cells. For example, a vector may comprise a coding sequence capable of being expressed in a target cell. As used herein, "vector construct," "expression vector," and "gene transfer vector," generally refer to any nucleic acid construct capable of directing the

expression of a gene of interest and which is useful in transferring the gene of interest into target cells. Thus, the term includes cloning and expression vehicles, as well as integrating vectors and non-integrating vectors. Vectors are thus capable of transferring nucleic acid sequences to target cells and, in some instances, are used to manipulate nucleic acid sequence, e.g., recombine nucleic acid sequences (i.e. to make recombinant nucleic acid sequences) and the like. For purposes of this disclosure examples of vectors include, but are not limited to, plasmids, phage, transposons, cosmids, virus, and the like.

**[0050]** An "expression cassette", as used herein, comprises any nucleic acid construct capable of directing the expression of any RNA transcript including gene/coding sequence of interest as well as non-translated RNAs. Such cassettes can be constructed into a "vector," "vector construct," "expression vector," or "gene transfer vector," in order to transfer the expression cassette into target cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors. A transcript of an expression cassette may be expressed stably or transiently and may be expressed from a cassette that integrates into the host genome (in a targeted or untargeted manner) or remain non-integrated as desired.

**[0051]** "Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression. As used herein, the terms "heterologous promoter" and "heterologous control regions" refer to promoters and other control regions that are not normally associated with a particular nucleic acid in nature. For example, a "transcriptional control region heterologous to a coding region" is a transcriptional control region that is not normally associated with the coding region in nature.

**[0052]** The term "immunological synapse" or "immune synapse" as used herein generally refers to the natural interface between two interacting immune cells of an adaptive immune response including, e.g., the interface between an antigen-presenting cell (APC) or target cell and an effector cell, e.g., a lymphocyte, an effector T cell, a natural killer cell, and the like. An immunological synapse between an APC and a T cell is generally initiated by the interaction of a T cell antigen receptor and major histocompatibility complex molecules, e.g., as described in Bromley et al., *Annu Rev Immunol.* 2001;19:375-96;

**[0053]** As used herein, the term "heterologous" used in reference to nucleic acid sequences, proteins or polypeptides, means that these molecules are not naturally occurring in the cell from which the heterologous nucleic acid sequence, protein or polypeptide was derived. For example, the nucleic acid sequence coding for a human polypeptide that is inserted into a cell that is not a human cell is a heterologous nucleic acid sequence in that particular context. Whereas heterologous nucleic acids may be derived from different organism or animal species, such nucleic acid need not be derived from separate organism species to be heterologous. For example, in some instances, a synthetic nucleic acid sequence or a polypeptide encoded therefrom may be heterologous to a cell into which it is introduced in that the cell did not previously contain the synthetic nucleic acid. As such, a synthetic nucleic acid

sequence or a polypeptide encoded therefrom may be considered heterologous to a human cell, e.g., even if one or more components of the synthetic nucleic acid sequence or a polypeptide encoded therefrom was originally derived from a human cell.

**[0054]** A "host cell," as used herein, denotes an *in vivo* or *in vitro* eukaryotic cell or a cell from a multicellular organism (e.g., a cell line) cultured as a unicellular entity, which eukaryotic cells can be, or have been, used as recipients for a nucleic acid (e.g., an expression vector that comprises a nucleotide sequence encoding a multimeric polypeptide of the present disclosure), and include the progeny of the original cell which has been genetically modified by the nucleic acid. It is understood that the progeny of a single cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation. A "recombinant host cell" (also referred to as a "genetically modified host cell") is a host cell into which has been introduced a heterologous nucleic acid, e.g., an expression vector. For example, a genetically modified eukaryotic host cell is genetically modified by virtue of introduction into a suitable eukaryotic host cell a heterologous nucleic acid, e.g., an exogenous nucleic acid that is foreign to the eukaryotic host cell, or a recombinant nucleic acid that is not normally found in the eukaryotic host cell.

**[0055]** In some instances, nucleic acid or amino acid sequences, including polypeptides and nucleic acids encoding polypeptides, are referred to based on "sequence similarity" or "sequence identity", e.g., as compared to one or more reference sequences. In other instances, a mutant or variant sequence may be referred to based on comparison to one or more reference sequences. For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

**[0056]** Where necessary or desired, optimal alignment of sequences for comparison can be conducted, for example, by the local homology algorithm of Smith and Waterman (Adv. Appl. Math. 2:482 (1981)), by the homology alignment algorithm of Needleman and Wunsch (J. Mol. Biol. 48:443-53 (1970)), by the search for similarity method of Pearson and Lipman (Proc. Natl. Acad. Sci. USA 85:2444-48 (1988)), by computerized implementations of these algorithms (e.g., GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection. (See generally Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, 4th ed., John Wiley and Sons, New York (1999)).

**[0057]** "T cell" includes all types of immune cells expressing CD3, including T-helper cells (CD4<sup>+</sup> cells), cytotoxic T-cells (CD8<sup>+</sup> cells), T-regulatory cells (Treg), and NK-T cells.

**[0058]** "Co-stimulatory ligand," as the term is used herein, includes a molecule on an antigen

presenting cell (e.g., an APC, dendritic cell, B cell, and the like) that specifically binds a cognate co-stimulatory molecule on a T cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A co-stimulatory ligand can include, but is not limited to, CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, Fas ligand (FasL), inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM), CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, HVEM, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, *inter alia*, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as, but not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds to CD83.

**[0059]** The terms "purifying", "isolating", and the like, refer to the removal of a desired substance, e.g., a recombinant protein, from a solution containing undesired substances, e.g., contaminates, or the removal of undesired substances from a solution containing a desired substance, leaving behind essentially only the desired substance. In some instances, a purified substance may be essentially free of other substances, e.g., contaminates. Purifying, as used herein, may refer to a range of different resultant purities, e.g., wherein the purified substance makes up more than 80% of all the substance in the solution, including more than 85%, more than 90%, more than 91%, more than 92%, more than 93%, more than 94%, more than 95%, more than 96%, more than 97%, more than 98%, more than 99%, more than 99.5%, more than 99.9%, and the like. As will be understood by those of skill in the art, generally, components of the solution itself, e.g., water or buffer, or salts are not considered when determining the purity of a substance.

**[0060]** As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, e.g., in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

**[0061]** The terms "individual," "subject," "host," and "patient," used interchangeably herein, refer to a mammal, including, but not limited to, murines (e.g., rats, mice), lagomorphs (e.g., rabbits), non-human primates, humans, canines, felines, ungulates (e.g., equines, bovines, ovines, porcines, caprines), etc.

**[0062]** A "therapeutically effective amount" or "efficacious amount" refers to the amount of an agent, or combined amounts of two agents, that, when administered to a mammal or other

subject for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" will vary depending on the agent(s), the disease and its severity and the age, weight, etc., of the subject to be treated.

**[0063]** Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, but is defined by the appended claims. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

**[0064]** Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

**[0065]** Unless defined otherwise, 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 invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described.

**[0066]** It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a multimeric polypeptide" includes a plurality of such polypeptides and reference to "the immunomodulatory polypeptide" includes reference to one or more immunomodulatory polypeptides and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

**[0067]** It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was

individually and explicitly disclosed herein.

**[0068]** The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

## DETAILED DESCRIPTION OF THE INVENTION

[0069] Herein is described a novel protein-based therapeutic platform that recapitulates a traditional immune response; an artificial immunological synapse for T cell activation (synTac). A novel fusion protein linking a costimulatory molecule to an MHC-epitope to allow for precise T cell engagement and clonal T cell activation, or inhibition, depending on the MOD molecule portion.

## MULTIMERIC POLYPEPTIDES

[0070] The present disclosure provides multimeric (e.g., heterodimeric, heterotrimeric) polypeptides. The present disclosure provides polyprotein precursors of a multimeric polypeptide of the present disclosure. The present disclosure provides precursor gene products, e.g., polyprotein precursors of a multimeric polypeptide of the present disclosure, and mRNA gene products encoding two or more polypeptide chains of a multimeric polypeptide of the present disclosure.

[0071] Also provided is a recombinant polypeptide construct comprising (i) a candidate epitope peptide bound by a first amino acid linker sequence contiguous with a sequence of amino acids comprising a sequence identical to a human native B2M peptide sequence contiguous with a second amino acid linker sequence contiguous with a T Cell modulatory domain peptide, wherein (i) is bound by one, or more than one, disulfide bond to (ii) a sequence of amino acids having the sequence of a MHC heavy chain contiguous with a third amino acid linker sequence contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain. In an embodiment, the recombinant polypeptide construct comprises

HPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQP  
KIVKWDRDMGGGSGGGSGGGSFTITAPKDLYVVEYGSNVTMECRFPVERE  
LDLLALVVYWEKEDEQVIQFVAGEEDLKPQHSNFRGRASLPKDQLLGNAALQITDVKL  
QDAGVYCCIISYGGADYKRITLKVNAPYRKINQRISVDPATSEHELICQAEGYPEAEVIWT  
NSDHQPVSGKRSVTSRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAELIPE  
LPAFHPPONRTSGSGATNFSSLKOAGDVEENPGPMSRSVALAVLALLSLSGLEAGSHSM

RYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSAASQRMEPRAPWIEQEGPEYWDGET  
 RKVKAHSQTHRVDLGLRG CYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDG  
 KDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLETCVEWLRRYLENGKE  
 TLQRTDAPKTHMTHHAVSDHEATLRCWALSFYP AEITLTWQRDGEDQTQDTELVETRP  
 AGDGT FQKWA AVV VPSGQE QRYTCHVQHEGLPKPLTLRWEPA AAGGDKTHTCPCPAP  
 ELLGGPSVFLFPPKPKD TL MISRTPEVTCVV DVSHEDPEVKFN WYV DGVEVHNAKTKP  
 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPVY  
 LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTPPVLDSDGSFFLYSKL  
 TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGSHHHHHHHH (SEQ ID  
 NO:6).

**[0072]** Also provided is recombinant polypeptide construct comprising (i) a candidate epitope peptide bound by a first amino acid linker sequence contiguous with a sequence of amino acids comprising a sequence identical to a human native B2M peptide sequence, wherein (i) is bound by one, or more than one, disulfide bond to (ii) a T Cell modulatory domain peptide contiguous with a second amino acid linker sequence contiguous with a sequence of amino acids having the sequence of a MHC heavy chain contiguous a third amino acid linker sequence contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain. In an embodiment, the recombinant polypeptide construct comprises  
 LLFGYPVYVGCGSGGGGGGGSIQRTPKIQVYSRHPAENGKSNFLNCYVSGF

HPSDIEVDLLKNGERIEKVEHSDLSFSKDWFSYLLYYTEFTPTEKDEYACRVNHVTLSQP  
 KIVKWD RMGGGGGGGGGGGGGGGGGGSGSGATNFSLLKQAGDVEENPGPMSRSV  
 ALAVLALLSLSGLEAFTITAPKDL YVVEYGSNVTMECRFPVERELDLLALVVYWEKEDE  
 QVIQFVAGEEDLKPQHSNFRGRASLPKDQLLKGNAAALQITDVKLQDAGVYCCISYGG  
 DYKRITLKVNAPYRKINQRISVDPATSEHELICQAEGYPEAEVIWTNSDHQPVSGKRSVTT  
 SRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAELIPEL PATHPPQNRTGGGG  
 SGGGGGGGGGGGGSGSHSMRYFFT SVSRPGRGEPRFIAVGYVDDTQFVRFDSAASQ  
 RMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGLRG CYNQSEAGSHTVQRMY  
 CDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQL  
 RAYLETCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYP AEIT  
 LTWQRDGEDQTQDTELVETRPAGDGT FQKWA AVV VPSGQE QRYTCHVQHEGLPKPLTL  
 RWEPA AAGGDKTHTCPCPAP ELLGGPSVFLFPPKPKD TL MISRTPEVTCVV DVSHEDP  
 EVKFN WYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA  
 LPAPIEKTISKAKGQPREPVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE  
 NNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK  
 GGSHHHHHHHH (SEQ ID NO:7).

**[0073]** Also provided is a protein comprising two of the recombinant polypeptide constructs described herein joined by one or more disulfide bonds between the respective immunoglobulin Fc domains thereof.

**[0074]** Also provided is a protein comprising two of the recombinant polypeptide constructs described herein joined by one or more disulfide bonds between the respective immunoglobulin Fc domains thereof.

**[0075]** This invention provides a synTac platform: an artificial immunological synapse for targeted T cell activation as defined by the appended claims.

**[0076]** In an embodiment, the beta 2 microglobulin has the same sequence as a human beta 2 microglobulin. In an embodiment, the Histocompatibility Complex heavy chain sequence has the same sequence as a human HLA-A sequence. In an embodiment, the Histocompatibility Complex heavy chain transmembrane domain has the same sequence as a human Major Histocompatibility Complex I (MHC I) heavy chain transmembrane domain. In an embodiment, the Histocompatibility Complex heavy chain transmembrane domain has the same sequence as a human Major Histocompatibility Complex II (MHC II) heavy chain transmembrane domain.

**[0077]** Also provided is a composition comprising a plurality of the constructs.

**[0078]** In an embodiment, the candidate epitope peptide is an 8, 9, 10, 11 or 12 amino acid peptide. In an embodiment, the candidate epitope peptide is 13, 14, 15, 16, or 17 amino acid peptide. In an embodiment, the candidate epitope peptide is a nonamer (9 amino acids in length).

**[0079]** The present disclosure provides multimeric polypeptides that comprise two or more (e.g., 2, 3, 4, or more) polypeptide chains. In some cases, a multimeric polypeptide of the present disclosure comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; ii) a first major histocompatibility complex (MHC) polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig scaffold, wherein the multimeric polypeptide comprises one or more immunomodulatory domains, where the one or more immunomodulatory domains is(are): A) at the C-terminus of the first polypeptide; B) at the N-terminus of the second polypeptide; C) at the C-terminus of the second polypeptide; or D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide.

**[0080]** In some cases, a multimeric polypeptide of the present disclosure comprises a first polypeptide and a second polypeptide, where the first polypeptide comprises, in order from amino terminus (N-terminus) to carboxyl terminus (C-terminus): a) an epitope (e.g., a T-cell epitope); b) a first major histocompatibility complex (MHC) polypeptide and c) an immunomodulatory polypeptide; and where the second polypeptide comprises, in order from

N-terminus to C-terminus: a) a second MHC polypeptide; and b) an immunoglobulin (Ig) Fc polypeptide. In other cases, a multimeric polypeptide of the present disclosure comprises a first polypeptide and a second polypeptide, where the first polypeptide comprises, in order from N-terminus to C-terminus: a) an epitope (e.g., a T-cell epitope); and b) a first MHC polypeptide; and where the second polypeptide comprises, in order from N-terminus to C-terminus: a) an immunomodulatory polypeptide; b) a second MHC polypeptide; and c) an Ig Fc polypeptide. In some instances, the first and the second MHC polypeptides are Class I MHC polypeptides; e.g., in some cases, the first MHC polypeptide is an MHC Class I  $\beta$ 2-microglobulin (B2M) polypeptide, and the second MHC polypeptide is an MHC Class I heavy chain (H chain). In other cases, the first and the second MHC polypeptides are Class II MHC polypeptides; e.g., in some cases, the first MHC polypeptide is an MHC Class II  $\alpha$ -chain polypeptide, and the second MHC polypeptide is an MHC Class II  $\beta$ -chain polypeptide. In other cases, the first polypeptide is an MHC Class II  $\beta$ -chain polypeptide, and the second MHC polypeptide is an MHC Class II  $\alpha$ -chain polypeptide. In some cases, the multimeric polypeptide includes two or more immunomodulatory polypeptides. Where a multimeric polypeptide of the present disclosure includes two or more immunomodulatory polypeptides, in some cases, the two or more immunomodulatory polypeptides are present in the same polypeptide chain, and may be in tandem. Where a multimeric polypeptide of the present disclosure includes two or more immunomodulatory polypeptides, in some cases, the two or more immunomodulatory polypeptides are present in separate polypeptides. In some cases, a multimeric polypeptide of the present disclosure is a heterodimer. In some cases, a multimeric polypeptide of the present disclosure is a trimeric polypeptide.

**[0081]** In some cases, a multimeric polypeptide of the present disclosure comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an Ig Fc polypeptide; and iii) an immunomodulatory domain. In some cases, a multimeric polypeptide of the present disclosure comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an immunomodulatory domain. In some cases, a multimeric polypeptide of the present disclosure comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an immunomodulatory domain; and ii) a second MHC polypeptide. In some cases, a multimeric polypeptide of the present disclosure comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; ii) a first MHC polypeptide; and iii) an immunomodulatory domain; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide. In some cases, where a multimeric polypeptide of the present disclosure comprises a non-Ig scaffold, the non-Ig scaffold is an XTEN peptide, a transferrin polypeptide, an Fc receptor polypeptide, an elastin-like polypeptide, a silk-like polypeptide, or a silk-elastin-like polypeptide.

**[0082]** In some cases, a multimeric polypeptide of the present disclosure is monovalent. In

some cases, a multimeric polypeptide of the present disclosure is multivalent. For example, depending on the Fc polypeptide present in a multimeric polypeptide of the present disclosure, the multimeric polypeptide can be a homodimer, where two molecules of the multimeric polypeptide are present in the homodimer, where the two molecules of the multimeric polypeptide can be disulfide linked to one another, e.g., via the Fc polypeptide present in the two molecules. As another example, a multimeric polypeptide of the present disclosure can comprise three, four, or five molecules of the multimeric polypeptide, where the molecules of the multimeric polypeptide can be disulfide linked to one another, e.g., via the Fc polypeptide present in the molecules.

### Linkers

**[0083]** A multimeric polypeptide of the present disclosure can include linker peptides interposed between, e.g., an epitope and an MHC polypeptide, between an MHC polypeptide and an immunomodulatory polypeptide, or between an MHC polypeptide and an Ig Fc polypeptide.

**[0084]** Suitable linkers (also referred to as "spacers") can be readily selected and can be of any of a number of suitable lengths, such as from 1 amino acid (e.g., Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and can be 1, 2, 3, 4, 5, 6, or 7 amino acids.

**[0085]** Exemplary linkers include glycine polymers (G)<sub>n</sub>, glycine-serine polymers (including, for example, (GS)<sub>n</sub>, (GSGGS)<sub>n</sub> (SEQ ID NO:8) and (GGGS)<sub>n</sub> (SEQ ID NO:9), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers can be used; both Gly and Ser are relatively unstructured, and therefore can serve as a neutral tether between components. Glycine polymers can be used; glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, Rev. Computational Chem. 11173-142 (1992)). Exemplary linkers can comprise amino acid sequences including, but not limited to, GGSG (SEQ ID NO:10), GGSGG (SEQ ID NO:11), GSGSG (SEQ ID NO:12), GSAGGG (SEQ ID NO:13), GGGSG (SEQ ID NO:14), GSSSG (SEQ ID NO:15), and the like.

**[0086]** In some cases, a linker polypeptide, present in a first polypeptide of a multimeric polypeptide of the present disclosure, includes a cysteine residue that can form a disulfide bond with a cysteine residue present in a second polypeptide of a multimeric polypeptide of the present disclosure. In some cases, for example, a suitable linker comprises the amino acid sequence GCGASGGGSGGGGS (SEQ ID NO:16).

### Epitopes

**[0087]** An epitope present in a multimeric polypeptide of the present disclosure can have a length of from about 4 amino acids to about 25 amino acids, e.g., the epitope can have a length of from 4 amino acids (aa) to 10 aa, from 10 aa to 15 aa, from 15 aa to 20 aa, or from 20 aa to 25 aa. For example, an epitope present in a multimeric polypeptide of the present disclosure can have a length of 4 amino acids (aa), 5 aa, 6 aa, 7, aa, 8 aa, 9 aa, 10 aa, 11 aa, 12 aa, 13 aa, 14 aa, 15 aa, 16 aa, 17 aa, 18 aa, 19 aa, 20 aa, 21 aa, 22 aa, 23 aa, 24 aa, or 25 aa. In some cases, an epitope present in a multimeric polypeptide of the present disclosure has a length of from 5 amino acids to 10 amino acids, e.g., 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, or 10 aa.

**[0088]** An epitope present in a multimeric polypeptide of the present disclosure is specifically bound by a T-cell, i.e., the epitope is specifically bound by an epitope-specific T cell. An epitope-specific T cell binds an epitope having a reference amino acid sequence, but does not substantially bind an epitope that differs from the reference amino acid sequence. For example, an epitope-specific T cell binds an epitope having a reference amino acid sequence, and binds an epitope that differs from the reference amino acid sequence, if at all, with an affinity that is less than  $10^{-6}$  M, less than  $10^{-5}$  M, or less than  $10^{-4}$  M. An epitope-specific T cell can bind an epitope for which it is specific with an affinity of at least  $10^{-7}$  M, at least  $10^{-8}$  M, at least  $10^{-9}$  M, or at least  $10^{-10}$  M.

**[0089]** Non-limiting examples of epitopes include, e.g., the human T-lymphotrophic virus-1 epitope LLFGYPVYY (SEQ ID NO:17); the tumor epitope KYQAVTTTL (SEQ ID NO:18); and the islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) epitope VYLKTNVFL (SEQ ID NO: 19) or TYLKTNLFL (SEQ ID NO:20). Yang et al. (2006) J. Immunol 176:2781.

### **MHC polypeptides**

**[0090]** As noted above, a multimeric polypeptide of the present disclosure includes MHC polypeptides. For the purposes of the instant disclosure, the term "major histocompatibility complex (MHC) polypeptides" is meant to include MHC polypeptides of various species, including human MHC (also referred to as human leukocyte antigen (HLA) polypeptides, rodent (e.g., mouse, rat, etc.) MHC polypeptides, and MHC polypeptides of other mammalian species (e.g., lagomorphs, non-human primates, canines, felines, ungulates (e.g., equines, bovines, ovines, caprines, etc.), and the like. The term "MHC polypeptide" is meant to include Class I MHC polypeptides (e.g.,  $\beta$ -2 microglobulin and MHC class I heavy chain) and MHC Class II polypeptides (e.g., MHC Class II  $\alpha$  polypeptide and MHC Class II  $\beta$  polypeptide).

**[0091]** As noted above, in some embodiments of a multimeric polypeptide of the present disclosure, the first and the second MHC polypeptides are Class I MHC polypeptides; e.g., in

some cases, the first MHC polypeptide is an MHC Class I  $\beta$ 2-microglobulin (B2M) polypeptide, and the second MHC polypeptide is an MHC Class I heavy chain (H chain). In other cases, the first and the second MHC polypeptides are Class II MHC polypeptides; e.g., in some cases, the first MHC polypeptide is an MHC Class II  $\alpha$ -chain polypeptide, and the second MHC polypeptide is an MHC Class II  $\beta$ -chain polypeptide. In other cases, the first polypeptide is an MHC Class II  $\beta$ -chain polypeptide, and the second MHC polypeptide is an MHC Class II  $\alpha$ -chain polypeptide.

**[0092]** In some cases, an MHC polypeptide of a multimeric polypeptide of the present disclosure is a human MHC polypeptide, where human MHC polypeptides are also referred to as "human leukocyte antigen" ("HLA") polypeptides. In some cases, an MHC polypeptide of a multimeric polypeptide of the present disclosure is a Class I HLA polypeptide, e.g., a  $\beta$ 2-microglobulin polypeptide, or a Class I HLA heavy chain polypeptide. Class I HLA heavy chain polypeptides include HLA-A heavy chain polypeptides, HLA-B heavy chain polypeptides, HLA-C heavy chain polypeptides, HLA-E heavy chain polypeptides, HLA-F heavy chain polypeptides, and HLA-G heavy chain polypeptides. In some cases, an MHC polypeptide of a multimeric polypeptide of the present disclosure is a Class II HLA polypeptide, e.g., a Class II HLA  $\alpha$  chain or a Class II HLA  $\beta$  chain. MHC Class II polypeptides include MCH Class II DP  $\alpha$  and  $\beta$  polypeptides, DM  $\alpha$  and  $\beta$  polypeptides, DOA  $\alpha$  and  $\beta$  polypeptides, DOB  $\alpha$  and  $\beta$  polypeptides, DQ  $\alpha$  and  $\beta$  polypeptides, and DR  $\alpha$  and  $\beta$  polypeptides.

**[0093]** As an example, an MHC Class I heavy chain polypeptide of a multimeric polypeptide of the present disclosure can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 25-365 of the amino acid sequence of the human HLA-A heavy chain polypeptide depicted in Figure 25A.

**[0094]** As an example, an MHC Class I heavy chain polypeptide of a multimeric polypeptide of the present disclosure can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 25-365 of the amino acid sequence of the following human HLA-A heavy chain amino acid sequence:

GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSAASQRMEPRAPWIEQEGPEY

WDGETRKVKAHSQTHRVDLGLTRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQ  
YAYDGKDYIALKEDLRSWTAADMAAQTCHKWEEAHVAEQLRAYLEGTCVEWLRRY  
LENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTE  
LVETRPAGDGTQKWAADVPSGQEQRYTCHVQHEGLPKPLTRWEP (SEQ ID NO:5).

**[0095]** As another example, an MHC Class I heavy chain polypeptide of a multimeric polypeptide of the present disclosure can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 25-362 of the amino acid sequence of the

human HLA-B heavy chain polypeptide depicted in Figure 25B.

**[0096]** As another example, an MHC Class I heavy chain polypeptide of a multimeric polypeptide of the present disclosure can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 25-362 of the amino acid sequence of the human HLA-C heavy chain polypeptide depicted in Figure 25C.

**[0097]** As another example, an MHC Class I heavy chain polypeptide of a multimeric polypeptide of the present disclosure can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

GPHSLRYFVTAVSRPGLGEPRFIAVGYVDDTQFVRFDSADNPRFEPRAPWMEQ

EGPEYWEEQTQRAKSDEQWFRVSLRTAQRYYNQSKGGSHTEQRMFGCDVGSDWRLLRGYQQFAYDGRDYIALNEDLKTWTAADTAALITRRKWEQAGDAEYYRAYLEGECVEWLRRYLELGNETLLRTDSPKAHVTYHPRSQVDVTLRCWALGFYPADITLTWQLNGEDLTQDMELVETRPAAGDGTFQKWAAGVPLGKEQNYTCHVHKGLPEPLTLRW (SEQ ID NO:22).

**[0098]** A  $\beta$ 2-microglobulin (B2M) polypeptide of a multimeric polypeptide of the present disclosure can be a human B2M polypeptide, a non-human primate B2M polypeptide, a murine B2M polypeptide, and the like. In some instances, a B2M polypeptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a B2M amino acid sequence depicted in FIG. 20.

**[0099]** In some cases, an MHC polypeptide comprises a single amino acid substitution relative to a reference MHC polypeptide (where a reference MHC polypeptide can be a wild-type MHC polypeptide), where the single amino acid substitution substitutes an amino acid with a cysteine (Cys) residue. Such cysteine residues, when present in an MHC polypeptide of a first polypeptide of a multimeric polypeptide of the present disclosure, can form a disulfide bond with a cysteine residue present in a second polypeptide chain of a multimeric polypeptide of the present disclosure.

**[0100]** In some cases, a first MHC polypeptide in a first polypeptide of a multimeric polypeptide of the present disclosure, and/or the second MHC polypeptide in the second polypeptide of a multimeric polypeptide of the present disclosure, includes an amino acid substitution to substitute an amino acid with a cysteine, where the substituted cysteine in the first MHC polypeptide forms a disulfide bond with a cysteine in the second MHC polypeptide, where a cysteine in the first MHC polypeptide forms a disulfide bond with the substituted cysteine in the second MHC polypeptide, or where the substituted cysteine in the first MHC polypeptide forms a disulfide bond with the substituted cysteine in the second MHC polypeptide.

**[0101]** For example, in some cases, one of following pairs of residues in an HLA  $\beta$ 2-microglobulin and an HLA Class I heavy chain is substituted with cysteines: 1) B2M residue 12, HLA Class I heavy chain residue 236; 2) B2M residue 12, HLA Class I heavy chain residue 237; 3) B2M residue 8, HLA Class I heavy chain residue 234; 4) B2M residue 10, HLA Class I heavy chain residue 235; 5) B2M residue 24, HLA Class I heavy chain residue 236; 6) B2M residue 28, HLA Class I heavy chain residue 232; 7) B2M residue 98, HLA Class I heavy chain residue 192; 8) B2M residue 99, HLA Class I heavy chain residue 234; 9) B2M residue 3, HLA Class I heavy chain residue 120; 10) B2M residue 31, HLA Class I heavy chain residue 96; 11) B2M residue 53, HLA Class I heavy chain residue 35; 12) B2M residue 60, HLA Class I heavy chain residue 96; 13) B2M residue 60, HLA Class I heavy chain residue 122; 14) B2M residue 63, HLA Class I heavy chain residue 27; 15) B2M residue Arg3, HLA Class I heavy chain residue Gly120; 16) B2M residue His31, HLA Class I heavy chain residue Gln96; 17) B2M residue Asp53, HLA Class I heavy chain residue Arg35; 18) B2M residue Trp60, HLA Class I heavy chain residue Gln96; 19) B2M residue Trp60, HLA Class I heavy chain residue Asp 122; 20) B2M residue Tyr63, HLA Class I heavy chain residue Tyr27; 21) B2M residue Lys6, HLA Class I heavy chain residue Glu232; 22) B2M residue Gln8, HLA Class I heavy chain residue Arg234; 23) B2M residue Tyr10, HLA Class I heavy chain residue Pro235; 24) B2M residue Ser11, HLA Class I heavy chain residue Gln242; 25) B2M residue Asn24, HLA Class I heavy chain residue Ala236; 26) B2M residue Ser28, HLA Class I heavy chain residue Glu232; 27) B2M residue Asp98, HLA Class I heavy chain residue His192; and 28) B2M residue Met99, HLA Class I heavy chain residue Arg234. The amino acid numbering of the MHC/HLA Class I heavy chain is in reference to the mature MHC/HLA Class I heavy chain, without a signal peptide. For example, in the amino acid sequence depicted in Figure 25A, which includes a signal peptide, Gly120 is Gly144; Gln96 is Gln120; etc.

### Immunomodulatory polypeptides

**[0102]** An immunomodulatory polypeptide of a multimeric polypeptide of the present disclosure can be an activating immunomodulatory polypeptide or an inhibitory immunomodulatory polypeptide. In some cases, a multimeric polypeptide of the present disclosure includes a single immunomodulatory polypeptide. In some cases, a multimeric polypeptide of the present disclosure includes two immunomodulatory polypeptides. In some cases, the two immunomodulatory polypeptides are in tandem in a polypeptide chain. In some cases, the two immunomodulatory polypeptides are in separate polypeptide chains. In some cases, the two immunomodulatory polypeptides are in separate polypeptide chains and are disulfide linked to one another.

**[0103]** An immunomodulatory polypeptide of a multimeric polypeptide of the present disclosure is in some cases a T-cell modulatory polypeptide. In some cases, the T-cell modulatory polypeptide is a stimulatory (activating) T-cell modulatory polypeptide. In some cases, the T-cell modulatory polypeptide is an inhibitory T-cell modulatory polypeptide. A T-cell modulatory polypeptide can be an antibody, a peptide ligand, a T-cell co-stimulatory polypeptide, a cytokine, or a toxin.

**[0104]** In some cases, an immunomodulatory polypeptide of a multimeric polypeptide of the present disclosure is an antibody-based or non-antibody-based recognition moiety that specifically binds a co-stimulatory polypeptide that is expressed on the surface of an epitope-specific T cell. Antibody-based recognition moieties include, e.g., antibodies; fragments of antibodies that retain specific binding to antigen, including, but not limited to, Fab, Fv, single-chain Fv (scFv), and Fd fragments; chimeric antibodies; humanized antibodies; single-chain antibodies (scAb), single domain antibodies (dAb); single domain heavy chain antibodies; single domain light chain antibodies; and the like. Suitable non-antibody-based recognition moieties include, e.g., affibodies; engineered Kunitz domains; monobodies (adnectins); anticalins; aptamers; designed ankyrin repeat domains (DARPins); a binding site of a cysteine-rich polypeptide (e.g., cysteine-rich knottin peptides); avimers; afflins; and the like. An antibody-based or non-antibody-based recognition moiety specifically binds co-stimulatory polypeptide that is expressed on the surface of an epitope-specific T cell, where such co-stimulatory polypeptides include, but are not limited to, CTLA4, PD1, ICOS, OX40, CD20, and 4-1BB. Co-stimulatory polypeptides that are expressed on the surface of an epitope-specific T cell are known in the art.

**[0105]** In some cases, an immunomodulatory polypeptide of a multimeric polypeptide of the present disclosure is a T-cell co-stimulatory polypeptide. In some cases, an immunomodulatory polypeptide of a multimeric polypeptide of the present disclosure is a T-cell co-stimulatory polypeptide and is a member of the tumor necrosis factor (TNF) superfamily; e.g., a FasL polypeptide, a 41BBL polypeptide, a CD40 polypeptide, an OX40L polypeptide, a CD30L polypeptide, a CD70 polypeptide, etc. In some cases, an immunomodulatory polypeptide of a multimeric polypeptide of the present disclosure is a T-cell co-stimulatory polypeptide and is a member of the immunoglobulin (Ig) superfamily; e.g., a CD7 polypeptide, a CD86 polypeptide, an ICAM polypeptide, etc.

**[0106]** Suitable immunomodulatory polypeptides of a multimeric polypeptide of the present disclosure include, but are not limited to, CD80 (B7-1), CD86 (B7-2), 4-1BBL, OX40L, ICOS-L, ICAM, PD-L1, FasL, and PD-L2. Suitable immunomodulatory polypeptides of a multimeric polypeptide of the present disclosure include, e.g., CD7, CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, and HVEM.

**[0107]** In some cases, a T-cell modulatory polypeptide of a multimeric polypeptide of the present disclosure is a PD-L1 polypeptide. In some cases, a PD-L1 polypeptide of a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 19-290 of a PD-L1 amino acid sequence depicted in Figure 26A or 26B.

**[0108]** In some cases, a T-cell modulatory polypeptide of a multimeric polypeptide of the present disclosure is a 4-1BBL polypeptide. In some cases, a 4-1BBL polypeptide of a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at

least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 50-254 of the 4-1BBL amino acid sequence depicted in Figure 27.

**[0109]** In some cases, a T-cell modulatory polypeptide of a multimeric polypeptide of the present disclosure is an ICOS-L polypeptide. In some cases, an ICOS-L polypeptide of a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 19-302 of the ICOS-L amino acid sequence depicted in Figure 28.

**[0110]** In some cases, a T-cell modulatory polypeptide of a multimeric polypeptide of the present disclosure is an OX40L polypeptide. In some cases, an OX40L polypeptide of a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 1-183 of the OX40L amino acid sequence depicted in Figure 29.

**[0111]** In some cases, a T-cell modulatory polypeptide of a multimeric polypeptide of the present disclosure is a PD-L2 polypeptide. In some cases, a PD-L2 polypeptide of a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 20-273 of the PD-L2 amino acid sequence depicted in Figure 30.

**[0112]** In some cases, a T-cell modulatory polypeptide of a multimeric polypeptide of the present disclosure is a CD80 (B7-1) polypeptide. In some cases, a CD80 polypeptide of a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 35-288 of the CD80 amino acid sequence depicted in Figure 31.

**[0113]** In some cases, a T-cell modulatory polypeptide of a multimeric polypeptide of the present disclosure is a CD86 polypeptide. In some cases, a CD86 polypeptide of a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 31-329 of the CD86 amino acid sequence depicted in Figure 32.

**[0114]** In some cases, a T-cell modulatory polypeptide of a multimeric polypeptide of the present disclosure is a FasL polypeptide. In some cases, a FasL polypeptide of a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 1-281 of the FasL amino acid sequence depicted

in Figure 33.

**[0115]** Further T cell modulatory domains (MODs) that can be employed in the invention include naturally occurring or synthetic human gene products (protein), affinity reagents (e.g., an antibody, antibody fragment, single chain Fvs, aptamers, nanobody) targeting a human gene product, including, but not limited to all secreted proteins arising from classical and non-classical (e.g., FGF2, IL1, S100A4) secretion mechanisms, and ecto-domains of all cell surface proteins anchored by naturally occurring genetically encoded protein segments (single or multiple membrane spans) or post-translational modifications such as GPI linkages). Any naturally occurring or synthetic affinity reagent (e.g., antibody, antibody fragment, single chain Fvs, aptamer, nanobody, lectin, etc) targeting a cell surface glycan or other post-translational modification (e.g., sulfation). Examples include, but are not limited to, members of the TNF/TNFR family (OX40L, ICOSL, FASL, LTA, LTB TRAIL, CD153, TNFSF9, RANKL, TWEAK, TNFSF13, TNFSF13b, TNFSF14, TNFSF15, TNFSF18, CD40LG, CD70) or affinity reagents directed at the TNF/TNFR family members; members of the Immunoglobulin superfamily (VISTA, PD1, PD-L1, PD-L2, B71, B72, CTLA4, CD28, TIM3, CD4, CD8, CD19, T cell receptor chains, ICOS, ICOS ligand, HHLA2, butyrophilins, BTLA, B7-H3, B7-H4, CD3, CD79a, CD79b, IgSF CAMS (including CD2, CD58, CD48, CD150, CD229, CD244, ICAM-1), Leukocyte immunoglobulin like receptors (LILR), killer cell immunoglobulin like receptors (KIR)), lectin superfamily members, selectins, cytokines/chemokine and cytokine/chemokine receptors, growth factors and growth factor receptors), adhesion molecules (integrins, fibronectins, cadherins), or ecto-domains of multi-span integral membrane protein, or affinity reagents directed at the Immunoglobulin superfamily and listed gene products. In addition, active homologs/orthologs of these gene products, including but not limited to, viral sequences (e.g., CMV, EBV), bacterial sequences, fungal sequences, eukaryotic pathogens (e.g., *Schistosoma*, *Plasmodium*, *Babesia*, *Eimeria*, *Theileria*, *Toxoplasma*, *Entamoeba*, *Leishmania*, and *Trypanosoma*), and mammalian -derived coding regions. In addition, a MOD may comprise a small molecules drug targeting a human gene product.

### Fc polypeptides

**[0116]** A multimeric polypeptide of the present disclosure comprises an Fc polypeptide, or another suitable scaffold polypeptide.

**[0117]** Suitable scaffold polypeptides include antibody-based scaffold polypeptides and non-antibody-based scaffolds. Non-antibody-based scaffolds include, e.g., albumin, an XTE<sup>N</sup> (extended recombinant) polypeptide, transferrin, an Fc receptor polypeptide, an elastin-like polypeptide (see, e.g., Hassouneh et al. (2012) Methods Enzymol. 502:215; e.g., a polypeptide comprising a pentapeptide repeat unit of (Val-Pro-Gly-X-Gly), where X is any amino acid other than proline), an albumin-binding polypeptide, a silk-like polypeptide (see, e.g., Valluzzi et al. (2002) Philos Trans R Soc Lond B Biol Sci. 357:165), a silk-elastin-like polypeptide (SELP; see, e.g., Megeed et al. (2002) Adv Drug Deliv Rev. 54:1075), and the like. Suitable XTE<sup>N</sup> polypeptides include, e.g., those disclosed in WO 2009/023270, WO 2010/091122, WO

2007/103515, US 2010/0189682, and US 2009/0092582; see also Schellenberger et al. (2009) *Nat Biotechnol.* 27:1186). Suitable albumin polypeptides include, e.g., human serum albumin.

**[0118]** Suitable scaffold polypeptides will in some cases be a half-life extending polypeptides. Thus, in some cases, a suitable scaffold polypeptide increases the *in vivo* half-life (e.g., the serum half-life) of the multimeric polypeptide, compared to a control multimeric polypeptide lacking the scaffold polypeptide. For example, in some cases, a scaffold polypeptide increases the *in vivo* half-life (e.g., the serum half-life) of the multimeric polypeptide, compared to a control multimeric polypeptide lacking the scaffold polypeptide, by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 50%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, at least about 25-fold, at least about 50-fold, at least about 100-fold, or more than 100-fold. As an example, in some cases, an Fc polypeptide increases the *in vivo* half-life (e.g., the serum half-life) of the multimeric polypeptide, compared to a control multimeric polypeptide lacking the Fc polypeptide, by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 50%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, at least about 25-fold, at least about 50-fold, at least about 100-fold, or more than 100-fold.

**[0119]** The Fc polypeptide of a multimeric polypeptide of the present disclosure can be a human IgG1 Fc, a human IgG2 Fc, a human IgG3 Fc, a human IgG4 Fc, etc. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to an amino acid sequence of an Fc region depicted in Figures 24A-C. In some cases, the Fc region comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the human IgG1 Fc polypeptide depicted in Figure 24A. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the human IgG2 Fc polypeptide depicted in Figure 24A; e.g., the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to amino acids 99-325 of the human IgG2 Fc polypeptide depicted in Figure 24A. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the human IgG3 Fc polypeptide depicted in Figure 24A; e.g., the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to amino acids 19-246 of the human IgG3 Fc polypeptide depicted in Figure 24A. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to amino acids 19-246 of the human IgG3 Fc polypeptide depicted in Figure 24A.

least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the human IgM Fc polypeptide depicted in FIG. 24B; e.g., the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to amino acids 1-276 to the human IgM Fc polypeptide depicted in FIG. 24B. In some cases, the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the human IgA Fc polypeptide depicted in Figure 24C; e.g., the Fc polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to amino acids 1-234 to the human IgA Fc polypeptide depicted in FIG. 24C.

### Additional polypeptides

**[0120]** A polypeptide chain of a multimeric polypeptide of the present disclosure can include one or more polypeptides in addition to those described above. Suitable additional polypeptides include epitope tags and affinity domains. The one or more additional polypeptide can be included at the N-terminus of a polypeptide chain of a multimeric polypeptide of the present disclosure, at the C-terminus of a polypeptide chain of a multimeric polypeptide of the present disclosure, or internally within a polypeptide chain of a multimeric polypeptide of the present disclosure.

#### *Epitope tag*

**[0121]** Suitable epitope tags include, but are not limited to, hemagglutinin (HA; e.g., YPYDVPDYA (SEQ ID NO:23); FLAG (e.g., DYKDDDDK (SEQ ID NO:24); c-myc (e.g., EQKLISEEDL; SEQ ID NO:25), and the like.

#### *Affinity domain*

**[0122]** Affinity domains include peptide sequences that can interact with a binding partner, e.g., such as one immobilized on a solid support, useful for identification or purification. DNA sequences encoding multiple consecutive single amino acids, such as histidine, when fused to the expressed protein, may be used for one-step purification of the recombinant protein by high affinity binding to a resin column, such as nickel sepharose. Exemplary affinity domains include His5 (HHHHH) (SEQ ID NO:26), HisX6 (HHHHHH) (SEQ ID NO:27), C-myc (EQKLISEEDL) (SEQ ID NO:25), Flag (DYKDDDDK) (SEQ ID NO:24), StrepTag (WSHPQFEK) (SEQ ID NO:28), hemagglutinin, e.g., HA Tag (YPYDVPDYA) (SEQ ID NO:23), glutathione-S-

transferase (GST), thioredoxin, cellulose binding domain, RYIRS (SEQ ID NO:30), Phe-His-His-Thr (SEQ ID NO:31), chitin binding domain, S-peptide, T7 peptide, SH2 domain, C-end RNA tag, WEAAAREACCRECCARA (SEQ ID NO:32), metal binding domains, e.g., zinc binding domains or calcium binding domains such as those from calcium-binding proteins, e.g., calmodulin, troponin C, calcineurin B, myosin light chain, recoverin, S-modulin, visinin, VILIP, neurocalcin, hippocalcin, frequenin, caltractin, calpain large-subunit, S100 proteins, parvalbumin, calbindin D9K, calbindin D28K, and calretinin, inteins, biotin, streptavidin, MyoD, Id, leucine zipper sequences, and maltose binding protein.

### Modifications

**[0123]** A multimeric polypeptide of the present disclosure can include one or more non-polypeptide moieties covalently linked to the multimeric polypeptide. Suitable non-polypeptide moieties include, e.g., biocompatible fatty acids and derivatives thereof; Hydroxy Alkyl Starch (HAS) e.g. Hydroxy Ethyl Starch (HES); poly(ethylene glycol); hyaluronic acid (HA); heparosan polymers (HEP); phosphorylcholine-based polymers; dextran; poly-sialic acids (PSA); and the like. In some cases, the non-polypeptide moiety increases the *in vivo* half-life of the multimeric polypeptide, compared to a control multimeric polypeptide that does not comprise the non-polypeptide moiety.

**[0124]** In some cases, a multimeric polypeptide of the present disclosure includes a detectable label. Suitable detectable labels include radioisotopes such as <sup>123</sup>I (iodine), <sup>18</sup>F (fluorine), <sup>99</sup>Tc (technetium), <sup>111</sup>In (indium), <sup>67</sup>Ga (gallium), radioactive Gd isotopes (<sup>153</sup>Gd); contrast agents such as gadolinium (Gd), dysprosium, and iron; an enzyme which generates a detectable product (e.g., luciferase,  $\beta$ -galactosidase, horse radish peroxidase, alkaline phosphatase, and the like); a fluorescent protein; a chromogenic protein, dye (e.g., fluorescein isothiocyanate, rhodamine, phycoerythrin, and the like); fluorescence emitting metals, e.g., <sup>152</sup>Eu, or others of the lanthanide series; chemiluminescent compounds, e.g., luminol, isoluminol, acridinium salts, and the like; bioluminescent compounds; and the like.

### Activity

**[0125]** Depending on the nature of the immunomodulatory ("MOD") polypeptide present in a multimeric polypeptide of the present disclosure, the multimeric polypeptide can activate or inhibit a target T cell. A multimeric polypeptide of the present disclosure selectively activates or inhibits a target T cell that is specific for the epitope present in the multimeric polypeptide. "Target T cells" include epitope-specific CD4 $^{+}$  T cells, epitope-specific CD8 $^{+}$  T cells. In some cases, the target CD4 $^{+}$  T cell is a helper T cell (e.g., a Th1, Th2, or Th17 cell). In some cases, the target CD4 $^{+}$  T cell is a CD4 $^{+}$ /CD25 $^{+}$ /FoxP3 $^{+}$  regulatory T (Treg) cell. In some cases, the target T cell is a CD8 $^{+}$  T cell and is a cytotoxic T cell. In some cases, the target T cell is a

memory T cell, which can be a CD4<sup>+</sup> T cell or a CD8<sup>+</sup> T cell, where memory T cells are generally CD45RO<sup>+</sup>. In some cases, the target T cell is an NK-T cell.

**[0126]** In some cases, a multimeric polypeptide of the present disclosure enhances T cell homing and trafficking. For example, in some cases, a multimeric polypeptide of the present disclosure, when contacted with a target T cell, increases extravasation of the target T cell to a treatment site. In some cases, a multimeric polypeptide of the present disclosure, when contacted with a target T cell, increases extravasation of the target T cell to a treatment site by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 75%, at least 2-fold, at least 5-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, at least 100-fold, or more than 100-fold, compared to the level of extravasation of the target T cell not contacted with the multimeric polypeptide. Increased extravasation can increase the number of T cells at a treatment site. In some cases, a multimeric polypeptide of the present disclosure, when contacted with a target T cell, increases the number of T cells at a treatment site.

**[0127]** In some cases, a multimeric polypeptide of the present disclosure increases the expression by a target T cell of one or more proteins that mediate or regulate lymphocyte trafficking by the target T cell. For example, in some cases, a multimeric polypeptide of the present disclosure, when contacted with a target T cell, increases the level of one or more adhesion molecules and/or chemokine receptor molecules in the target T cell. For example, in some cases, a multimeric polypeptide of the present disclosure, when contacted with a target T cell, increases the expression of one or more adhesion molecules and/or chemokine receptor molecules by the target T cell by at least 2-fold, at least 5-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, at least 100-fold, or more than 100-fold, compared to the level of the adhesion molecule and/or chemokine receptor molecule produced by the target T cell not contacted with the multimeric polypeptide. Examples of adhesion molecules include adhesion molecules produced by CD8 T cells, where examples of such adhesion molecules include, but are not limited to, CD44, LFA-1, and VLA-4. Examples of chemokine receptors include chemokine receptors produced by CD8 T cells, where examples of such chemokine receptors include, but are not limited to, CCR5, CCR7 and CXCR3.

**[0128]** In some cases, a multimeric polypeptide of the present disclosure results in the generation of memory T cells capable of rapid cytotoxic responses against a previously experienced epitope. For example, in some cases, a multimeric polypeptide of the present disclosure, when contacted with a target T cell, results in the generation of memory T cells comprising 0.5% or more of the antigen-specific T cell pool. For example, in some cases, a multimeric polypeptide of the present disclosure, when contacted with a target T cell, results in the generation of memory T cells comprising 0.5% or more, 1% or more, 2% or more, 3% or more, 4% or more, 5% or more, 10% or more, 15% or more, or 20% or more, of the antigen-specific T cell pool. An example of a cell surface marker of T memory cells is CD45RO.

**[0129]** In some cases, a multimeric polypeptide of the present disclosure increases proliferation of a target T cell. For example, in some cases, a multimeric polypeptide of the

present disclosure, when contacted with a target T cell, increases proliferation of the target T cell by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 75%, at least 2-fold, at least 5-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, at least 100-fold, or more than 100-fold, compared to the proliferation of the target T cell not contacted with the multimeric polypeptide.

**[0130]** In some cases, a multimeric polypeptide of the present disclosure increases cytotoxic activity of a T cell toward a target cell. For example, in some cases, a multimeric polypeptide of the present disclosure, when contacted with a target T cell, increases cytotoxic activity of the T cell toward a target cell by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 75%, at least 2-fold, at least 5-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, at least 100-fold, or more than 100-fold, compared to the cytotoxic activity of the T cell toward the target cell not contacted with the multimeric polypeptide. Targets of T cells include virus-infected cells, cancer cells, and the like.

**[0131]** In some cases, a multimeric polypeptide of the present disclosure increases cytokine production by a target T cell. For example, in some cases, a multimeric polypeptide of the present disclosure, when contacted with a target T cell, increases cytokine production by the T cell by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 75%, at least 2-fold, at least 5-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, at least 100-fold, or more than 100-fold, compared to the level of cytokine produced by the target T cell not contacted with the multimeric polypeptide. Examples of cytokines include cytokines produced by Th1 cells, e.g., IL-2, IFN- $\gamma$ , and TNF- $\alpha$ ; cytokines produced by Th17 cells, e.g., IL-17, IL-21, and IL-22; cytokines produced by Treg cells, e.g., TGF- $\beta$ , IL-35, and IL-10.

**[0132]** In some cases, a multimeric polypeptide of the present disclosure inhibits cytokine production by a target T cell. For example, in some cases, a multimeric polypeptide of the present disclosure, when contacted with a target T cell, inhibits cytokine production by a target T cell by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, or more than 90%, compared to the level of cytokine produced by the target T cell not contacted with the multimeric polypeptide. Examples of cytokines include cytokines produced by Th2 cells, e.g., IL-4, IL-5, IL-6, IL-10, and IL-13.

### Exemplary embodiments

**[0133]** Non-limiting examples of a multimeric polypeptide of the present disclosure include:

1. 1) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a 4-BBL polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii)

an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

2. 2) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a PD-L1 polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

3. 3) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) ICOS-L polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a

linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

4. 4) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) an OX40L polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;
5. 5) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a CD80 polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one

another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

6. 6) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a CD86 polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

7. 7) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a PD-L2 polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide

and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

8. 8) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; and ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) i) a 4-BBL polypeptide; ii) an MHC Class I heavy chain polypeptide; and iii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

9. 9) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; and ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) i) a PD-L1 polypeptide; ii) an MHC Class I heavy chain polypeptide; and iii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present

in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

10. 10) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; and ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) i) an ICOS-L polypeptide; ii) an MHC Class I heavy chain polypeptide; and iii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;
11. 11) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; and ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) i) an OX40L polypeptide; ii) an MHC Class I heavy chain polypeptide; and iii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide

include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

12. 12) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; and ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) i) a CD80 polypeptide; ii) an MHC Class I heavy chain polypeptide; and iii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;
13. 13) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; and ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) i) a CD86 polypeptide; ii) an MHC Class I heavy chain polypeptide; and iii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc

polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

14. 14) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; and ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) i) a PD-L2 polypeptide; ii) an MHC Class I heavy chain polypeptide; and iii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;
15. 15) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; and ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) i) a FasL polypeptide; ii) an MHC Class I heavy chain polypeptide; and iii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM

Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

16. 16) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) two 4-BBL polypeptides in tandem; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;
17. 17) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) two PD-L1 polypeptides in tandem; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide.

In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

18. 18) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) two ICOS-L polypeptides in tandem; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;
19. 19) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) two OX40L polypeptides in tandem; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide.

In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

20. 20) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) two CD80 polypeptides in tandem; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

21. 21) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) two CD86 polypeptides in tandem; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide.

In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

22. 22) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) two PD-L2 polypeptides in tandem; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

23. 23) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) two FasL polypeptides in tandem; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide.

In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

24. 24) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a first 4-1BBL polypeptide; b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide; and c) a third polypeptide comprising a second 4-1BBL polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another; and the first and the third polypeptides are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the first polypeptide and the third polypeptide are disulfide linked to one another via a cysteine residue present in (or substituted into) the first and the second 4-1BBL polypeptides. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

25. 25) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a first PD-L1 polypeptide; b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide; and c) a third polypeptide comprising a second PD-L1 polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another; and the first and the third polypeptides are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in

the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the first polypeptide and the third polypeptide are disulfide linked to one another via a cysteine residue present in (or substituted into) the first and the second PD-L1 polypeptides. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

26. 26) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a first ICOS-L polypeptide; b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide; and c) a third polypeptide comprising a second ICOS-L polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another; and the first and the third polypeptides are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the first polypeptide and the third polypeptide are disulfide linked to one another via a cysteine residue present in (or substituted into) the first and the second ICOS-L polypeptides. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;
27. 27) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a first OX40L polypeptide; b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii)

an Ig Fc polypeptide; and c) a third polypeptide comprising a second OX40L polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another; and the first and the third polypeptides are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the first polypeptide and the third polypeptide are disulfide linked to one another via a cysteine residue present in (or substituted into) the first and the second OX40L polypeptides. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

28. 28) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a first CD80 polypeptide; b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide; and c) a third polypeptide comprising a second CD80 polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another; and the first and the third polypeptides are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the first polypeptide and the third polypeptide are disulfide linked to one another via a cysteine residue present in (or substituted into) the first and the second CD80 polypeptides. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In

some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

29. 29) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a first CD86 polypeptide; b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide; and c) a third polypeptide comprising a second CD86 polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another; and the first and the third polypeptides are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the first polypeptide and the third polypeptide are disulfide linked to one another via a cysteine residue present in (or substituted into) the first and the second CD86 polypeptides. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide;

30. 30) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a CD80 polypeptide; b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide; and c) a third polypeptide comprising a CD86 polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another; and the first and the third polypeptides are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present

in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the first polypeptide and the third polypeptide are disulfide linked to one another via a cysteine residue present in (or substituted into) the CD80 polypeptide and the CD86 polypeptides. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide; and

31. 31) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a first PD-L2 polypeptide; b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide; and c) a third polypeptide comprising a second PD-L2 polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another; and the first and the third polypeptides are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the first polypeptide and the third polypeptide are disulfide linked to one another via a cysteine residue present in (or substituted into) the first and the second PD-L2 polypeptides. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide; and
32. 32) a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from

N-terminus to C-terminus: i) a T-cell epitope; ii) an MHC Class I  $\beta$ 2-microglobulin polypeptide; and iii) a first FasL polypeptide; b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an MHC Class I heavy chain polypeptide; and ii) an Ig Fc polypeptide; and c) a third polypeptide comprising a second FasL polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another; and the first and the third polypeptides are disulfide linked to one another. In some cases, the first polypeptide comprises a linker polypeptide between the epitope and the  $\beta$ 2-microglobulin polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the linker polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide. In some cases, the first polypeptide and the second polypeptide are disulfide linked to one another via a cysteine residue present in the MHC Class I  $\beta$ 2-microglobulin polypeptide, and a cysteine residue present in the MHC Class I heavy chain polypeptide; in some of these embodiments, the MHC Class I  $\beta$ 2-microglobulin polypeptide and/or the MHC Class I heavy chain polypeptide include an amino acid substitution to provide a cysteine that participates in the disulfide bond. In some cases, the first polypeptide and the third polypeptide are disulfide linked to one another via a cysteine residue present in (or substituted into) the first and the second FasL polypeptides. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG2 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgG3 Fc polypeptide. In some cases, the Ig Fc polypeptide is an IgA Fc polypeptide or an IgM Fc polypeptide. In some cases, MHC Class II polypeptides are used in place of the MHC Class I polypeptides. In some cases, the multimeric polypeptide includes an epitope tag and/or an affinity domain C-terminal to the Fc polypeptide.

#### POLYPROTEIN PRECURSORS

**[0134]** This disclosure provides a recombinant polypeptide comprising a sequence of amino acids identical to a first B2M leader sequence contiguous with a candidate epitope peptide contiguous with a first amino acid linker sequence contiguous with a sequence of amino acids identical to a human native B2M peptide sequence contiguous with a second amino acid linker sequence contiguous with a T cell modulatory domain peptide sequence contiguous with a third amino acid linker contiguous with a second B2M leader sequence contiguous with a sequence of amino acids identical to a MHC heavy chain contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain.

**[0135]** The first amino acid may be any sequence of amino acids 50 amino acids or less to a minimum of 5 amino acids. The second amino acid linker may be any sequence of amino acids 70 amino acids or less to a minimum of 5 amino acids. The third amino acid linker may be a

viral 2A peptide, or a peptide with known protease cleavage ability (e.g., a furin cleavage site, Tobacco Etch Virus [TEV] sequence, Precision protease site, or thrombin protease). For example, the first amino acid comprises GGGSGGGGSGGGS (SEQ ID NO:1). In another example, the second amino acid linker comprises GGGSGGGGSGGGSGGGS (SEQ ID NO:2). In another example, the third amino acid linker comprises SGSGATNFSLLKQAGDVEENPGP (SEQ ID NO:3).

**[0136]** This disclosure also provides recombinant polypeptide comprising a sequence of amino acids identical to a first B2M leader sequence contiguous with a candidate epitope peptide contiguous with a first amino acid linker sequence contiguous with a sequence of amino acids identical to a human native B2M peptide sequence contiguous with a second amino acid linker sequence contiguous with a second B2M leader sequence contiguous with a T cell modulatory domain peptide sequence contiguous with a third amino acid linker contiguous with a sequence of amino acids identical to a MHC heavy chain contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain.

#### Linkers

**[0137]** The first amino acid may be any sequence of amino acids 50 amino acids or less to a minimum of 5 amino acids. The second amino acid linker may be any sequence of amino acids 70 amino acids or less to a minimum of 5 amino acids. The third amino acid linker may be a viral 2A peptide, or a peptide with known protease cleavage ability (e.g., a furin cleavage site, Tobacco Etch Virus [TEV] sequence, Precision protease site, or thrombin protease). For example, the first amino acid comprises GGGSGGGGSGGGS (SEQ ID NO:1). In another example, the second amino acid linker comprises GGGSGGGGSGGGSGGGS (SEQ ID NO:2). In a further example, the third amino acid linker comprises SGSGATNFSLLKQAGDVEENPGP (SEQ ID NO:3).

**[0138]** In an example of the recombinant polypeptides, the third amino acid linker is self-cleaving. In an example of the recombinant polypeptides, the second amino acid linker is self-cleaving. In an example of the recombinant polypeptides, the self-cleaving peptide is a viral 2A peptide or has the sequence thereof. In an embodiment, the viral 2A peptide is a porcine teschovirus-1 (P2A), foot- and-mouth disease virus (F2A), *Thosaea asigna* virus (T2A), equine rhinitis A virus (E2A) or viral porcine teschovirus-1 (P2A) peptide, or has the sequence of one thereof. Alternatively, this can also be delivered as two separate plasmids (or viruses) removing the 2A sequence entirely.

**[0139]** The proteolytically cleavable linker can include a protease recognition sequence recognized by a protease selected from the group consisting of alanine carboxypeptidase, *Armillaria mellea* astacin, bacterial leucyl aminopeptidase, cancer procoagulant, cathepsin B, clostripain, cytosol alanyl aminopeptidase, elastase, endoproteinase Arg-C, enterokinase, gastricsin, gelatinase, Gly-X carboxypeptidase, glycyl endopeptidase, human rhinovirus 3C protease, hypodermin C, IgA-specific serine endopeptidase, leucyl aminopeptidase, leucyl

endopeptidase, lysC, lysosomal pro-X carboxypeptidase, lysyl aminopeptidase, methionyl aminopeptidase, myxobacter, nardilysin, pancreatic endopeptidase E, picornain 2A, picornain 3C, proendopeptidase, prolyl aminopeptidase, proprotein convertase I, proprotein convertase II, russellysin, saccharopepsin, semenogelase, T-plasminogen activator, thrombin, tissue kallikrein, tobacco etch virus (TEV), togavirin, tryptophanyl aminopeptidase, U-plasminogen activator, V8, venombin A, venombin AB, and Xaa-pro aminopeptidase. In some cases, the proteolytically cleavable linker can include a protease recognition sequence recognized by a host enzyme, e.g., an enzyme naturally produced by the host cell.

**[0140]** For example, the proteolytically cleavable linker can comprise a matrix metalloproteinase cleavage site, e.g., a cleavage site for a MMP selected from collagenase-1, -2, and -3 (MMP-1, -8, and -13), gelatinase A and B (MMP-2 and -9), stromelysin 1, 2, and 3 (MMP-3, -10, and -11), matrilysin (MMP-7), and membrane metalloproteinases (MT1-MMP and MT2-MMP). For example, the cleavage sequence of MMP-9 is Pro-X-X-Hy (wherein, X represents an arbitrary residue; Hy, a hydrophobic residue), e.g., Pro-X-X-Hy-(Ser/Thr), e.g., Pro-Leu/Gln-Gly-Met-Thr-Ser (SEQ ID NO:33) or Pro-Leu/Gln-Gly-Met-Thr (SEQ ID NO:21). Another example of a protease cleavage site is a plasminogen activator cleavage site, e.g., a uPA or a tissue plasminogen activator (tPA) cleavage site. Specific examples of cleavage sequences of uPA and tPA include sequences comprising Val-Gly-Arg. Another example of a protease cleavage site that can be included in a proteolytically cleavable linker is a tobacco etch virus (TEV) protease cleavage site, e.g., ENLYTQS (SEQ ID NO:34), where the protease cleaves between the glutamine and the serine. Another example of a protease cleavage site that can be included in a proteolytically cleavable linker is an enterokinase cleavage site, e.g., DDDDK (SEQ ID NO:35), where cleavage occurs after the lysine residue. Another example of a protease cleavage site that can be included in a proteolytically cleavable linker is a thrombin cleavage site, e.g., LVPR (SEQ ID NO:36). Another example of a protease cleavage site that can be included in a proteolytically cleavable linker is a furin cleavage site, e.g., Arg-X-(Arg/Lys)-Arg, where X is any amino acid. Additional suitable linkers comprising protease cleavage sites include linkers comprising one or more of the following amino acid sequences: LEVLFQGP (SEQ ID NO:37), cleaved by PreScission protease (a fusion protein comprising human rhinovirus 3C protease and glutathione-S-transferase; Walker et al. (1994) *Biotechnol.* 12:601); a thrombin cleavage site, e.g., CGLVPAGSGP (SEQ ID NO:38); SLLKSRMVPNFN (SEQ ID NO:39) or SLLIARRMPNFN (SEQ ID NO:40), cleaved by cathepsin B; SKLVQASASGVN (SEQ ID NO:41) or SSYLKASDAPDN (SEQ ID NO:42), cleaved by an Epstein-Barr virus protease; RPKPQQFFGLMN (SEQ ID NO:43) cleaved by MMP-3 (stromelysin); SLRPLALWRSFN (SEQ ID NO:44) cleaved by MMP-7 (matrilysin); SPQGIAGQRNFN (SEQ ID NO:45) cleaved by MMP-9; DVDERDVRGFASFL (SEQ ID NO:46) cleaved by a thermolysin-like MMP; SLPLGLWAPNFN (SEQ ID NO:47) cleaved by matrix metalloproteinase 2(MMP-2); SLLIFRSWANFN (SEQ ID NO:48) cleaved by cathepsin L; SGVVIATVIVIT (SEQ ID NO:49) cleaved by cathepsin D; SLGPQGIWGQFN (SEQ ID NO:50) cleaved by matrix metalloproteinase 1(MMP-1); KKSPGRVVGGSV (SEQ ID NO:51) cleaved by urokinase-type plasminogen activator; PQGLLGAPGILG (SEQ ID NO:52) cleaved by membrane type 1 matrixmetalloproteinase (MT-MMP); HGPEGLRVGFYESDVMGRGHARLVHVEEPHT (SEQ ID NO:53) cleaved by stromelysin 3 (or

MMP-11), thermolysin, fibroblast collagenase and stromelysin-1; GPQGLAGQRGIV (SEQ ID NO:54) cleaved by matrix metalloproteinase 13 (collagenase-3); GGSGQRGRKALE (SEQ ID NO:55) cleaved by tissue-type plasminogen activator(tPA); SLSALLSSDIFN (SEQ ID NO:56) cleaved by human prostate-specific antigen; SLPRFKIIGGFN (SEQ ID NO:57) cleaved by kallikrein (hK3); SLLGIAVPGNFN (SEQ ID NO:58) cleaved by neutrophil elastase; and FFKNIVTPRTPP (SEQ ID NO:59) cleaved by calpain (calcium activated neutral protease). Additional examples suitable proteolytically cleavable linkers include: 1) ATNFSLKQAGDVEENPGP (SEQ ID NO:60); 2) EGRGSLLTCGDVEENPGP (SEQ ID NO:61); 3) QCTNYALLKLAGDVESNPGP (SEQ ID NO:62); and 4) VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:63). Additional examples suitable proteolytically cleavable linkers include: 1) GSGATNFSLKQAGDVEENPGP (SEQ ID NO:64); 2) GSGEGRGSLLTCGDVEENPGP (SEQ ID NO:65); 3) GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO:66); and 4) GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:67).

**[0141]** Examples of suitable linkers include 2A linkers (for example T2A), 2A-like linkers or functional equivalents thereof and combinations thereof. In some embodiments, the linkers include the picornaviral 2A-like linker, CHYSEL sequences of porcine teschovirus (P2A), Thosea asigna virus (T2A), and combinations, variants, and functional equivalents thereof. In other embodiments, the linker sequences may comprise Asp-Val/Ile-Glu-X-Asn-Pro-Gly<sup>2A</sup>-Pro<sup>2B</sup> motif, which results in cleavage between the 2A glycine and the 2B proline. For the purposes of the present disclosure, P2A (GSGATNFSLKQAGDVEENPGP (SEQ ID NO:64)), T2A (GSGEGRGSLLTCGDVEENPGP (SEQ ID NO:65)), E2A (GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO:66)), and F2A (GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:67)) can be considered as either "proteolytic cleavage sites" or "ribosome skipping signals" (CHYSEL). See, e.g., Kim et al. (2011) PLoS ONE 6:e18556. The mechanism by which the encoded polypeptides are generated as two polypeptide chains may be by self cleaving of the linker, by ribosome skipping, or translational shunting. Regardless of the mechanism, the at least two polypeptide chains of a multimeric polypeptide of the present disclosure can be produced using a P2A, T2A, E2A, or F2A sequence. Suitable linkers include polypeptides comprising an amino acid sequence such as GSGATNFSLKQAGDVEENPGP (SEQ ID NO:64), GSGEGRGSLLTCGDVEENPGP (SEQ ID NO:65), GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO:66), GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:67), or an amino acid sequence having from 1 to 5 amino acid substitutions relative to an amino acid sequence set forth in SEQ ID NOs:64-67 (e.g., an amino acid sequence having from 1 to 5 conservative amino acid substitutions relative to an amino acid sequence set forth in SEQ ID NOs:64-67). Suitable linkers include polypeptides comprising an amino acid sequence such as GSGATNFSLKQAGDVEENPGP (SEQ ID NO:64), GSGEGRGSLLTCGDVEENPGP (SEQ ID NO:65), GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO:66), GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:67), or an amino acid sequence having from 1 to 10 amino acid substitutions relative to an amino acid sequence set forth in SEQ ID NOs:64-67 (e.g., an amino acid sequence having from 1 to 10 conservative amino acid substitutions relative to an amino acid sequence set forth in SEQ ID NOs:64-67).

**Epitopes**

**[0142]** In an embodiment of the recombinant polypeptides, the candidate epitope comprises 7-20 amino acids. In an embodiment of the recombinant polypeptides, the epitope peptide is 5-20 amino acids for MHC class I. In an embodiment, the epitope peptide is 8-11 amino acids for MHC class I. In an embodiment, the epitope peptide is 5-40 amino acids for MHC class II. In an embodiment, the epitope peptide is 13-17 amino acids for MHC class II. In an embodiment, the epitope peptide is any naturally occurring or mutant human sequence, or any pathogen-derived sequence.

**MHC polypeptides**

**[0143]** In an embodiment of the recombinant polypeptides, the first and/or second B2M leader sequence has the sequence of human B2M leader sequence.

**[0144]** In some cases, a leader peptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following human B2M leader sequence: MSRSVALAVLALLSLSGLEA (SEQ ID NO:68).

**[0145]** In some instances, a B2M leader sequence as described herein may be a mammalian B2M leader sequence including but not limited to, e.g., a human B2M leader sequence, a primate B2M leader sequence, a rodent B2M leader sequence, and the like. In some instances, a B2M leader as described herein comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity with one of the B2M leader sequences depicted in FIG. 20.

**[0146]** In an embodiment, the B2M comprises the sequence:  
IQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSF  
SKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO:4).

**[0147]** In some instances, a B2M polypeptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a B2M amino acid sequence depicted in FIG. 20.

**[0148]** In an embodiment of the recombinant polypeptides, the MHC heavy chain is a human MHC heavy chain. In an embodiment of the recombinant polypeptides, the MHC heavy chain is an MHC I molecule. Exemplary MHC I heavy chains include the alpha chain of HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-K and HLA-L. In an embodiment of the recombinant

polypeptides, the MHC heavy chain is an HLA-A02:01. In an embodiment, the HLA is HLA-A02.

In an embodiment, the HLA-A02 comprises the sequence:

GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSAASQRMEPRAPWIEQE

GPEYWDGETRKVKAHSQTHRVDLGLRGYYNQSEAGSHTVQRMYGCDVGSDWRFLR

GYHQQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEW

LRRYLENGKETLQRTDAPKTHMTHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQT

QDTELVETRPAGDGTQKWAAVVPSGQEQRYTCHVQHEGLPKPLTRWEP (SEQ ID

NO:5).

**[0149]** In an embodiment of the recombinant polypeptides, the MHC heavy chain is an MHC II molecule. Exemplary MHC II heavy chains include those of HLA-D.

**[0150]** In an embodiment of the recombinant polypeptides, the recombinant polypeptide further comprises a mutation in a human native B2M peptide sequence thereof and in the Heavy Chain sequence thereof so as to effect a disulfide bond between the B2M peptide sequence and Heavy Chain sequence.

**[0151]** In an embodiment of the recombinant polypeptides, the recombinant polypeptide the Heavy Chain sequence is an HLA and the disulfide bond links one of the following pairs of residues:

B2M residue 12, HLA residue 236;

B2M residue 12, HLA residue 237;

B2M residue 8, HLA residue 234;

B2M residue 10, HLA residue 235;

B2M residue 24, HLA residue 236;

B2M residue 28, HLA residue 232;

B2M residue 98, HLA residue 192;

B2M residue 99, HLA residue 234;

B2M residue 3, HLA residue 120;

B2M residue 31, HLA residue 96;

B2M residue 53, HLA residue 35;

B2M residue 60, HLA residue 96;

B2M residue 60, HLA residue 122;

B2M residue 63, HLA residue 27;  
B2M residue Arg3, HLA residue Gly120;  
B2M residue His31, HLA residue Gln96;  
B2M residue Asp53, HLA residue Arg35;  
B2M residue Trp60, HLA residue Gln96;  
B2M residue Trp60, HLA residue Asp122;  
B2M residue Tyr63, HLA residue Tyr27;  
B2M residue Lys6, HLA residue Glu232;  
B2M residue Gln8, HLA residue Arg234;  
B2M residue Tyr10, HLA residue Pro235;  
B2M residue Ser11, HLA residue Gln242;  
B2M residue Asn24, HLA residue Ala236;  
B2M residue Ser28, HLA residue Glu232;  
B2M residue Asp98, HLA residue His192; and  
B2M residue Met99, HLA residue Arg234

(See SEQ ID NO:s 4 and 5 for B2M and HLA sequences).

**[0152]** In an embodiment of the recombinant polypeptides, the Heavy Chain sequence is an HLA and wherein the disulfide bond links one of the following pairs of residues:

first linker position Gly 2, Heavy Chain (HLA) position Tyr 84;  
Light Chain (B2M) position Arg 12, HLA Ala236; and/or  
B2M residue Arg12, HLA residue Gly237.

#### *Fc polypeptides*

**[0153]** In an embodiment of the recombinant polypeptides, the immunoglobulin Fc domain is an IgG Fc domain. In an embodiment of the recombinant polypeptides, the immunoglobulin Fc

domain is an IgA Fc domain. In an embodiment of the recombinant polypeptides, the immunoglobulin Fc domain is an IgM Fc domain. In an embodiment of the recombinant polypeptides, the immunoglobulin Fc domain is a human immunoglobulin Fc domain. In an embodiment of the recombinant polypeptides, the immunoglobulin Fc domain is an IgG1 Fc domain.

### **Immunomodulatory polypeptides**

**[0154]** In an embodiment of the recombinant polypeptides, the T cell modulatory domain is an inhibitory domain.

**[0155]** In an embodiment of the recombinant polypeptides, the T cell modulatory domain is a stimulating domain.

**[0156]** In an embodiment of the recombinant polypeptides, the T cell modulatory domain is an antibody, and antibody fragment, a peptide ligand, a T cell costimulatory peptide, a cytokine or a toxin.

**[0157]** In an embodiment of the recombinant polypeptides, the T cell modulatory domain comprises a PD-L1 peptide, the Ig variable domain of a PD-L1 peptide, the T cell modulatory domain comprises 4-1BBL, the T cell modulatory domain comprises B7-1W88A, or the T cell modulatory domain comprises anti-CD28 single chain Fv.

**[0158]** Further T cell modulatory domains (MODs) that can be employed in the invention include naturally occurring or synthetic human gene products (protein), affinity reagents (e.g., an antibody, antibody fragment, single chain Fvs, aptamers, nanobody) targeting a human gene product, including, but not limited to all secreted proteins arising from classical and non-classical (e.g., FGF2, IL1, S100A4) secretion mechanisms, and ecto-domains of all cell surface proteins anchored by naturally occurring genetically encoded protein segments (single or multiple membrane spans) or post-translational modifications such as GPI linkages). Any naturally occurring or synthetic affinity reagent (e.g., antibody, antibody fragment, single chain Fvs, aptamer, nanobody, lectin, etc) targeting a cell surface glycan or other post-translational modification (e.g., sulfation). Examples include, but are not limited to, members of the TNF/TNFR family (OX40L, ICOSL, FASL, LTA, LTB TRAIL, CD153, TNFSF9, RANKL, TWEAK, TNFSF13, TNFSF13b, TNFSF14, TNFSF15, TNFSF18, CD40LG, CD70) or affinity reagents directed at the TNF/TNFR family members; members of the Immunoglobulin superfamily (VISTA, PD1, PD-L1, PD-L2, B71, B72, CTLA4, CD28, TIM3, CD4, CD8, CD19, T cell receptor chains, ICOS, ICOS ligand, HHLA2, butyrophilins, BTLA, B7-H3, B7-H4, CD3, CD79a, CD79b, IgSF CAMS (including CD2, CD58, CD48, CD150, CD229, CD244, ICAM-1), Leukocyte immunoglobulin like receptors (LILR), killer cell immunoglobulin like receptors (KIR)), lectin superfamily members, selectins, cytokines/chemokine and cytokine/chemokine receptors, growth factors and growth factor receptors), adhesion molecules (integrins, fibronectins, cadherins), or ecto-domains of multi-span intergral membrane protein, or affinity reagents

directed at the Immunoglobulin superfamily and listed gene products. In addition, active homologs/orthologs of these gene products, including but not limited to, viral sequences (e.g., CMV, EBV), bacterial sequences, fungal sequences, eukaryotic pathogens (e.g., *Schistosoma*, *Plasmodium*, *Babesia*, *Eimeria*, *Theileria*, *Toxoplasma*, *Entamoeba*, *Leishmania*, and *Trypanosoma*), and mammalian -derived coding regions. In addition, a MOD may comprise a small molecules drug targeting a human gene product.

**Additional polypeptides**

**[0159]** In an embodiment of the recombinant polypeptides, they further comprise a His-8 tag contiguous with the C-terminal thereof.

**NUCLEIC ACIDS**

**[0160]** A nucleic acid is provided encoding any of the recombinant polypeptides described herein. In an embodiment, the nucleic acid is a DNA. In an embodiment, the nucleic acid is a cDNA. In an embodiment, the nucleic acid is an RNA. In an embodiment, the nucleic acid is an mRNA.

**[0161]** In an embodiment, the recombinant nucleic acid is a vector. In an embodiment, the vector is a viral vector. In an embodiment, the viral vector is a lentiviral vector.

**[0162]** The present disclosure provides nucleic acids comprising nucleotide sequences encoding a multimeric polypeptide of the present disclosure. In some cases, the individual polypeptide chains of a multimeric polypeptide of the present disclosure are encoded in separate nucleic acids. In some cases, all polypeptide chains of a multimeric polypeptide of the present disclosure are encoded in a single nucleic acid. In some cases, a first nucleic acid comprises a nucleotide sequence encoding a first polypeptide of a multimeric polypeptide of the present disclosure; and a second nucleic acid comprises a nucleotide sequence encoding a second polypeptide of a multimeric polypeptide of the present disclosure. In some cases, single nucleic acid comprises a nucleotide sequence encoding a first polypeptide of a multimeric polypeptide of the present disclosure and a second polypeptide of a multimeric polypeptide of the present disclosure. In some cases, a nucleic acid comprises a nucleotide sequence encoding a polyprotein precursor, as described above.

**Separate nucleic acids encoding individual polypeptide chains of a multimeric polypeptide**

**[0163]** The present disclosure provides nucleic acids comprising nucleotide sequences encoding a multimeric polypeptide of the present disclosure. As noted above, in some cases,

the individual polypeptide chains of a multimeric polypeptide of the present disclosure are encoded in separate nucleic acids. In some cases, nucleotide sequences encoding the separate polypeptide chains of a multimeric polypeptide of the present disclosure are operably linked to transcriptional control elements, e.g., promoters, such as promoters that are functional in a eukaryotic cell, where the promoter can be a constitutive promoter or an inducible promoter.

**[0164]** The present disclosure provides a first nucleic acid and a second nucleic acid, where the first nucleic acid comprises a nucleotide sequence encoding a first polypeptide of a multimeric polypeptide of the present disclosure, where the first polypeptide comprises, in order from N-terminus to C-terminus: a) an epitope (e.g., a T-cell epitope); b) a first MHC polypeptide; and c) an immunomodulatory polypeptide; and where the second nucleic acid comprises a nucleotide sequence encoding a second polypeptide of a multimeric polypeptide of the present disclosure, where the second polypeptide comprises, in order from N-terminus to C-terminus: a) a second MHC polypeptide; and b) an Ig Fc polypeptide. Suitable T-cell epitopes, MHC polypeptides, immunomodulatory polypeptides, and Ig Fc polypeptides, are described above. In some cases, the nucleotide sequences encoding the first and the second polypeptides are operably linked to transcriptional control elements. In some cases, the transcriptional control element is a promoter that is functional in a eukaryotic cell. In some cases, the nucleic acids are present in separate expression vectors.

**[0165]** The present disclosure provides a first nucleic acid and a second nucleic acid, where the first nucleic acid comprises a nucleotide sequence encoding a first polypeptide of a multimeric polypeptide of the present disclosure, where the first polypeptide comprises, in order from N-terminus to C-terminus: a) an epitope (e.g., a T-cell epitope); and b) a first MHC polypeptide; and where the second nucleic acid comprises a nucleotide sequence encoding a second polypeptide of a multimeric polypeptide of the present disclosure, where the second polypeptide comprises, in order from N-terminus to C-terminus: a) an immunomodulatory polypeptide; b) a second MHC polypeptide; and c) an Ig Fc polypeptide. Suitable T-cell epitopes, MHC polypeptides, immunomodulatory polypeptides, and Ig Fc polypeptides, are described above. In some cases, the nucleotide sequences encoding the first and the second polypeptides are operably linked to transcriptional control elements. In some cases, the transcriptional control element is a promoter that is functional in a eukaryotic cell. In some cases, the nucleic acids are present in separate expression vectors.

**Nucleic acid encoding two or more polypeptides present in a multimeric polypeptide**

**[0166]** The present disclosure provides a nucleic acid comprising nucleotide sequences encoding at least the first polypeptide and the second polypeptide of a multimeric polypeptide of the present disclosure. In some cases, where a multimeric polypeptide of the present disclosure includes a first, second, and third polypeptide, the nucleic acid includes a nucleotide sequence encoding the first, second, and third polypeptides. In some cases, the nucleotide sequences encoding the first polypeptide and the second polypeptide of a multimeric

polypeptide of the present disclosure includes a proteolytically cleavable linker interposed between the nucleotide sequence encoding the first polypeptide and the nucleotide sequence encoding the second polypeptide. In some cases, the nucleotide sequences encoding the first polypeptide and the second polypeptide of a multimeric polypeptide of the present disclosure includes an internal ribosome entry site (IRES) interposed between the nucleotide sequence encoding the first polypeptide and the nucleotide sequence encoding the second polypeptide. In some cases, the nucleotide sequences encoding the first polypeptide and the second polypeptide of a multimeric polypeptide of the present disclosure includes a ribosome skipping signal (or cis-acting hydrolase element, CHYSEL) interposed between the nucleotide sequence encoding the first polypeptide and the nucleotide sequence encoding the second polypeptide. Examples of nucleic acids are described below, where a proteolytically cleavable linker is provided between nucleotide sequences encoding the first polypeptide and the second polypeptide of a multimeric polypeptide of the present disclosure; in any of these embodiments, an IRES or a ribosome skipping signal can be used in place of the nucleotide sequence encoding the proteolytically cleavable linker.

**[0167]** In some cases, a first nucleic acid (e.g., a recombinant expression vector, an mRNA, a viral RNA, etc.) comprises a nucleotide sequence encoding a first polypeptide chain of a multimeric polypeptide of the present disclosure; and a second nucleic acid (e.g., a recombinant expression vector, an mRNA, a viral RNA, etc.) comprises a nucleotide sequence encoding a second polypeptide chain of a multimeric polypeptide of the present disclosure. In some cases, the nucleotide sequence encoding the first polypeptide, and the second nucleotide sequence encoding the second polypeptide, are each operably linked to transcriptional control elements, e.g., promoters, such as promoters that are functional in a eukaryotic cell, where the promoter can be a constitutive promoter or an inducible promoter.

**[0168]** The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a recombinant polypeptide, where the recombinant polypeptide comprises, in order from N-terminus to C-terminus: a) an epitope (e.g., a T-cell epitope); b) a first MHC polypeptide; c) an immunomodulatory polypeptide; d) a proteolytically cleavable linker; e) a second MHC polypeptide; and f) an immunoglobulin (Ig) Fc polypeptide. The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a recombinant polypeptide, where the recombinant polypeptide comprises, in order from N-terminus to C-terminus: a) a first leader peptide; b) the epitope; c) the first MHC polypeptide; d) the immunomodulatory polypeptide; e) the proteolytically cleavable linker; f) a second leader peptide; g) the second MHC polypeptide; and h) the Ig Fc polypeptide. The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a recombinant polypeptide, where the recombinant polypeptide comprises, in order from N-terminus to C-terminus: a) an epitope; b) a first MHC polypeptide; c) a proteolytically cleavable linker; d) an immunomodulatory polypeptide; e) a second MHC polypeptide; and f) an Ig Fc polypeptide. In some cases, the first leader peptide and the second leader peptide is a  $\beta$ 2-M leader peptide. In some cases, the nucleotide sequence is operably linked to a transcriptional control element. In some cases, the transcriptional control element is a promoter that is functional in a eukaryotic cell.

**[0169]** Suitable MHC polypeptides are described above. In some cases, the first MHC polypeptide is a  $\beta$ 2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide. In some cases, the  $\beta$ 2-microglobulin polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4. In some cases, the MHC class I heavy chain polypeptide is an HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-K, or HLA-L heavy chain. In some cases, the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:5. In some cases, the first MHC polypeptide is an MHC Class II alpha chain polypeptide; and wherein the second MHC polypeptide is an MHC class II beta chain polypeptide.

**[0170]** Suitable Fc polypeptides are described above. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide, an IgG2 Fc polypeptide, an IgG3 Fc polypeptide, an IgG4 Fc polypeptide, an IgA Fc polypeptide, or an IgM Fc polypeptide. In some cases, the Ig Fc polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to an amino acid sequence depicted in Figures 24A-24C.

**[0171]** Suitable immunomodulatory polypeptides are described above. In some cases, the immunomodulatory polypeptide is selected from a 4-1BBL polypeptide, a B7-1 polypeptide; a B7-2 polypeptide, an ICOS-L polypeptide, an OX-40L polypeptide, a CD80 polypeptide, a CD86 polypeptide, a PD-L1 polypeptide, a FasL polypeptide, and a PD-L2 polypeptide. In some cases, the immunomodulatory polypeptide is selected from a CD7, CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, and HVEM.

**[0172]** Suitable proteolytically cleavable linkers are described above. In some cases, the proteolytically cleavable linker comprises an amino acid sequence selected from: a) LEVLFQGP (SEQ ID NO:37); b) ENLYTQS (SEQ ID NO:34); c) DDDDK (SEQ ID NO:35); d) LVPR (SEQ ID NO:36); and e) GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:64).

**[0173]** In some cases, a linker between the epitope and the first MHC polypeptide comprises a first Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, such that the first and the second Cys residues provide for a disulfide linkage between the linker and the second MHC polypeptide. In some cases, first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, such that the first Cys residue and the second Cys residue provide for a disulfide linkage between the first MHC polypeptide and the second MHC polypeptide.

#### Recombinant expression vectors

**[0174]** The present disclosure provides recombinant expression vectors comprising nucleic acids of the present disclosure. In some cases, the recombinant expression vector is a non-

viral vector. In some embodiments, the recombinant expression vector is a viral construct, e.g., a recombinant adeno-associated virus construct (see, e.g., U.S. Patent No. 7,078,387), a recombinant adenoviral construct, a recombinant lentiviral construct, a recombinant retroviral construct, a non-integrating viral vector, etc.

**[0175]** Suitable expression vectors include, but are not limited to, viral vectors (e.g. viral vectors based on vaccinia virus; poliovirus; adenovirus (see, e.g., Li et al., Invest Ophthalmol Vis Sci 35:2543 2549, 1994; Borras et al., Gene Ther 6:515 524, 1999; Li and Davidson, PNAS 92:7700 7704, 1995; Sakamoto et al., H Gene Ther 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (see, e.g., Ali et al., Hum Gene Ther 9:81 86, 1998, Flannery et al., PNAS 94:6916 6921, 1997; Bennett et al., Invest Ophthalmol Vis Sci 38:2857 2863, 1997; Jomary et al., Gene Ther 4:683 690, 1997, Rolling et al., Hum Gene Ther 10:641 648, 1999; Ali et al., Hum Mol Genet 5:591 594, 1996; Srivastava in WO 93/09239, Samulski et al., J. Vir. (1989) 63:3822-3828; Mendelson et al., Virol. (1988) 166:154-165; and Flotte et al., PNAS (1993) 90:10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, e.g., Miyoshi et al., PNAS 94:10319 23, 1997; Takahashi et al., J Virol 73:7812 7816, 1999); a retroviral vector (e.g., Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, a lentivirus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like.

**[0176]** Numerous suitable expression vectors are known to those of skill in the art, and many are commercially available. The following vectors are provided by way of example; for eukaryotic host cells: pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, and pSVLSV40 (Pharmacia). However, any other vector may be used so long as it is compatible with the host cell.

**[0177]** Depending on the host/vector system utilized, any of a number of suitable transcription and translation control elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector (see e.g., Bitter et al. (1987) Methods in Enzymology, 153:516-544).

**[0178]** In some embodiments, a nucleotide sequence encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide is operably linked to a control element, e.g., a transcriptional control element, such as a promoter. The transcriptional control element may be functional in either a eukaryotic cell, e.g., a mammalian cell; or a prokaryotic cell (e.g., bacterial or archaeal cell). In some embodiments, a nucleotide sequence encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide is operably linked to multiple control elements that allow expression of the nucleotide sequence encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide in both prokaryotic and eukaryotic cells.

**[0179]** Non-limiting examples of suitable eukaryotic promoters (promoters functional in a eukaryotic cell) include those from cytomegalovirus (CMV) immediate early, herpes simplex

virus (HSV) thymidine kinase, early and late SV40, long terminal repeats (LTRs) from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. The expression vector may also contain a ribosome binding site for translation initiation and a transcription terminator. The expression vector may also include appropriate sequences for amplifying expression.

#### GENETICALLY MODIFIED HOST CELLS

**[0180]** A cell is provided transformed with a nucleic acid encoding any of the recombinant polypeptides described herein. Examples of cells that can be transformed with a nucleic acid encoding any of the recombinant polypeptides include isolated mammalian cells, including but not limited to Human Embryonic Kidney (HEK), Chinese Hamster Ovary (CHO), NS0 (murine myeloma) cells, human amniocytic cells (CAP, CAP-T), yeast cells (including, but not limited to, *S. cerevisiae*, *Pichia pastoris*), plant cells (including, but not limited to, Tobacco NT1, BY-2), insect cells (including but not limited to SF9, S2, SF21, Tni (e.g. High 5)) or bacterial cells (including, but not limited to, *E. coli*).

**[0181]** The present disclosure provides a genetically modified host cell, where the host cell is genetically modified with a nucleic acid of the present disclosure.

**[0182]** Suitable host cells include eukaryotic cells, such as yeast cells, insect cells, and mammalian cells. In some cases, the host cell is a cell of a mammalian cell line. Suitable mammalian cell lines include human cell lines, non-human primate cell lines, rodent (e.g., mouse, rat) cell lines, and the like. Suitable mammalian cell lines include, but are not limited to, HeLa cells (e.g., American Type Culture Collection (ATCC) No. CCL-2), CHO cells (e.g., ATCC Nos. CRL9618, CCL61, CRL9096), 293 cells (e.g., ATCC No. CRL-1573), Vero cells, NIH 3T3 cells (e.g., ATCC No. CRL-1658), Huh-7 cells, BHK cells (e.g., ATCC No. CCL10), PC12 cells (ATCC No. CRL1721), COS cells, COS-7 cells (ATCC No. CRL1651), RAT1 cells, mouse L cells (ATCC No. CCL1.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, and the like.

**[0183]** In some cases, the host cell is a mammalian cell that has been genetically modified such that it does not synthesize endogenous MHC  $\beta$ 2-M.

#### METHODS OF PRODUCING A MULTIMERIC POLYPEPTIDE

**[0184]** The present disclosure provides methods of producing a multimeric polypeptide of the present disclosure. The methods generally involve culturing, in a culture medium, a host cell that is genetically modified with a recombinant expression vector comprising a nucleotide sequence encoding the multimeric polypeptide; and isolating the multimeric polypeptide from the genetically modified host cell and/or the culture medium. A host cell that is genetically

modified with a recombinant expression vector comprising a nucleotide sequence encoding the multimeric polypeptide is also referred to as an "expression host." As noted above, in some cases, the individual polypeptide chains of a multimeric polypeptide of the present disclosure are encoded in separate recombinant expression vectors. In some cases, all polypeptide chains of a multimeric polypeptide of the present disclosure are encoded in a single recombinant expression vector.

**[0185]** Isolation of the multimeric polypeptide from the expression host cell (e.g., from a lysate of the expression host cell) and/or the culture medium in which the host cell is cultured, can be carried out using standard methods of protein purification.

**[0186]** For example, a lysate may be prepared of the expression host and the lysate purified using high performance liquid chromatography (HPLC), exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. Alternatively, where the multimeric polypeptide is secreted from the expression host cell into the culture medium, the multimeric polypeptide can be purified from the culture medium using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. In some cases, the compositions which are used will comprise at least 80% by weight of the desired product, at least about 85% by weight, at least about 95% by weight, or at least about 99.5% by weight, in relation to contaminants related to the method of preparation of the product and its purification. The percentages can be based upon total protein.

**[0187]** In some cases, e.g., where the multimeric polypeptide comprises an affinity tag, the multimeric polypeptide can be purified using an immobilized binding partner of the affinity tag.

## COMPOSITIONS

**[0188]** The present disclosure provides compositions, including pharmaceutical compositions, comprising a multimeric polypeptide of the present disclosure. The present disclosure provides compositions, including pharmaceutical compositions, comprising a nucleic acid or a recombinant expression vector of the present disclosure.

### Compositions comprising a multimeric polypeptide

**[0189]** A composition of the present disclosure can comprise, in addition to a multimeric polypeptide of the present disclosure, one or more of: a salt, e.g., NaCl, MgCl, KCl, MgSO<sub>4</sub>, etc.; a buffering agent, e.g., a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), etc.; a solubilizing agent; a detergent, e.g., a non-ionic detergent such as Tween-20, etc.; a protease inhibitor; glycerol;

and the like.

**[0190]** The composition may comprise a pharmaceutically acceptable excipient, a variety of which are known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, "Remington: The Science and Practice of Pharmacy", 19th Ed. (1995), or latest edition, Mack Publishing Co; A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy", 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H.C. Ansel et al., eds 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A.H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

**[0191]** A pharmaceutical composition can comprise a multimeric polypeptide of the present disclosure, and a pharmaceutically acceptable excipient. In some cases, a subject pharmaceutical composition will be suitable for administration to a subject, e.g., will be sterile. For example, in some embodiments, a subject pharmaceutical composition will be suitable for administration to a human subject, e.g., where the composition is sterile and is free of detectable pyrogens and/or other toxins.

**[0192]** The protein compositions may comprise other components, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium, carbonate, and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, hydrochloride, sulfate salts, solvates (e.g., mixed ionic salts, water, organics), hydrates (e.g., water), and the like.

**[0193]** For example, compositions may include aqueous solution, powder form, granules, tablets, pills, suppositories, capsules, suspensions, sprays, and the like. The composition may be formulated according to the various routes of administration described below.

**[0194]** Where a multimeric polypeptide of the present disclosure is administered as an injectable (e.g. subcutaneously, intraperitoneally, and/or intravenous) directly into a tissue, a formulation can be provided as a ready-to-use dosage form, or as non-aqueous form (e.g. a reconstitutable storage-stable powder) or aqueous form, such as liquid composed of pharmaceutically acceptable carriers and excipients. The protein-containing formulations may also be provided so as to enhance serum half-life of the subject protein following administration. For example, the protein may be provided in a liposome formulation, prepared as a colloid, or other conventional techniques for extending serum half-life. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al. 1980 Ann. Rev. Biophys. Bioeng. 9:467, U.S. Pat. Nos. 4,235,871, 4,501,728 and 4,837,028. The preparations may also be provided in controlled release or slow-release forms.

**[0195]** Other examples of formulations suitable for parenteral administration include isotonic sterile injection solutions, anti-oxidants, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. For example, a subject pharmaceutical composition can be present in a container, e.g., a sterile container, such as a syringe. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets.

**[0196]** The concentration of a multimeric polypeptide of the present disclosure in a formulation can vary widely (e.g., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight) and will usually be selected primarily based on fluid volumes, viscosities, and patient-based factors in accordance with the particular mode of administration selected and the patient's needs.

**[0197]** The present disclosure provides a container comprising a composition of the present disclosure, e.g., a liquid composition. The container can be, e.g., a syringe, an ampoule, and the like. In some cases, the container is sterile. In some cases, both the container and the composition are sterile.

#### Compositions comprising a nucleic acid or a recombinant expression vector

**[0198]** The present disclosure provides compositions, e.g., pharmaceutical compositions, comprising a nucleic acid or a recombinant expression vector of the present disclosure. A wide variety of pharmaceutically acceptable excipients is known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy", 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H. C. Ansel et al., eds 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A. H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

**[0199]** A composition of the present disclosure can include: a) a subject nucleic acid or recombinant expression vector; and b) one or more of: a buffer, a surfactant, an antioxidant, a hydrophilic polymer, a dextrin, a chelating agent, a suspending agent, a solubilizer, a thickening agent, a stabilizer, a bacteriostatic agent, a wetting agent, and a preservative. Suitable buffers include, but are not limited to, (such as N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane (BIS-Tris), N-(2-hydroxyethyl)piperazine-N'-3-propanesulfonic acid (EPPS or HEPPS), glycylglycine, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 3-(N-morpholino)propane sulfonic acid (MOPS), piperazine-N,N'-bis(2-ethane-sulfonic acid) (PIPES), sodium

bicarbonate, 3-(N-tris(hydroxymethyl)-methyl-amino)-2-hydroxy-propanesulfonic acid) TAPSO, (N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), N-tris(hydroxymethyl)methylglycine (Tricine), tris(hydroxymethyl)-aminomethane (Tris), etc.). Suitable salts include, e.g., NaCl, MgCl<sub>2</sub>, KCl, MgSO<sub>4</sub>, etc.

**[0200]** A pharmaceutical formulation of the present disclosure can include a nucleic acid or recombinant expression vector of the present disclosure in an amount of from about 0.001% to about 90% (w/w). In the description of formulations, below, "subject nucleic acid or recombinant expression vector" will be understood to include a nucleic acid or recombinant expression vector of the present disclosure. For example, in some embodiments, a subject formulation comprises a nucleic acid or recombinant expression vector of the present disclosure.

**[0201]** A subject nucleic acid or recombinant expression vector can be admixed, encapsulated, conjugated or otherwise associated with other compounds or mixtures of compounds; such compounds can include, e.g., liposomes or receptor-targeted molecules. A subject nucleic acid or recombinant expression vector can be combined in a formulation with one or more components that assist in uptake, distribution and/or absorption.

**[0202]** A subject nucleic acid or recombinant expression vector composition can be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. A subject nucleic acid or recombinant expression vector composition can also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

**[0203]** A formulation comprising a subject nucleic acid or recombinant expression vector can be a liposomal formulation. As used herein, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes that can interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH sensitive or negatively charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes can be used to deliver a subject nucleic acid or recombinant expression vector.

**[0204]** Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. Liposomes and their uses are further described in U.S. Pat. No. 6,287,860.

**[0205]** The formulations and compositions of the present disclosure may also include surfactants. The use of surfactants in drug products, formulations and in emulsions is well known in the art. Surfactants and their uses are further described in U.S. Pat. No. 6,287,860.

**[0206]** In one embodiment, various penetration enhancers are included, to effect the efficient delivery of nucleic acids. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs. Penetration enhancers may be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants. Penetration enhancers and their uses are further described in U.S. Pat. No. 6,287,860.

**[0207]** Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets, or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Suitable oral formulations include those in which a subject antisense nucleic acid is administered in conjunction with one or more penetration enhancers surfactants and chelators. Suitable surfactants include, but are not limited to, fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Suitable bile acids/salts and fatty acids and their uses are further described in U.S. Pat. No. 6,287,860. Also suitable are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. An exemplary suitable combination is the sodium salt of lauric acid, capric acid, and UDCA. Further penetration enhancers include, but are not limited to, polyoxyethylene-9-lauryl ether, and polyoxyethylene-20-cetyl ether. Suitable penetration enhancers also include propylene glycol, dimethylsulfoxide, triethanolamine, N,N-dimethylacetamide, N,N-dimethylformamide, 2-pyrrolidone and derivatives thereof, tetrahydrofurfuryl alcohol, and AZONE™.

#### METHODS OF MODULATING T CELL ACTIVITY

**[0208]** Also provided is a method of inhibiting a T cell clone which recognizes an epitope peptide comprising contacting a T cell of the clone with a recombinant peptide as described herein, wherein the recombinant peptide comprises the epitope peptide and comprises a T cell modulatory domain which is an inhibitory domain, in an amount effective to inhibit a T cell clone.

**[0209]** Also provided is a method of stimulating a T cell clone which recognizes an epitope peptide comprising contacting a T cell of the clone with a recombinant peptide as described herein, wherein the recombinant peptide comprises the epitope peptide and comprises a T cell modulatory domain which is an stimulatory domain, in an amount effective to stimulate a T cell clone.

**[0210]** The present disclosure provides a method of selectively modulating the activity of an

epitope-specific T cell, the method comprising contacting the T cell with a multimeric polypeptide of the present disclosure, where contacting the T cell with a multimeric polypeptide of the present disclosure selectively modulates the activity of the epitope-specific T cell. In some cases, the contacting occurs *in vitro*. In some cases, the contacting occurs *in vivo*. In some cases, the contacting occurs *ex vivo*.

**[0211]** In some cases, e.g., where the target T cell is a CD8<sup>+</sup> T cell, the multimeric polypeptide comprises Class I MHC polypeptides (e.g.,  $\beta$ 2-microglobulin and Class I MHC heavy chain). In some cases, e.g., where the target T cell is a CD4<sup>+</sup> T cell, the multimeric polypeptide comprises Class II MHC polypeptides (e.g., Class II MHC  $\alpha$  chain; Class II MHC  $\beta$  chain).

**[0212]** Where a multimeric polypeptide of the present disclosure includes an immunomodulatory polypeptide that is an activating polypeptide, contacting the T cell with the multimeric polypeptide activates the epitope-specific T cell. In some instances, the epitope-specific T cell is a T cell that is specific for an epitope present on a cancer cell, and contacting the epitope-specific T cell with the multimeric polypeptide increases cytotoxic activity of the T cell toward the cancer cell. In some instances, the epitope-specific T cell is a T cell that is specific for an epitope present on a cancer cell, and contacting the epitope-specific T cell with the multimeric polypeptide increases the number of the epitope-specific T cells.

**[0213]** In some instances, the epitope-specific T cell is a T cell that is specific for an epitope present on a virus-infected cell, and contacting the epitope-specific T cell with the multimeric polypeptide increases cytotoxic activity of the T cell toward the virus-infected cell. In some instances, the epitope-specific T cell is a T cell that is specific for an epitope present on a virus-infected cell, and contacting the epitope-specific T cell with the multimeric polypeptide increases the number of the epitope-specific T cells.

**[0214]** Where a multimeric polypeptide of the present disclosure includes an immunomodulatory polypeptide that is an inhibiting polypeptide, contacting the T cell with the multimeric inhibits the epitope-specific T cell. In some instances, the epitope-specific T cell is a self-reactive T cell that is specific for an epitope present in a self antigen, and the contacting reduces the number of the self-reactive T cells.

## TREATMENT METHODS

**[0215]** Also provided is a method of treating an autoimmune disorder by inhibiting a self-reactive T cell clone which recognizes an epitope peptide comprising contacting a T cell of the clone with a recombinant peptide as described herein, wherein the recombinant peptide comprises the epitope peptide and comprises a T cell modulatory domain which is an inhibitory domain, in an amount effective to treat an autoimmune disorder.

**[0216]** Also provided is a method of treating a cancer by stimulating a T cell clone which

recognizes an epitope peptide on a cancer comprising contacting a T cell of the clone with a recombinant peptide as described herein, wherein the recombinant peptide comprises the epitope peptide and comprises a T cell modulatory domain which is an stimulatory domain, in an amount effective to treat the cancer.

**[0217]** In an embodiment, the cells transformed to express a recombinant polypeptide of the invention are isolated suspension-adapted cells. In an embodiment of the plurality of said isolated suspension-adapted cells, or of the recombinant nucleic acid, the nucleic acid comprises DNA.

**[0218]** In an embodiment, the T-cells comprise peripheral T-cells obtained from a subject. In an embodiment, the T-cells comprise T-cells in a subject. In an embodiment, the T-cells comprise peripheral T-cells in a subject. In an embodiment of the methods herein, the subject is human.

**[0219]** The present disclosure provides a method of selectively modulating the activity of an epitope-specific T cell in an individual, the method comprising administering to the individual an amount of the multimeric polypeptide of the present disclosure, or one or more nucleic acids encoding the multimeric polypeptide, effective to selectively modulate the activity of an epitope-specific T cell in an individual. In some cases, a treatment method of the present disclosure comprises administering to an individual in need thereof one or more recombinant expression vectors comprising nucleotide sequences encoding a multimeric polypeptide of the present disclosure. In some cases, a treatment method of the present disclosure comprises administering to an individual in need thereof one or more mRNA molecules comprising nucleotide sequences encoding a multimeric polypeptide of the present disclosure. In some cases, a treatment method of the present disclosure comprises administering to an individual in need thereof a multimeric polypeptide of the present disclosure.

**[0220]** The present disclosure provides a method of selectively modulating the activity of an epitope-specific T cell in an individual, the method comprising administering to the individual an effective amount of a multimeric polypeptide of the present disclosure, or one or more nucleic acids (e.g., expression vectors; mRNA; etc.) comprising nucleotide sequences encoding the multimeric polypeptide, where the multimeric polypeptide selectively modulates the activity of the epitope-specific T cell in the individual. Selectively modulating the activity of an epitope-specific T cell can treat a disease or disorder in the individual. Thus, the present disclosure provides a treatment method comprising administering to an individual in need thereof an effective amount of a multimeric polypeptide of the present disclosure.

**[0221]** In some cases, the immunomodulatory polypeptide is an activating polypeptide, and the multimeric polypeptide activates the epitope-specific T cell. In some cases, the epitope is a cancer-associated epitope, and the multimeric polypeptide increases the activity of a T cell specific for the cancer-associate epitope.

**[0222]** The present disclosure provides a method of treating cancer in an individual, the

method comprising administering to the individual an effective amount of a multimeric polypeptide of the present disclosure, or one or more nucleic acids (e.g., expression vectors; mRNA; etc.) comprising nucleotide sequences encoding the multimeric polypeptide, where the multimeric polypeptide comprises a T-cell epitope that is a cancer epitope, and where the multimeric polypeptide comprises a stimulatory immunomodulatory polypeptide. In some cases, an "effective amount" of a multimeric polypeptide is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of cancer cells in the individual. For example, in some cases, an "effective amount" of a multimeric polypeptide of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of cancer cells in the individual by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, compared to the number of cancer cells in the individual before administration of the multimeric polypeptide, or in the absence of administration with the multimeric polypeptide. In some cases, an "effective amount" of a multimeric polypeptide of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of cancer cells in the individual to undetectable levels. In some cases, an "effective amount" of a multimeric polypeptide of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof, reduces the tumor mass in the individual. For example, in some cases, an "effective amount" of a multimeric polypeptide of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof, reduces the tumor mass in the individual by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, compared to the tumor mass in the individual before administration of the multimeric polypeptide, or in the absence of administration with the multimeric polypeptide. In some cases, an "effective amount" of a multimeric polypeptide of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof, increases survival time of the individual. For example, in some cases, an "effective amount" of a multimeric polypeptide of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof, increases survival time of the individual by at least 1 month, at least 2 months, at least 3 months, from 3 months to 6 months, from 6 months to 1 year, from 1 year to 2 years, from 2 years to 5 years, from 5 years to 10 years, or more than 10 years, compared to the expected survival time of the individual in the absence of administration with the multimeric polypeptide.

**[0223]** In some instances, the epitope-specific T cell is a T cell that is specific for an epitope present on a virus-infected cell, and contacting the epitope-specific T cell with the multimeric polypeptide increases cytotoxic activity of the T cell toward the virus-infected cell. In some instances, the epitope-specific T cell is a T cell that is specific for an epitope present on a virus-infected cell, and contacting the epitope-specific T cell with the multimeric polypeptide increases the number of the epitope-specific T cells.

**[0224]** Thus, the present disclosure provides a method of treating a virus infection in an individual, the method comprising administering to the individual an effective amount of a

multimeric polypeptide of the present disclosure, or one or more nucleic acids comprising nucleotide sequences encoding the multimeric polypeptide, where the multimeric polypeptide comprises a T-cell epitope that is a viral epitope, and where the multimeric polypeptide comprises a stimulatory immunomodulatory polypeptide. In some cases, an "effective amount" of a multimeric polypeptide is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of virus-infected cells in the individual. For example, in some cases, an "effective amount" of a multimeric polypeptide of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of virus-infected cells in the individual by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, compared to the number of virus-infected cells in the individual before administration of the multimeric polypeptide, or in the absence of administration with the multimeric polypeptide. In some cases, an "effective amount" of a multimeric polypeptide of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of virus-infected cells in the individual to undetectable levels.

**[0225]** Thus, the present disclosure provides a method of treating an infection in an individual, the method comprising administering to the individual an effective amount of a multimeric polypeptide of the present disclosure, or one or more nucleic acids comprising nucleotide sequences encoding the multimeric polypeptide, where the multimeric polypeptide comprises a T-cell epitope that is a pathogen-associated epitope, and where the multimeric polypeptide comprises a stimulatory immunomodulatory polypeptide. In some cases, an "effective amount" of a multimeric polypeptide is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of pathogens in the individual. For example, in some cases, an "effective amount" of a multimeric polypeptide of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of pathogens in the individual by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, compared to the number of pathogens in the individual before administration of the multimeric polypeptide, or in the absence of administration with the multimeric polypeptide. In some cases, an "effective amount" of a multimeric polypeptide of the present disclosure is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of pathogens in the individual to undetectable levels. Pathogens include viruses, bacteria, protozoans, and the like.

**[0226]** In some cases, the immunomodulatory polypeptide is an inhibitory polypeptide, and the multimeric polypeptide inhibits activity of the epitope-specific T cell. In some cases, the epitope is a self-epitope, and the multimeric polypeptide selectively inhibits the activity of a T cell specific for the self-epitope.

**[0227]** The present disclosure provides a method of treating an autoimmune disorder in an individual, the method comprising administering to the individual an effective amount of a multimeric polypeptide of the present disclosure, or one or more nucleic acids comprising

nucleotide sequences encoding the multimeric polypeptide, where the multimeric polypeptide comprises a T-cell epitope that is a self epitope, and where the multimeric polypeptide comprises an inhibitory immunomodulatory polypeptide. In some cases, an "effective amount" of a multimeric polypeptide is an amount that, when administered in one or more doses to an individual in need thereof, reduces the number self-reactive T cells by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%, compared to number of self-reactive T cells in the individual before administration of the multimeric polypeptide, or in the absence of administration with the multimeric polypeptide. In some cases, an "effective amount" of a multimeric polypeptide is an amount that, when administered in one or more doses to an individual in need thereof, reduces production of Th2 cytokines in the individual. In some cases, an "effective amount" of a multimeric polypeptide is an amount that, when administered in one or more doses to an individual in need thereof, ameliorates one or more symptoms associated with an autoimmune disease in the individual.

**[0228]** As noted above, in some cases, in carrying out a subject treatment method, a multimeric polypeptide of the present disclosure is administered to an individual in need thereof, as the polypeptide *per se*. In other instances, in carrying out a subject treatment method, one or more nucleic acids comprising nucleotide sequences encoding a multimeric polypeptide of the present disclosure is/are administering to an individual in need thereof. Thus, in other instances, one or more nucleic acids of the present disclosure, e.g., one or more recombinant expression vectors of the present disclosure, is/are administered to an individual in need thereof.

### Formulations

**[0229]** Suitable formulations are described above, where suitable formulations include a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: a) a multimeric polypeptide of the present disclosure; and b) a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: a) a nucleic acid comprising a nucleotide sequence encoding a multimeric polypeptide of the present disclosure; and b) a pharmaceutically acceptable excipient; in some instances, the nucleic acid is an mRNA. In some cases, a suitable formulation comprises: a) a first nucleic acid comprising a nucleotide sequence encoding the first polypeptide of a multimeric polypeptide of the present disclosure; b) a second nucleic acid comprising a nucleotide sequence encoding the second polypeptide of a multimeric polypeptide of the present disclosure; and c) a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: a) a recombinant expression vector comprising a nucleotide sequence encoding a multimeric polypeptide of the present disclosure; and b) a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: a) a first recombinant expression vector comprising a nucleotide sequence encoding the first polypeptide of a multimeric polypeptide of the present disclosure; b) a second recombinant expression vector comprising a nucleotide sequence encoding the second polypeptide of a multimeric polypeptide of the present disclosure; and c) a pharmaceutically

acceptable excipient.

**[0230]** Suitable pharmaceutically acceptable excipients are described above.

**Dosages**

**[0231]** A suitable dosage can be determined by an attending physician or other qualified medical personnel, based on various clinical factors. As is well known in the medical arts, dosages for any one patient depend upon many factors, including the patient's size, body surface area, age, the particular polypeptide or nucleic acid to be administered, sex of the patient, time, and route of administration, general health, and other drugs being administered concurrently. A multimeric polypeptide of the present disclosure may be administered in amounts between 1 ng/kg body weight and 20 mg/kg body weight per dose, e.g. between 0.1 mg/kg body weight to 10 mg/kg body weight, e.g. between 0.5 mg/kg body weight to 5 mg/kg body weight; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. If the regimen is a continuous infusion, it can also be in the range of 1  $\mu$ g to 10 mg per kilogram of body weight per minute.

**[0232]** In some cases, a suitable dose of a multimeric polypeptide of the present disclosure is from 0.01  $\mu$ g to 100 g per kg of body weight, from 0.1  $\mu$ g to 10 g per kg of body weight, from 1  $\mu$ g to 1 g per kg of body weight, from 10  $\mu$ g to 100 mg per kg of body weight, from 100  $\mu$ g to 10 mg per kg of body weight, or from 100  $\mu$ g to 1 mg per kg of body weight. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the administered agent in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein a multimeric polypeptide of the present disclosure is administered in maintenance doses, ranging from 0.01  $\mu$ g to 100 g per kg of body weight, from 0.1  $\mu$ g to 10 g per kg of body weight, from 1  $\mu$ g to 1 g per kg of body weight, from 10  $\mu$ g to 100 mg per kg of body weight, from 100  $\mu$ g to 10 mg per kg of body weight, or from 100  $\mu$ g to 1 mg per kg of body weight.

**[0233]** Those of skill will readily appreciate that dose levels can vary as a function of the specific multimeric polypeptide, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

**[0234]** In some embodiments, multiple doses of a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure are administered. The frequency of administration of a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure can vary depending on any of a variety of factors, e.g., severity of the symptoms, etc. For example, in some embodiments, a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a

recombinant expression vector of the present disclosure is administered once per month, twice per month, three times per month, every other week (qow), once per week (qw), twice per week (biw), three times per week (tiw), four times per week, five times per week, six times per week, every other day (qod), daily (qd), twice a day (qid), or three times a day (tid).

**[0235]** The duration of administration of a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure, e.g., the period of time over which a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure is administered, can vary, depending on any of a variety of factors, e.g., patient response, etc. For example, a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure can be administered over a period of time ranging from about one day to about one week, from about two weeks to about four weeks, from about one month to about two months, from about two months to about four months, from about four months to about six months, from about six months to about eight months, from about eight months to about 1 year, from about 1 year to about 2 years, or from about 2 years to about 4 years, or more.

#### Routes of administration

**[0236]** An active agent (a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure) is administered to an individual using any available method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic and localized routes of administration.

**[0237]** Conventional and pharmaceutically acceptable routes of administration include intratumoral, peritumoral, intramuscular, intratracheal, intracranial, subcutaneous, intradermal, topical application, intravenous, intraarterial, rectal, nasal, oral, and other enteral and parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the multimeric polypeptide and/or the desired effect. A multimeric polypeptide of the present disclosure, or a nucleic acid or recombinant expression vector of the present disclosure, can be administered in a single dose or in multiple doses.

**[0238]** In some embodiments, a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure is administered intravenously. In some embodiments, a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure is administered intramuscularly. In some embodiments, a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure is administered locally. In some embodiments, a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure is administered

intratumorally. In some embodiments, a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure is administered peritumorally. In some embodiments, a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure is administered intracranially. In some embodiments, a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure is administered subcutaneously.

**[0239]** In some embodiments, a multimeric polypeptide of the present disclosure is administered intravenously. In some embodiments, a multimeric polypeptide of the present disclosure is administered intramuscularly. In some embodiments, a multimeric polypeptide of the present disclosure is administered locally. In some embodiments, a multimeric polypeptide of the present disclosure is administered intratumorally. In some embodiments, a multimeric polypeptide of the present disclosure is administered peritumorally. In some embodiments, a multimeric polypeptide of the present disclosure is administered intracranially. In some embodiments, a multimeric polypeptide is administered subcutaneously.

**[0240]** A multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the disclosure include, but are not necessarily limited to, enteral, parenteral, or inhalational routes.

**[0241]** Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, intratumoral, peritumoral, and intravenous routes, i.e., any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of a multimeric polypeptide of the present disclosure, a nucleic acid of the present disclosure, or a recombinant expression vector of the present disclosure. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

#### **Subjects suitable for treatment**

**[0242]** Subjects suitable for treatment with a method of the present disclosure include individuals who have cancer, including individuals who have been diagnosed as having cancer, individuals who have been treated for cancer but who failed to respond to the treatment, and individuals who have been treated for cancer and who initially responded but subsequently became refractory to the treatment. Subjects suitable for treatment with a method of the present disclosure include individuals who have an infection (e.g., an infection with a pathogen such as a bacterium, a virus, a protozoan, etc.), including individuals who have been

diagnosed as having an infection, and individuals who have been treated for an infection but who failed to respond to the treatment. Subjects suitable for treatment with a method of the present disclosure include individuals who have bacterial infection, including individuals who have been diagnosed as having a bacterial infection, and individuals who have been treated for a bacterial infection but who failed to respond to the treatment. Subjects suitable for treatment with a method of the present disclosure include individuals who have a viral infection, including individuals who have been diagnosed as having a viral infection, and individuals who have been treated for a viral infection but who failed to respond to the treatment. Subjects suitable for treatment with a method of the present disclosure include individuals who have an autoimmune disease, including individuals who have been diagnosed as having an autoimmune disease, and individuals who have been treated for a autoimmune disease but who failed to respond to the treatment.

**[0243]** All combinations of the various elements described herein are within the scope of the invention as defined by the appended claims unless otherwise indicated herein or otherwise clearly contradicted by context.

**[0244]** This invention will be better understood from the Experimental Details, which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as defined by the appended claims.

## EXAMPLES

**[0245]** The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

### EXAMPLE 1: GENERATION OF SYNTAC HETERODIMERS

**[0246]** Aspects of the instant disclosure pertain to a novel protein based therapeutic platform, "synTac," which mimics the interaction specificity and regulatory signals of the immunological synapse. SynTac is a fusion protein linking a costimulatory molecule to an MHC-epitope

allowing for precise T cell engagement and clonal T cell activation or inhibition (FIG. 1), a soluble version of the body's natural response. In this way, synTac combines the best of epitopes, bispecific antibodies, soluble costimulatory molecules and ADCs. SynTac allows for highly specific cell targeting through the MHC-epitope, with a "single chain fusion" design disallowing cross presentation of the free epitope (FIG. 2A-2C). A T cell modulatory domain (alternatively described herein as "MOD") is also covalently attached, which elicits either activation or inhibition depending on the nature of the costimulatory engagement. This elicits an antigen-specific, not global, T cell response. Notably, the MOD can include any known antibody, antibody fragment, costimulatory molecule, or other literature-validated payload (cytokines, toxins, etc.), and does not need to be internalized to exert an effect on the T cell. Moreover, both targets are present on the surface of the same cell eliminating the "spacing problem" of traditional bispecific antibodies.

**[0247]** In one example, the strategy exploits an Fc-fusion construction, (a non-limiting example is set forth in FIG. 2A-2C), to increase the valency, stability and therapeutic window of the associated products. Briefly, the Fc region is a native covalent homo-dimer and stabilized through two disulfide bonds illustrated as two thin lines in FIG. 2A-2C. The presence of the Fc domain is known to prolong therapeutic activity by increasing plasma half-life, owing to its interaction with the neonatal Fc-receptor as well as to the slower renal clearance for larger sized bivalent molecules [23, 24]. From a biophysical perspective, the Fc domain folds independently and can improve the solubility and stability of the partner molecule both *in vitro* and *in vivo* [25], and the Fc region allows for easy cost-effective purification by protein-A/G affinity chromatography during production [26]. FIG. 2A shows a single chain peptide MHC protein (single chain trimer [27]) linked at its carboxy terminus to an IgG Fc region. As depicted, these single chain constructions are limited with respect to ones ability to extend the system through alternative protein linkages (such as the MOD). Specifically, linkages are preferably restricted to a region C-terminal of the MHC, depicted by dashed lines in FIG. 2A (which herein is termed direct linkage). MHC I or MHC II molecules can be used. Expression of constructs using a direct linkage approach is highly dependent on the MOD being used. A solution to this, disclosed herein, is to split the construct into respective heavy and light chains and fuse both peptides and proteins to various ends (FIG. 2B and FIG. 2C). One construction results in an amino-terminal association of the peptide to the light chain (beta 2 microglobulin) followed by a carboxy terminal extension of the light chain to the MOD effector molecule (FIG. 2B). In this scenario the heavy chain (HLA-molecule) is fused to the Fc region. All components associate during production within eukaryotic cells (e.g., HEK, CHO) and self assemble. Constructs are held together covalently through disulfide bridges. An alternative orientation (FIG. 2C) places the MOD amino-terminal of the Fc fused heavy chain with the peptide still linked to the B2M light chain. Again all components self assemble and form stable covalent interactions through disulfide bonds. Traditional bispecific antibodies often attempt to bridge two cells by dimerizing one amino terminal Fc payload with one carboxy terminal Fc payload. In contrast, a construction disclosed herein orients two different protein payloads, an MHC-epitope targeting mechanism and a MOD effector, to the surface of the same cell, similar to a CH1 - light chain interaction found within traditional antibodies. Further, the use of Fc fusions allows tailored engagement of associated effector functions, such as antibody-dependent cell-

mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) or phagocytosis, by modulation of the binding affinities to Fc receptors through mutations [28].

**[0248]** A design for two base synTac molecules is presented in FIG. 3A-3B. Briefly, this construct utilizes a native human B2M leader sequence to allow for efficient secretion and ER processing immediately followed by a candidate epitope (labeled as peptide). Once in the ER the leader sequence is fully removed and allows for the presentation of the peptide in the MHC binding pocket. For a "light chain" linkage (LC, FIG. 3A), this is coupled to the native B2M molecule through linker L1 and the MOD through linker L2. This entire cassette is linked to another B2M leader sequence, the MHC heavy chain (e.g. human HLA-A02:01 or murine H-2Kd in the examples), and an Fc domain (either human IgG1 or murine IgG2a) by a viral porcine teschovirus-1 (P2A) "self-cleaving" peptide to allow for stoichiometric expression of each chain. The P2A peptide was chosen as this has the highest reported "cleavage" efficiency of all viral 2A peptides expressed in mammalian cells [29]. The "heavy chain" (HC, FIG. 3B) linkage is similar however the viral P2A peptide now follows the B2M and the MOD follows the second leader peptide, leading to the protein construct shown in FIG. 2C. Both constructs can terminate in an 8x His tag for ease of purification.

**[0249]** *Specialized Expression Cells:* Although both chains are expressed and co-localize to the ER, owing to the P2A linkage, there was some concern that endogenous B2M from the expression host (suspension adapted HEK293 cells) could out-compete the recombinant version as HEK293 cells natively express HLA and B2M molecules. This would result in either decreased stability (e.g., manifesting in decreased overall yields) or a highly undesirable heterogeneous protein sample. To avoid this complication, the CRISPR/CAS system was leveraged to knock out native B2M from the HEK cell pool [30]. Briefly, guide RNA was designed against endogenous B2M, transfected along with a plasmid encoding CRISPR/CAS and allowed to culture for three days. The cultured cells were surface stained against anti-B2M and counter selected (sorted on loss of fluorescence) by fluorescence activated cell sorting (FACS). The sorted cells were allowed to recover and subjected to two more rounds of staining, counter-sorting and recovery (3 rounds in total) to ensure efficient (~100%) knock-out. As illustrated in FIG. 4, the final pool was quality checked by monitoring surface expression of B2M via FACS, suggesting complete ablation of the endogenous B2M protein. Experiments leveraging next generation sequencing to quantify the knock-out percentages at a genomic level are then performed. The resulting HEK-293-B2M-KO line (termed HEK-KO) was used for all subsequent experiments.

**[0250]** *Engineered Disulfide Bonds:* To increase protein stability and circumvent the complications associated with potential peptide transfer to cellular MHC molecules (cross-presentation) and B2M release, single chain constructs are generally employed [27, 31]. However these single chain constructions (shown in FIG. 2A) are limited with respect to an ability to extend the system through alternative protein linkages (such as the MOD). A solution is to split the construct into respective heavy and light chains analogous to previous efforts [32] but now fuse both peptides and proteins to various ends as described (FIG. 2B and 2C). However, in the final construct, to retain the stability afforded by traditional single chain

systems, the option of engineering disulfide bridges between the heavy and light chains was investigated (illustrated as S-S in FIG. 2), as seen in disulfide trapped single chain trimers [dt-SCT] [33]. Notably, as initial synTac production attempts utilizing the dt-SCT disulfide schema resulted in low levels of expression, and this being further dependent on the peptide being presented, the dt-SCT disulfide configuration was deemed not ideal for use in split protein systems. Thus, it was sought to identify alternative positions to engineer disulfide bridges better suited for split protein systems, such as synTac. Two positions were chosen from the light chain (2, 12) each with a disulfide bond potential for two positions in the heavy chain (119, 120 and 236, 237 respectively, from analysis of PDB 2X4R). Notably, these positions are highly conserved residues not known to interact with the peptide binding groove [34], TCR complex [35] or CD8 coreceptor [36]. High-level expression was demonstrated for one construct (H236-L12, with H referring to the heavy chain position and L referring to the light, labeled as synTac 18 in FIG. 5A-5B) with modest expression for a second (H237-L12, synTac 17 FIG. 5A-5B). The dt-SCT disulfide schema was used as a positive control (labeled as synTac 2). A high molecular weight moiety was formed as seen by non-reducing PAGE gels suggesting stable disulfide bond formation (FIG. 5A). All expressing constructs were scaled up to the 100 ml scale, purified and activity tested through binding of cognate TCR expressed on the surface of HEK cells (termed HEK-A6), as monitored by FACS fluorescence, suggesting proper folding and activity (FIG. 5B). Cells expressing non-cognate TCR (termed HEK-AS01) were used as a negative control. Additional constructs have been generated bearing only a C-terminal 8X His tag (monovalent).

**[0251] SynTac controls:** Previous work has focused on autoimmune diabetes [37], and a disease-relevant model system, specifically autoreactive CD8+ 8.3 T cells isolated from the pancreatic islets of a nonobese diabetic (NOD) mouse, has been used. Building on this work, synTac constructs were generated bearing a peptide composed of residues 206 to 214 of islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP206-214) presented by the murine class-I H-2Kd allele (termed IGRP) known to interact with 8.3 T cells. A control synTac presenting the tumor-derived peptide (KYQAVTTTL, SEQ ID NO:18), which is not recognized by 8.3 T cells, was prepared in an identical fashion (e.g., murine H-2Kd presentation) and designated TUM. To determine the degree to which the system can tolerate multiple HLA alleles (e.g., murine H2-Kd, human HLA-A02, etc.), a third synTac variant was constructed bearing a previously validated human HLA-A02 restricted epitope (Human T-lymphotropic virus, Tax 11-19) and termed HTLV. To allow for targeted T cell depletion, initial synTac constructs used a light chain linkage format and carried a PD-L1 MOD domain (schematically illustrated in FIG. 2B). Each synTac variant (IGRP, TUM and HTLV) showed positive expression profiles in HEK-KO cells, non-reducing SDS page results shown in FIG. 6A. To examine the generality of the expression system, IGRP based synTac constructs with variant MOD domains were explored, including two MODs for T cell stimulation (i.e., humanized anti-CD28 single chain Fv and the extracellular domain of TNF ligand 4-1BBL), and another two MODs allowing for T cell inhibition (a single point mutant of B7-1 [W88A], known to bind only to CTLA4 [38] and a truncated variant of PD-L1 [Ig variable domain only]). All constructs expressed well in HEK-KO cells, FIG. 6B. The ability to express synTac proteins leveraging a heavy chain linkage format was further explored (schematically illustrated in FIG.

2C). For these an IGRP epitope was used as the targeting peptide and PD-L1 or humanized anti CD28 scFv as the MOD, again showing positive expression profiles in HEK-KO cells (FIG. 6C). These were subsequently produced at a scale of 1L or more and purified to homogeneity through both Ni<sup>2+</sup> IMAC and size exclusion in an endotoxin free environment. All IGRP and TUM constructs were utilized in T cell proliferation assays and HTLV constructs for the TCR-synTac-PD1 bridging experiments below.

**[0252] TCR-synTac-PD1 Bridging:** While the solution profile following size exclusion is indicative of a well-folded protein, it is desirable to validate the integrity of each synTac component (both the MHC-epitope targeting mechanism and MOD) prior to employing these reagents in activity assays. The previously described HEK-A6 cells were used as a positive control and cells expressing a non-cognate TCR (AS01, responsive to an HLA-A0201-restricted Epstein-Bar virus epitope) were generated and used as a negative control along with untransduced parental cells, termed HEK-AS01 and PARENTAL respectively. TCR expression was confirmed by mCerulean fluorescence (TCR fusion reporter) and surface staining for the TCR signaling complex (CD5ε expression proxy). HEK-A6 cells were challenged with non-fluorescent purified HTLV-PD-L1 synTac variants and incubated with its cognate receptor PD1 fused to murine IgG2a. The PD-1-Fc fusion was detected using a FITC labeled anti-mouse secondary antibody. FITC fluorescence (i.e. 'bridging') was dependent on cognate TCR surface expression as shown in FIG. 7A-7B. In particular, FITC fluorescence was not observed when challenged against non-cognate TCR bearing HEK cells or parental cells (HEK-AS01, PARENTAL), when challenged against FITC-PD1-Fc only or when the MOD was absent.

**[0253] SynTac in action: T cell Assays.** As proof of concept for the targeting power of the synTac platform, an inhibitory synTac construct was tested in a T cell suppression assay. It was hypothesized that a light chain version of synTac IGRP fused to PD-L1 would specifically suppress IGRP206-214-specific T cells. CD8+ splenocytes were purified from a nonobese diabetic mouse transgenic for the 8.3 T cell receptor. This splenocyte subset contains primarily CD8+ T cells which are specific for the IGRP206-214 peptide in the context of H-2Kd. These CD8+ T cells were then cultured in the presence of immobilized anti-CD3 antibody, a treatment known to stimulate polyclonal T cell activation, and treated stimulated cultures with soluble versions of either synTac IGRP-PD-L1 or synTac TUM-PD-L1 to examine the antigen specificity of any suppressive effect. A version of synTac IGRP without PD-L1 served as an effector control for the MOD domain. Before seeding, cells were labeled with carboxyfluorescein succinimidyl ester (CFSE), a fluorescent cytosolic dye whose intensity halves with each cell division, in order to monitor the extent of T cell activation-induced cellular proliferation. After a 5 day culture period, cells were harvested and examined using flow cytometry for viability and proliferation. Supernatants were also examined for the expression of the CD8+ T cell effector cytokines IPN $\gamma$  and TNF $\alpha$  using a multiplexed flow cytometric bead assay. All CD8+ T cell activation parameters examined were suppressed in an antigen-specific and effector (i.e. MOD) domain-dependent manner, shown in FIG. 8A-8D. That is, IGRP-PD-L1 synTac was highly suppressive relative to either TUM-PD-L1 synTac or IGRP-(without PD-L1) indicating that the activity of synTac was dependent on both the peptide-MHC and MOD domains (FIG. 8D). SynTac was able to suppress IPN $\gamma$  secretion by approximately 100 fold and resulted in the

death of the vast majority of cells, suggesting that synTac bearing PDL1 as a MOD domain is capable of functionally suppressing as well as eliminating targeted specificities.

**[0254] Affinity Attenuation:** A possible issue with the use of the PD-1/PDL-1 system as a modulating domain is that PD-L1 has the potential to bind more than one receptor, with concomitant differences in downstream signaling. PD-L1 has been shown to bind to both B7-1 and PD-1. To avoid the complication of off-target binding, single point mutants may be used that bind only the desired target, PD-1 (e.g., specifically G119D and G119R, and others as discussed herein) while retaining their T cell inhibitory potential when tested as independent Fc fusions. Notably, the mutant PD-L1 Fc fusions alone can be useful reagents for immunomodulation. In the context of synTac, these mutants offer a range of PD-1 binding affinities. IGRP based synTac fusion proteins bearing the G119D and G119R mutants have been produced.

**[0255] Modular Design:** Soluble monovalent MHC molecules have an intrinsically low affinity for their cognate T cell receptors and thus have not been useful reagents for diagnostic or therapeutic purposes. While dimeric MHC complexes have been used in various systems to visualize antigen specific T cells [39], higher avidity MHC tetramers and higher order multimers are more commonly used [40]. It is clear from the present work that the current dimeric synTac construction provides for high level expression of well folded protein and elicits targeted T cell responses, however in select cases it may be desirable to extend the synTac technology by increasing valency to enhance T cell targeting potential. To that end, synTac variants were designed again bearing an IGRP targeting mechanism, with the PD-L1 MOD as a light chain linkage in the context of an IgA and IgM Fc region. Through covalent association with the J-chain through disulfide bridges, the IgA and IgM backbone allows for tetramer and decamer based presentation respectively. Lentivirus was generated, HEK-KO cells transduced and expression tested, supporting an initial ability to express these reagents. If desired, one can link the MOD directly to the J-chain, as an N-terminal, C-terminal or dual fusion to change the valency of MOD to targeting molecule. Further, owing to the flexibility of the synTac configuration, one can present multiple peptide epitopes or MODs simultaneously (e.g., tri-specificity) by using a dual heavy chain/light chain linkage. In addition, other MODs include but are not limited to 4-1BBL and anti-CD28 for activation and B7W for inhibition. Select constructs can leverage additional targeting epitopes. Moreover, synTac variants with higher levels of valency (IgA and IgM) can be used as well as non-stoichiometrically linked MODs (e.g., J-chain linkages) as described.

#### **EXAMPLE 2: GENERATION OF TRIMERIC SYNTAC POLYPEPTIDES**

**[0256] Stimulatory MOD (4-1BBL) receptor trimeric expression:** Initial efforts to generate active 4-1BBL bearing synTacs leveraged the light chain linkage variant (FIG. 3A). This was expressed as a single transfection (all pieces encoded on a single plasmid), split by a viral P2A sequence and resulted in highly expressed well-folded protein (FIG. 6B, lane 5). Gel filtration profiles coupled with multi-angle light scattering (MALS) data, suggested the initial version to

be a well-folded dimer (as illustrated in FIG. 10B, FIG. 9B). It has been observed that 4-1BBL, a TNF family ligand, requires trimerization (e.g., three copies of the same protein, homotrimer) for full activity. To achieve trimerization, the 4-1BBL bearing synTac construct along with "free" 4-1BBL (4-1BBL alone having no affinity tag [residues 50-254, including the membrane proximal and TNF homology domains, FIG. 10A; FIG. 9A]) were both expressed in the same cell (e.g., co-expression) to allow for native assembly and trimerization, as illustrated in FIG. 10C (original synTac construct in BLACK, Free BBL in GRAY) (co-expression of FIG. 9A and FIG. 9B constructs). Gel filtration chromatography coupled with multi-angle light scattering (MALS) data supports that the new version is the desired trimer (FIG. 11A-11B, labeled as synTac number 40 + 51). As described below (*MOD optimization*) the 4-1BBL constructs can be further optimized to further improve expression and purification profiles and increase stability and reproducibility.

**[0257] Stimulatory MOD receptor binding and human/mouse cross reactivity:** While the solution profile following size exclusion is indicative of a well-folded protein, it is desirable to validate the integrity of each synTac component (both the MHC-epitope targeting mechanism and MOD) prior to employing these reagents in activity assays. This particular targeting mechanism (IGRP peptide in the context of murine Kd) has been thoroughly validated (FIG. 7A-7B), thus the extent of 4-1BBL receptor binding was further investigated. To that end, Protein A microbeads were coated to saturation with recombinant human or mouse 4-1BB-Fc fusion protein (from commercial sources). 4-1BB coated microbeads were then used to bind synTac constructs bearing 4-1BB ligand (dimeric and trimeric versions) as the comodulatory domain, followed by a fluorescent detection antibody specific for the synTac heavy chain isotype. The extent of specific binding of synTac 4-1BBL to bead-borne 4-1BB was then measured by high throughput flow cytometry. Using this system, the degree of cross reactivity and relative affinities of 4-1BBL for both human AND murine 4-1BB was explored in the context of the synTac scaffold. 4-1BBL bearing synTacs (termed Trimer, Dimer) were shown to bind cognate receptor, but not "receptor-less" (termed no MOD) Fc bound microbeads, suggesting a well-folded and active protein reagent (FIG. 12). Further, the timer bound in an affinity range expected for dual trimeric engagement with the original dimer, showing a 10 fold reduction in binding affinity, again supporting dimeric presentation. MOD-less synTac (labeled as no MOD) was used as a negative control, showing no binding for 4-1BBL receptors. Notably, all constructs bind to both murine and human receptors (cross-react) and will thus allow for direct extension to *in vivo* murine trials.

**[0258] *In vitro* T cell stimulation assays:** In order to test the activity of the 4-1BBL synTacs, CD8 splenocytes were first purified from 8.3 TCR transgenic NOD mice and fluorescently labeled with CFSE to track proliferation before being treated *in vitro* with either soluble IGRP-41BBL synTac (dimer and trimer) or soluble TUM-41BBL synTac (FIG. 13). Control treatments were media alone or immobilized anti-CD3. After 4 days in culture, cells were examined by FACS for viability (DAPI exclusion) and proliferation (CFSE dilution). Supernatants were examined for IPNy and TNF $\alpha$  levels by a flow cytometric ELISA. As in the case of syntac-PDL1 (FIG. 8A-8D), the *in vitro* activity of syntac-41BBL was highly antigen-specific, resulting in much greater viability, proliferation, and cytokine release in the case of syntac IGRP-41BBL versus

TUM-41BBL. As expected, trimeric 4-1BBL was necessary for full activity (e.g., proliferation, viability and cytokine release). In addition, responses to IGRP-41BBL compared favorably to the immobilized anti-CD3 benchmark, suggesting that soluble syntac-41BBL can mediate high levels of T cell activation. All further related experiments described herein utilized trimeric syntac-41BBL.

**[0259] *In vivo T cell stimulation - single dose:*** Whether syntac-41BBL could exert similar effects on T cell activation *in vivo* was further examined. NOD mice were treated with syntac IGRP-41BBL versus syntac TUM-41BBL and the frequencies of IGRP specific CD8<sup>+</sup> T cells in the spleen were determined. Unlike TCR transgenic NOD mice, in which most T cells are of a defined clonotype, standard NOD mice have highly diverse TCR repertoires and are a better approximation of a 'natural' immune repertoire. NOD mice were injected intraperitoneally with syntac IGRP-41BBL, syntac TUM-41BBL or PBS and sacrificed 6 days post injection. Splenocytes were then examined via flow cytometry for relative frequencies of IGRP-specific CD8 T cells using an appropriate peptide-MHC pentamer stain. IGRP-41BBL treatment was associated with a much higher frequency of IGRP-specific CD8 T cells versus controls. In addition, *in-vivo* expanded IGRP-specific cells were capable of producing IPN<sub>y</sub> when re-stimulated *in vitro*. These results support the ability of syntac-41BBL to expand functional CD8 effector T cells in an antigen-specific manner (FIG. 14).

**[0260] *In vivo T cell stimulation - multi dose:*** The effect of altered treatment regimen on *in vivo* T cell activation was examined, with particular attention to an established tumor antigen, the "TUM" nonamer peptide. NOD mice were treated with syntac IGRP-41BBL versus syntac TUM-41BBL using three doses (as compared to the previous single dose) over a period of two weeks. The frequencies of IGRP- or TUM-specific CD8 T cells were determined. NOD mice were injected intraperitoneally with syntac IGRP-41BBL, syntac TUM-41BBL or PBS and sacrificed 7 days post injection. Blood (PBMC's) and splenocytes were then examined via flow cytometry for relative frequencies of IGRP- or TUM-specific CD8 T cells using an appropriate peptide-MHC pentamer stain. Again IGRP-41BBL treatment was associated with a much higher frequency of IGRP-specific CD8 T cells, while TUM-41BBL treatment was associated with a much higher frequency of TUM-specific CD8 T cells, versus irrelevant antigen and PBS controls (FIG. 15). A similar pattern was observed in the spleen. These results support the ability of a multidose syntac-41BBL regimen to expand functional CD8 effector T cells in an antigen-specific manner, including the antigen-specific expansion of rare-tumor specific T cells.

**[0261] *In vivo T cell inhibition:*** Non-obese diabetic (NOD) mice were injected intraperitoneally with syntac IGRP-PDL1, syntac TUM-PDL1, or PBS. Six days post injection, pancreata were dissociated and pancreatic cells were examined via flow cytometry for relative frequencies of IGRP-specific CD8<sup>+</sup> T cells, using an appropriate peptide-MHC pentamer stain. As shown in FIG. 23, IGRP-PDL1 treatment was associated with a much lower frequency of IGRP-specific CD8<sup>+</sup> T cells, compared to the control syntac TUM-PDL1- and PBS-treated mice. These data demonstrate antigen-specific *in vivo* depletion following a single dose of syntac.

**[0262]** *MOD Optimization:* Over the course of experimentation, it was observed that most of the target protein (4-1BBL trimeric synTac) displayed characteristics of higher order multimers in size exclusion chromatography and would degrade with time, likely through release/exchange of "free" BBL. Thus a 4-1BBL backbone with increased stability and ease of production was sought with an emphasis on covalent assembly of 4-1BBL. Toward that end the use of engineered disulfide bonds within the TNF homology domain of 4-1BBL (FIG. 16A; Disulfides indicated with arrows; FIG. 9C-9E) were explored. From analysis of the X-ray structure (PDB 2X29), three potential pairs of residues were chosen which have likely disulfide bond potential and are unlikely to interfere with receptor binding. Two native residues in each construct were replaced with cysteine residues (Q94C:P245C), Q94C:P242C, and Q89C:L115C, termed synTac 69, 70 and 71 respectively), expressed in human cells with a "free" nontagged version harboring the same mutations (termed 98, 99, 100 respectively) to allow for covalent locking and the degree of disulfide bonding was observed by non-reduced SDS PAGE analysis (FIG. 18; the following co-expression constructs are termed DL1 (disulfide lock-1, synTac 69/98), DL2 (70/99) and DL3 (71/100)). All three constructs expressed well, allowed for disulfide locking (FIG. 18) and bound to receptor (FIG. 17). While these covalent "disulfide-locked" variants of synTac-4-1BBL address the stability issues discussed, co-expression of "free" BBL (co-expression) is still required to allow for trimerization which can complicate the production and biomanufacture of stimulatory synTacs. One solution to this obstacle was found to be expression of the 4-1BBL TNF homology domain as a single contiguous construct, termed single chain trimer (4-1BBL-SCT, FIG. 16B; FIG. 9F). Specifically, three copies of 4-1BBL residues 80-246 (TNF homology domain only) were held covalently by two (G4S)<sub>5</sub> linker sequences (FIG. 16B, linkers illustrated as curved lines; FIG. 9F). Expression and gel filtration coupled with multi-angle light scattering (MALS) data supports that the new version is the desired covalent single chain trimer (FIG. 18) and bound well to 4-1BBL receptor (FIG. 17).

**[0263]** While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the invention as defined by the appended claims. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective of the present invention within the scope as defined by the appended claim..

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### [0265]

<110> Almo, Steven C

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Garforth, Scott J

<120> Syntac Polypeptides and Uses Thereof

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35 40 45

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 130 135 140

Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val  
 145 150 155 160

Glu Glu Asn Pro Gly Pro Met Ser Arg Ser Val Ala Leu Ala Val Leu  
 165 170 175

Ala Leu Leu Ser Leu Ser Gly Leu Glu Ala Phe Thr Ile Thr Ala Pro  
 180 185 190

Lys Asp Leu Tyr Val Val Glu Tyr Gly Ser Asn Val Thr Met Glu Cys  
 195 200 205

Arg Phe Pro Val Glu Arg Glu Leu Asp Leu Leu Ala Leu Val Val Tyr  
 210 215 220

Trp Glu Lys Glu Asp Glu Gln Val Ile Gln Phe Val Ala Gly Glu Glu  
 225 230 235 240

Asp Leu Lys Pro Gln His Ser Asn Phe Arg Gly Arg Ala Ser Leu Pro  
 245 250 255

Lys Asp Gln Leu Leu Lys Gly Asn Ala Ala Leu Gln Ile Thr Asp Val  
 260 265 270

Lys Leu Gln Asp Ala Gly Val Tyr Cys Cys Ile Ile Ser Tyr Gly Gly  
 275 280 285

Ala Asp Tyr Lys Arg Ile Thr Leu Lys Val Asn Ala Pro Tyr Arg Lys  
 290 295 300

Ile Asn Gln Arg Ile Ser Val Asp Pro Ala Thr Ser Glu His Glu Leu  
 305 310 315 320

Ile Cys Gln Ala Glu Gly Tyr Pro Glu Ala Glu Val Ile Trp Thr Asn  
 325 330 335

Ser Asp His Gln Pro Val Ser Gly Lys Arg Ser Val Thr Thr Ser Arg  
 340 345 350

Thr Glu Gly Met Leu Leu Asn Val Thr Ser Ser Leu Arg Val Asn Ala  
 355 360 365

Thr Ala Asn Asp Val Phe Tyr Cys Thr Phe Trp Arg Ser Gln Pro Gly  
 370 375 380

Gln Asn His Thr Ala Glu Leu Ile Ile Pro Glu Leu Pro Ala Thr His  
 385 390 395 400

Pro Pro Gln Asn Arg Thr Gly Gly Gly Ser Gly Gly Gly Ser  
 405 410 415

Gly Gly Gly Ser Gly Gly Gly Ser Gly Ser His Ser Met Arg  
 420 425 430

Tyr Phe Phe Thr Ser Val Ser Arg Pro Gly Arg Gly Glu Pro Arg Phe  
 435 440 445

Ile Ala Val Gly Tyr Val Asp Asp Thr Gln Phe Val Arg Phe Asp Ser  
 450 455 460

Asp Ala Ala Ser Gln Arg Met Glu Pro Arg Ala Pro Trp Ile Glu Gln  
 465 470 475 480

Glu Gly Pro Glu Tyr Trp Asp Gly Glu Thr Arg Lys Val Lys Ala His  
 485 490 495

Ser Gln Thr His Arg Val Asp Leu Gly Thr Leu Arg Gly Cys Tyr Asn  
 500 505 510

Gln Ser Glu Ala Gly Ser His Thr Val Gln Arg Met Tyr Gly Cys Asp  
 515 520 525

Val Gly Ser Asp Trp Arg Phe Leu Arg Gly Tyr His Gln Tyr Ala Tyr  
 530 535 540

Asp Gly Lys Asp Tyr Ile Ala Leu Lys Glu Asp Leu Arg Ser Trp Thr  
 545 550 555 560

Ala Ala Asp Met Ala Ala Gln Thr Thr Lys His Lys Trp Glu Ala Ala  
 565 570 575

His Val Ala Glu Gln Leu Arg Ala Tyr Leu Glu Gly Thr Cys Val Glu  
 580 585 590

Trp Ile Arg Arg Tyr Leu Glu Asn Gly Lys Glu Thr Leu Gln Arg Thr  
 595 600 605

Asp Ala Pro Lys Thr His Met Thr His His Ala Val Ser Asp His Glu  
 610 615 620

Ala Thr Leu Arg Cys Trp Ala Leu Ser Phe Tyr Pro Ala Glu Ile Thr  
 625 630 635 640

Leu Thr Trp Gln Arg Asp Gly Glu Asp Gln Thr Gln Asp Thr Glu Leu  
 645 650 655

Val Glu Thr Arg Pro Ala Gly Asp Gly Thr Phe Gln Lys Trp Ala Ala  
 660 665 670

Val Val Val Pro Ser Gly Gln Glu Gln Arg Tyr Thr Cys His Val Gln  
 675 680 685

His Glu Gly Leu Pro Lys Pro Leu Thr Leu Arg Trp Glu Pro Ala Ala  
 690 695 700

Ala Gly Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
 705 710 715 720

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 725 730 735

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
 740 745 750

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
 755 760 765

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
 770 775 780

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
 785 790 795 800

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
 805 810 815

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
 820 825 830

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
 835 840 845

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
 850 855 860

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
 865 870 875 880

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
 885 890 895

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
 900 905 910

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
915 920 925

Ser Leu Ser Pro Gly Lys Gly Gly Ser His His His His His His  
930 935 940

His  
945

<210> 8

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<220>

<221> REPEAT

<222> (1)..(5)

<223> Repeat n times where n is an integer of at least 1

<400> 8

Gly Ser Gly Gly Ser  
1 5

<210> 9

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<220>

<221> REPEAT

<222> (1)..(4)

<223> Repeat n times where n is an integer of at least 1

<400> 9

Gly Gly Gly Ser  
1

<210> 10

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 10  
Gly Gly Ser Gly  
1

<210> 11  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 11  
Gly Gly Ser Gly Gly  
1 5

<210> 12  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 12  
Gly Ser Gly Ser Gly  
1 5

<210> 13  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 13  
Gly Ser Gly Gly Gly  
1 5

<210> 14  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 14  
Gly Gly Gly Ser Gly  
1 5

<210> 15  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 15  
Gly Ser Ser Ser Gly  
1 5

<210> 16  
<211> 15  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 16  
Gly Cys Gly Ala Ser Gly Gly Gly Ser Gly Gly Gly Ser  
1 5 10 15

<210> 17  
<211> 9  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 17  
Leu Leu Phe Gly Tyr Pro Val Tyr Val  
1 5

<210> 18  
<211> 9  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 18  
Lys Tyr Gln Ala Val Thr Thr Thr Leu  
1 5

<210> 19  
<211> 9  
<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 19

Val Tyr Leu Lys Thr Asn Val Phe Leu  
1 5

<210> 20

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 20

Thr Tyr Leu Lys Thr Asn Leu Phe Leu  
1 5

<210> 21

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<220>

<221> MISC\_FEATURE

<222> (2)..(2)

<223> X can be L or Q

<400> 21

Pro Xaa Gly Met Thr  
1 5

<210> 22

<211> 274

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 22

Gly Pro His Ser Leu Arg Tyr Phe Val Thr Ala Val Ser Arg Pro Gly  
1 5 10 15

Leu Gly Glu Pro Arg Phe Ile Ala Val Gly Tyr Val Asp Asp Thr Gln  
20 25 30

Phe Val Arg Phe Asp Ser Asp Ala Asp Asn Pro Arg Phe Glu Pro Arg  
 35 40 45

Ala Pro Trp Met Glu Gln Glu Gly Pro Glu Tyr Trp Glu Glu Gln Thr  
 50 55 60

Gln Arg Ala Lys Ser Asp Glu Gln Trp Phe Arg Val Ser Leu Arg Thr  
 65 70 75 80

Ala Gln Arg Tyr Tyr Asn Gln Ser Lys Gly Gly Ser His Thr Phe Gln  
 85 90 95

Arg Met Phe Gly Cys Asp Val Gly Ser Asp Trp Arg Leu Leu Arg Gly  
 100 105 110

Tyr Gln Gln Phe Ala Tyr Asp Gly Arg Asp Tyr Ile Ala Leu Asn Glu  
 115 120 125

Asp Leu Lys Thr Trp Thr Ala Ala Asp Thr Ala Ala Leu Ile Thr Arg  
 130 135 140

Arg Lys Trp Glu Gln Ala Gly Asp Ala Glu Tyr Tyr Arg Ala Tyr Leu  
 145 150 155 160

Glu Gly Glu Cys Val Glu Trp Leu Arg Arg Tyr Leu Glu Leu Gly Asn  
 165 170 175

Glu Thr Leu Leu Arg Thr Asp Ser Pro Lys Ala His Val Thr Tyr His  
 180 185 190

Pro Arg Ser Gln Val Asp Val Thr Leu Arg Cys Trp Ala Leu Gly Phe  
 195 200 205

Tyr Pro Ala Asp Ile Thr Leu Thr Trp Gln Leu Asn Gly Glu Asp Leu  
 210 215 220

Thr Gln Asp Met Glu Leu Val Glu Thr Arg Pro Ala Gly Asp Gly Thr  
 225 230 235 240

Phe Gln Lys Trp Ala Ala Val Val Pro Leu Gly Lys Glu Gln Asn  
 245 250 255

Tyr Thr Cys His Val His His Lys Gly Leu Pro Glu Pro Leu Thr Leu  
 260 265 270

Arg Trp

<210> 23

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 23

Tyr Pro Tyr Asp Val Pro Asp Tyr Ala  
1 5

<210> 24

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 24

Asp Tyr Lys Asp Asp Asp Asp Lys  
1 5

<210> 25

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 25

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu  
1 5 10

<210> 26

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 26

His His His His His  
1 5

<210> 27

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 27

His His His His His His

1 5

<210> 28

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 28

Trp Ser His Pro Gln Phe Glu Lys  
1 5

<210> 29

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 29

Tyr Pro Tyr Asp Val Pro Asp Tyr Ala  
1 5

<210> 30

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 30

Arg Tyr Ile Arg Ser  
1 5

<210> 31

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 31

Phe His His Thr  
1

<210> 32

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 32

Trp Glu Ala Ala Ala Arg Glu Ala Cys Cys Arg Glu Cys Cys Ala Arg  
1 5 10 15

Ala

<210> 33

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<220>

<221> SITE

<222> (2)..(2)

<223> X can be L or Q

<400> 33

Pro Xaa Gly Met Thr Ser  
1 5

<210> 34

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 34

Glu Asn Ieu Tyr Thr Gln Ser  
1 5

<210> 35

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 35

Asp Asp Asp Asp Lys  
1 5

1 5

<210> 36

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 36

Leu Val Pro Arg  
1

<210> 37

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 37

Leu Glu Val Leu Phe Gln Gly Pro  
1 5

<210> 38

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 38

Cys Gly Leu Val Pro Ala Gly Ser Gly Pro  
1 5 10

<210> 39

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 39

Ser Leu Leu Lys Ser Arg Met Val Pro Asn Phe Asn  
1 5 10

<210> 40

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 40

Ser Leu Leu Ile Ala Arg Arg Met Pro Asn Phe Asn  
1 5 10

<210> 41

<211> 12

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<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 41

Ser Lys Leu Val Gln Ala Ser Ala Ser Gly Val Asn  
1 5 10

<210> 42

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 42

Ser Ser Tyr Leu Lys Ala Ser Asp Ala Pro Asp Asn  
1 5 10

<210> 43

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 43

Arg Pro Lys Pro Gln Gln Phe Phe Gly Leu Met Asn  
1 5 10

<210> 44

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 44

Ser Leu Arg Pro Leu Ala Leu Trp Arg Ser Phe Asn  
1 5 10

<210> 45

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 45

Ser Pro Gln Gly Ile Ala Gly Gln Arg Asn Phe Asn  
1 5 10

<210> 46

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 46

Asp Val Asp Glu Arg Asp Val Arg Gly Phe Ala Ser Phe Leu  
1 5 10

<210> 47

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 47

Ser Leu Pro Leu Gly Leu Trp Ala Pro Asn Phe Asn  
1 5 10

<210> 48

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 48  
Ser Leu Leu Ile Phe Arg Ser Trp Ala Asn Phe Asn  
1 5 10

<210> 49  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 49  
Ser Gly Val Val Ile Ala Thr Val Ile Val Ile Thr  
1 5 10

<210> 50  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
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<400> 50  
Ser Leu Gly Pro Gln Gly Ile Trp Gly Gln Phe Asn  
1 5 10

<210> 51  
<211> 12  
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<220>  
<223> Synthetic peptide

<400> 51  
Lys Lys Ser Pro Gly Arg Val Val Gly Gly Ser Val  
1 5 10

<210> 52  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 52  
Pro Gln Gly Leu Leu Gly Ala Pro Gly Ile Leu Gly  
1 5 10

<210> 53  
<211> 31  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 53  
His Gly Pro Glu Gly Leu Arg Val Gly Phe Tyr Glu Ser Asp Val Met  
1 5 10 15

Gly Arg Gly His Ala Arg Leu Val His Val Glu Glu Pro His Thr  
20 25 30

<210> 54  
<211> 12  
<212> PRT  
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<220>  
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<400> 54  
Gly Pro Gln Gly Leu Ala Gly Gln Arg Gly Ile Val  
1 5 10

<210> 55  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
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<400> 55  
Gly Gly Ser Gly Gln Arg Gly Arg Lys Ala Leu Glu  
1 5 10

<210> 56  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 56  
Ser Leu Ser Ala Leu Leu Ser Ser Asp Ile Phe Asn  
1 5 10

<210> 57

<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 57  
Ser Leu Pro Arg Phe Lys Ile Ile Gly Gly Phe Asn  
1 5 10

<210> 58  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 58  
Ser Leu Leu Gly Ile Ala Val Pro Gly Asn Phe Asn  
1 5 10

<210> 59  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 59  
Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro  
1 5 10

<210> 60  
<211> 19  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 60  
Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val Glu Glu Asn  
1 5 10 15  
  
Pro Gly Pro

<210> 61  
<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 61

Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro  
1 5 10 15

Gly Pro

<210> 62

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 62

Gln Cys Thr Asn Tyr Ala Leu Leu Lys Leu Ala Gly Asp Val Glu Ser  
1 5 10 15

Asn Pro Gly Pro  
20

<210> 63

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 63

Val Lys Gln Thr Leu Asn Phe Asp Leu Leu Lys Leu Ala Gly Asp Val  
1 5 10 15

Glu Ser Asn Pro Gly Pro  
20

<210> 64

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 64

Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val  
1 5 10 15

10 20

Glu Glu Asn Pro Gly Pro  
20

<210> 65  
<211> 21  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 65  
Gly Ser Gly Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu  
1 5 10 15

Glu Asn Pro Gly Pro  
20

<210> 66  
<211> 23  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 66  
Gly Ser Gly Gln Cys Thr Asn Tyr Ala Leu Leu Lys Leu Ala Gly Asp  
1 5 10 15

Val Glu Ser Asn Pro Gly Pro  
20

<210> 67  
<211> 25  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Synthetic peptide

<400> 67  
Gly Ser Gly Val Lys Gln Thr Leu Asn Phe Asp Leu Leu Lys Leu Ala  
1 5 10 15

Gly Asp Val Glu Ser Asn Pro Gly Pro  
20 25

<210> 68  
<211> 20  
<212> PRT

<213> Homo sapiens

<400> 68

Met	Ser	Arg	Ser	Val	Ala	Leu	Ala	Val	Leu	Ala	Leu	Leu	Ser	Leu	Ser
1															
														15	

Gly	Leu	Glu	Ala												
															20

<210> 69

<211> 225

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic polypeptide

<400> 69

Met	Ser	Arg	Ser	Val	Ala	Leu	Ala	Val	Leu	Ala	Leu	Leu	Ser	Leu	Ser
1															
														15	

Gly	Leu	Glu	Ala	Ala	Cys	Pro	Trp	Ala	Val	Ser	Gly	Ala	Arg	Ala	Ser
															30

Pro	Gly	Ser	Ala	Ala	Ser	Pro	Arg	Leu	Arg	Glu	Gly	Pro	Glu	Leu	Ser
															45

Pro	Asp	Asp	Pro	Ala	Gly	Leu	Leu	Asp	Leu	Arg	Gln	Gly	Met	Phe	Ala
															50
															55
															60

Gln	Leu	Val	Ala	Gln	Asn	Val	Leu	Leu	Ile	Asp	Gly	Pro	Leu	Ser	Trp
															80

Tyr	Ser	Asp	Pro	Gly	Leu	Ala	Gly	Val	Ser	Leu	Thr	Gly	Gly	Leu	Ser
															85
															90
															95

Tyr	Lys	Glu	Asp	Thr	Lys	Glu	Leu	Val	Val	Ala	Lys	Ala	Gly	Val	Tyr
															100
															105
															110

Tyr	Val	Phe	Phe	Gln	Leu	Glu	Leu	Arg	Arg	Val	Val	Ala	Gly	Glu	Gly
															115
															120
															125

Ser	Gly	Ser	Val	Ser	Leu	Ala	Leu	His	Leu	Gln	Pro	Leu	Arg	Ser	Ala
															130
															135
															140

Ala	Gly	Ala	Ala	Ala	Leu	Ala	Leu	Thr	Val	Asp	Leu	Pro	Pro	Ala	Ser
															145
															150
															155
															160

Ser	Glu	Ala	Arg	Asn	Ser	Ala	Phe	Gly	Phe	Gln	Gly	Arg	Leu	Leu	His
															165
															170
															175

Leu	Ser	Ala	Gly	Gln	Arg	Leu	Gly	Val	His	Leu	His	Thr	Glu	Ala	Arg
															180
															185
															190

Ala	Arg	His	Ala	Trp	Gln	Leu	Thr	Gln	Gly	Ala	Thr	Val	Leu	Gly	Leu
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

195

200

205

Phe Arg Val Thr Pro Glu Ile Pro Ala Gly Leu Pro Ser Pro Arg Ser  
 210 215 220

Glu  
 225

&lt;210&gt; 70

&lt;211&gt; 939

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Synthetic polypeptide

&lt;400&gt; 70

Met Ser Arg Ser Val Ala Leu Ala Val Leu Ala Leu Leu Ser Leu Ser  
 1 5 10 15

Gly Leu Glu Ala Val Tyr Leu Lys Thr Asn Val Phe Leu Gly Cys Gly  
 20 25 30

Ala Ser Gly Gly Gly Ser Gly Gly Gly Ser Met Ile Gln Lys  
 35 40 45

Thr Pro Gln Ile Gln Val Tyr Ser Arg His Pro Pro Glu Asn Gly Lys  
 50 55 60

Pro Asn Ile Leu Asn Cys Tyr Val Thr Gln Phe His Pro Pro His Ile  
 65 70 75 80

Glu Ile Gln Met Leu Lys Asn Gly Lys Lys Ile Pro Lys Val Glu Met  
 85 90 95

Ser Asp Met Ser Phe Ser Lys Asp Trp Ser Phe Tyr Ile Leu Ala His  
 100 105 110

Thr Glu Phe Thr Pro Thr Glu Thr Asp Thr Tyr Ala Cys Arg Val Lys  
 115 120 125

His Ala Ser Met Ala Glu Pro Lys Thr Val Tyr Trp Asp Arg Asp Met  
 130 135 140

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly  
 145 150 155 160

Gly Gly Gly Ser Gly Gly Gly Ser Ala Cys Pro Trp Ala Val Ser  
 165 170 175

Gly Ala Arg Ala Ser Pro Gly Ser Ala Ala Ser Pro Arg Leu Arg Glu  
 180 185 190

Gly Pro Glu Leu Ser Pro Asp Asp Pro Ala Gly Leu Leu Asp Leu Arg  
 195 200 205

Gln Gly Met Phe Ala Gln Leu Val Ala Gln Asn Val Leu Leu Ile Asp  
210 215 220

Gly Pro Leu Ser Trp Tyr Ser Asp Pro Gly Leu Ala Gly Val Ser Leu  
225 230 235 240

Thr Gly Gly Leu Ser Tyr Lys Glu Asp Thr Lys Glu Leu Val Val Ala  
245 250 255

Lys Ala Gly Val Tyr Tyr Val Phe Phe Gln Leu Glu Leu Arg Arg Val  
260 265 270

Val Ala Gly Glu Gly Ser Gly Ser Val Ser Leu Ala Leu His Leu Gln  
275 280 285

Pro Leu Arg Ser Ala Ala Gly Ala Ala Ala Leu Ala Leu Thr Val Asp  
290 295 300

Leu Pro Pro Ala Ser Ser Glu Ala Arg Asn Ser Ala Phe Gly Phe Gln  
305 310 315 320

Gly Arg Leu Leu His Leu Ser Ala Gly Gln Arg Leu Gly Val His Leu  
325 330 335

His Thr Glu Ala Arg Ala Arg His Ala Trp Gln Leu Thr Gln Gly Ala  
340 345 350

Thr Val Leu Gly Leu Phe Arg Val Thr Pro Glu Ile Pro Ala Gly Leu  
355 360 365

Pro Ser Pro Arg Ser Glu Ser Gly Ser Gly Ala Thr Asn Phe Ser Leu  
370 375 380

Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ser Arg  
385 390 395 400

Ser Val Ala Leu Ala Val Leu Ala Leu Leu Ser Leu Ser Gly Leu Glu  
405 410 415

Ala Gly Pro His Ser Leu Arg Tyr Phe Val Thr Ala Val Ser Arg Pro  
420 425 430

Gly Leu Gly Glu Pro Arg Phe Ile Ala Val Gly Tyr Val Asp Asp Thr  
435 440 445

Gln Phe Val Arg Phe Asp Ser Asp Ala Asp Asn Pro Arg Phe Glu Pro  
450 455 460

Arg Ala Pro Trp Met Glu Gln Glu Gly Pro Glu Tyr Trp Glu Glu Gln  
465 470 475 480

Thr Gln Arg Ala Lys Ser Asp Glu Gln Trp Phe Arg Val Ser Leu Arg  
485 490 495

Thr Ala Gln Arg Cys Tyr Asn Gln Ser Lys Gly Gly Ser His Thr Phe  
500 505 510

Gln Arg Met Phe Gly Cys Asp Val Gly Ser Asp Trp Arg Leu Leu Arg  
 515 520 525

Gly Tyr Gln Gln Phe Ala Tyr Asp Gly Arg Asp Tyr Ile Ala Leu Asn  
 530 535 540

Glu Asp Leu Lys Thr Trp Thr Ala Ala Asp Thr Ala Ala Leu Ile Thr  
 545 550 555 560

Arg Arg Lys Trp Glu Gln Ala Gly Asp Ala Glu Tyr Tyr Arg Ala Tyr  
 565 570 575

Leu Glu Gly Glu Cys Val Glu Trp Leu Arg Arg Tyr Leu Glu Leu Gly  
 580 585 590

Asn Glu Thr Leu Leu Arg Thr Asp Ser Pro Lys Ala His Val Thr Tyr  
 595 600 605

His Pro Arg Ser Gln Val Asp Val Thr Leu Arg Cys Trp Ala Leu Gly  
 610 615 620

Phe Tyr Pro Ala Asp Ile Thr Leu Thr Trp Gln Leu Asn Gly Glu Asp  
 625 630 635 640

Leu Thr Gln Asp Met Glu Leu Val Glu Thr Arg Pro Ala Gly Asp Gly  
 645 650 655

Thr Phe Gln Lys Trp Ala Ala Val Val Val Pro Leu Gly Lys Glu Gln  
 660 665 670

Asn Tyr Thr Cys His Val His His Lys Gly Leu Pro Glu Pro Leu Thr  
 675 680 685

Leu Arg Trp Ala Ala Ala Gly Gly Pro Arg Gly Pro Thr Ile Lys Pro  
 690 695 700

Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser  
 705 710 715 720

Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu  
 725 730 735

Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro  
 740 745 750

Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala  
 755 760 765

Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val  
 770 775 780

Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe  
 785 790 795 800

Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr  
 805 810 815

Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu

820 825 830

Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys  
835 840 845

Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn  
850 855 860

Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp  
865 870 875 880

Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys  
885 890 895

Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly  
900 905 910

Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys  
915 920 925

Gly Gly Ser His His His His His His His His  
930 935

<210> 71

<211> 939

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic polypeptide

<400> 71

Met Ser Arg Ser Val Ala Leu Ala Val Leu Ala Leu Leu Ser Leu Ser  
1 5 10 15

Gly Leu Glu Ala Val Tyr Leu Lys Thr Asn Val Phe Leu Gly Cys Gly  
20 25 30

Ala Ser Gly Gly Gly Ser Gly Gly Gly Ser Met Ile Gln Lys  
35 40 45

Thr Pro Gln Ile Gln Val Tyr Ser Arg His Pro Pro Glu Asn Gly Lys  
50 55 60

Pro Asn Ile Leu Asn Cys Tyr Val Thr Gln Phe His Pro Pro His Ile  
65 70 75 80

Glu Ile Gln Met Leu Lys Asn Gly Lys Lys Ile Pro Lys Val Glu Met  
85 90 95

Ser Asp Met Ser Phe Ser Lys Asp Trp Ser Phe Tyr Ile Leu Ala His  
100 105 110

Thr Glu Phe Thr Pro Thr Glu Thr Asp Thr Tyr Ala Cys Arg Val Lys  
 115 120 125

His Ala Ser Met Ala Glu Pro Lys Thr Val Tyr Trp Asp Arg Asp Met  
 130 135 140

Gly Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly 145 150 155 160

Gly Gly Gly Ser Gly Gly Ser Ala Cys Pro Trp Ala Val Ser  
 165 170 175

Gly Ala Arg Ala Ser Pro Gly Ser Ala Ala Ser Pro Arg Leu Arg Glu  
 180 185 190

Gly Pro Glu Leu Ser Pro Asp Asp Pro Ala Gly Leu Leu Asp Leu Arg  
 195 200 205

Gln Gly Met Phe Ala Cys Leu Val Ala Gln Asn Val Leu Leu Ile Asp  
 210 215 220

Gly Pro Leu Ser Trp Tyr Ser Asp Pro Gly Leu Ala Gly Val Ser Leu  
 225 230 235 240

Thr Gly Gly Leu Ser Tyr Lys Glu Asp Thr Lys Glu Leu Val Val Ala  
 245 250 255

Lys Ala Gly Val Tyr Val Phe Phe Gln Leu Glu Leu Arg Arg Val  
 260 265 270

Val Ala Gly Glu Gly Ser Gly Ser Val Ser Leu Ala Leu His Leu Gln  
 275 280 285

Pro Leu Arg Ser Ala Ala Gly Ala Ala Leu Ala Leu Thr Val Asp  
 290 295 300

Leu Pro Pro Ala Ser Ser Glu Ala Arg Asn Ser Ala Phe Gly Phe Gln  
 305 310 315 320

Gly Arg Leu Leu His Leu Ser Ala Gly Gln Arg Leu Gly Val His Leu  
 325 330 335

His Thr Glu Ala Arg Ala Arg His Ala Trp Gln Leu Thr Gln Gly Ala  
 340 345 350

Thr Val Leu Gly Leu Phe Arg Val Thr Pro Glu Ile Cys Ala Gly Leu  
 355 360 365

Pro Ser Pro Arg Ser Glu Ser Gly Ser Gly Ala Thr Asn Phe Ser Leu  
 370 375 380

Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ser Arg  
 385 390 395 400

Ser Val Ala Leu Ala Val Leu Ala Leu Ser Leu Ser Gly Leu Glu  
405 410 415

Ala Gly Pro His Ser Leu Arg Tyr Phe Val Thr Ala Val Ser Arg Pro  
420 425 430

Gly Ile Gly Glu Pro Arg Phe Ile Ala Val Gly Tyr Val Asp Asp Thr  
435 440 445

Gln Phe Val Arg Phe Asp Ser Asp Ala Asp Asn Pro Arg Phe Glu Pro  
450 455 460

Arg Ala Pro Trp Met Glu Gln Glu Gly Pro Glu Tyr Trp Glu Glu Gln  
465 470 475 480

Thr Gln Arg Ala Lys Ser Asp Glu Gln Trp Phe Arg Val Ser Leu Arg  
485 490 495

Thr Ala Gln Arg Cys Tyr Asn Gln Ser Lys Gly Gly Ser His Thr Phe  
500 505 510

Gln Arg Met Phe Gly Cys Asp Val Gly Ser Asp Trp Arg Ile Leu Arg  
515 520 525

Gly Tyr Gln Gln Phe Ala Tyr Asp Gly Arg Asp Tyr Ile Ala Leu Asn  
530 535 540

Glu Asp Leu Lys Thr Trp Thr Ala Ala Asp Thr Ala Ala Leu Ile Thr  
545 550 555 560

Arg Arg Lys Trp Glu Gln Ala Gly Asp Ala Glu Tyr Tyr Arg Ala Tyr  
565 570 575

Leu Glu Gly Glu Cys Val Glu Trp Leu Arg Arg Tyr Leu Glu Leu Gly  
580 585 590

Asn Glu Thr Leu Leu Arg Thr Asp Ser Pro Lys Ala His Val Thr Tyr  
595 600 605

His Pro Arg Ser Gln Val Asp Val Thr Leu Arg Cys Trp Ala Leu Gly  
610 615 620

Phe Tyr Pro Ala Asp Ile Thr Leu Thr Trp Gln Leu Asn Gly Glu Asp  
625 630 635 640

Leu Thr Gln Asp Met Glu Leu Val Glu Thr Arg Pro Ala Gly Asp Gly  
645 650 655

Thr Phe Gln Lys Trp Ala Ala Val Val Pro Leu Gly Lys Glu Gln  
660 665 670

Asn Tyr Thr Cys His Val His His Lys Gly Leu Pro Glu Pro Leu Thr  
675 680 685

Leu Arg Trp Ala Ala Ala Gly Gly Pro Arg Gly Pro Thr Ile Lys Pro  
690 695 700

Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser  
 705 710 715 720

Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu  
 725 730 735

Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro  
 740 745 750

Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala  
 755 760 765

Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val  
 770 775 780

Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe  
 785 790 795 800

Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr  
 805 810 815

Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu  
 820 825 830

Pro Pro Pro Glu Glu Glu Met Thr Lys Gln Val Thr Leu Thr Cys  
 835 840 845

Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn  
 850 855 860

Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp  
 865 870 875 880

Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys  
 885 890 895

Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly  
 900 905 910

Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys  
 915 920 925

Gly Gly Ser His His His His His His His His  
 930 935

<210> 72

<211> 225

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic polypeptide

<400> 72

Met Ser Arg Ser Val Ala Leu Ala Val Leu Ala Leu Leu Ser Leu Ser  
 1 5 10 15

Gly Leu Glu Ala Ala Cys Pro Trp Ala Val Ser Gly Ala Arg Ala Ser  
 20 25 30

Pro Gly Ser Ala Ala Ser Pro Arg Leu Arg Glu Gly Pro Glu Leu Ser  
 35 40 45

Pro Asp Asp Pro Ala Gly Leu Leu Asp Leu Arg Gln Gly Met Phe Ala  
 50 55 60

Cys Leu Val Ala Gln Asn Val Leu Leu Ile Asp Gly Pro Leu Ser Trp  
 65 70 75 80

Tyr Ser Asp Pro Gly Leu Ala Gly Val Ser Leu Thr Gly Gly Leu Ser  
 85 90 95

Tyr Lys Glu Asp Thr Lys Glu Leu Val Val Ala Lys Ala Gly Val Tyr  
 100 105 110

Tyr Val Phe Phe Gln Leu Glu Leu Arg Arg Val Val Ala Gly Glu Gly  
 115 120 125

Ser Gly Ser Val Ser Leu Ala Leu His Leu Gln Pro Leu Arg Ser Ala  
 130 135 140

Ala Gly Ala Ala Ala Leu Ala Leu Thr Val Asp Leu Pro Pro Ala Ser  
 145 150 155 160

Ser Glu Ala Arg Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His  
 165 170 175

Leu Ser Ala Gly Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg  
 180 185 190

Ala Arg His Ala Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu  
 195 200 205

Phe Arg Val Thr Pro Glu Ile Cys Ala Gly Leu Pro Ser Pro Arg Ser  
 210 215 220

Glu  
 225

<210> 73

<211> 939

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic polypeptide

<400> 73

Met Ser Arg Ser Val Ala Leu Ala Val Leu Ala Leu Leu Ser Leu Ser  
 1 5 10 15

Gly Leu Glu Ala Val Tyr Leu Lys Thr Asn Val Phe Leu Gly Cys Gly  
 20 25 30

Ala Ser Gly Gly Gly Ser Gly Gly Gly Ser Met Ile Gln Lys  
 35 40 45

Thr Pro Gln Ile Gln Val Tyr Ser Arg His Pro Pro Glu Asn Gly Lys  
 50 55 60

Pro Asn Ile Leu Asn Cys Tyr Val Thr Gln Phe His Pro Pro His Ile  
 65 70 75 80

Glu Ile Gln Met Leu Lys Asn Gly Lys Lys Ile Pro Lys Val Glu Met  
 85 90 95

Ser Asp Met Ser Phe Ser Lys Asp Trp Ser Phe Tyr Ile Leu Ala His  
 100 105 110

Thr Glu Phe Thr Pro Thr Glu Thr Asp Thr Tyr Ala Cys Arg Val Lys  
 115 120 125

His Ala Ser Met Ala Glu Pro Lys Thr Val Tyr Trp Asp Arg Asp Met  
 130 135 140

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly  
 145 150 155 160

Gly Gly Gly Ser Gly Gly Ser Ala Cys Pro Trp Ala Val Ser  
 165 170 175

Gly Ala Arg Ala Ser Pro Gly Ser Ala Ala Ser Pro Arg Leu Arg Glu  
 180 185 190

Gly Pro Glu Leu Ser Pro Asp Asp Pro Ala Gly Leu Leu Asp Leu Arg  
 195 200 205

Gln Gly Met Phe Ala Cys Leu Val Ala Gln Asn Val Leu Leu Ile Asp  
 210 215 220

Gly Pro Leu Ser Trp Tyr Ser Asp Pro Gly Leu Ala Gly Val Ser Leu  
 225 230 235 240

Thr Gly Gly Leu Ser Tyr Lys Glu Asp Thr Lys Glu Leu Val Val Ala  
 245 250 255

Lys Ala Gly Val Tyr Tyr Val Phe Phe Gln Leu Glu Leu Arg Arg Val  
 260 265 270

Val Ala Gly Glu Gly Ser Gly Ser Val Ser Leu Ala Leu His Leu Gln  
 275 280 285

Pro Leu Arg Ser Ala Ala Gly Ala Ala Ala Leu Leu Thr Val Asp  
 290 295 300

Leu Pro Pro Ala Ser Ser Glu Ala Arg Asn Ser Ala Phe Gly Phe Gln  
 305 310 315 320  
  
 Gly Arg Leu Leu His Leu Ser Ala Gly Gln Arg Leu Gly Val His Leu  
 325 330 335  
  
 His Thr Glu Ala Arg Ala Arg His Ala Trp Gln Leu Thr Gln Gly Ala  
 340 345 350  
  
 Thr Val Leu Gly Leu Phe Arg Val Thr Cys Glu Ile Pro Ala Gly Leu  
 355 360 365  
  
 Pro Ser Pro Arg Ser Glu Ser Gly Ser Gly Ala Thr Asn Phe Ser Leu  
 370 375 380  
  
 Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ser Arg  
 385 390 395 400  
  
 Ser Val Ala Leu Ala Val Leu Ala Leu Ser Leu Ser Gly Leu Glu  
 405 410 415  
  
 Ala Gly Pro His Ser Leu Arg Tyr Phe Val Thr Ala Val Ser Arg Pro  
 420 425 430  
  
 Gly Leu Gly Glu Pro Arg Phe Ile Ala Val Gly Tyr Val Asp Asp Thr  
 435 440 445  
  
 Gln Phe Val Arg Phe Asp Ser Asp Ala Asp Asn Pro Arg Phe Glu Pro  
 450 455 460  
  
 Arg Ala Pro Trp Met Glu Gln Glu Gly Pro Glu Tyr Trp Glu Glu Gln  
 465 470 475 480  
  
 Thr Gln Arg Ala Lys Ser Asp Glu Gln Trp Phe Arg Val Ser Leu Arg  
 485 490 495  
  
 Thr Ala Gln Arg Cys Tyr Asn Gln Ser Lys Gly Gly Ser His Thr Phe  
 500 505 510  
  
 Gln Arg Met Phe Gly Cys Asp Val Gly Ser Asp Trp Arg Leu Leu Arg  
 515 520 525  
  
 Gly Tyr Gln Gln Phe Ala Tyr Asp Gly Arg Asp Tyr Ile Ala Leu Asn  
 530 535 540  
  
 Glu Asp Leu Lys Thr Trp Thr Ala Ala Asp Thr Ala Ala Leu Ile Thr  
 545 550 555 560  
  
 Arg Arg Lys Trp Glu Gln Ala Gly Asp Ala Glu Tyr Tyr Arg Ala Tyr  
 565 570 575  
  
 Leu Glu Gly Glu Cys Val Glu Trp Leu Arg Arg Tyr Leu Glu Leu Gly  
 580 585 590  
  
 Asn Glu Thr Leu Leu Arg Thr Asp Ser Pro Lys Ala His Val Thr Tyr  
 595 600 605

His Pro Arg Ser Gln Val Ser Val Thr Leu Arg Gln Tyr Ala Thr Glu

Asp Phe Arg Ser Gln Val Asp Val Thr Leu Arg Cys Tyr Ala Leu Gly

610 615 620

Phe Tyr Pro Ala Asp Ile Thr Leu Thr Trp Gln Leu Asn Gly Glu Asp  
625 630 635 640

Leu Thr Gln Asp Met Glu Leu Val Glu Thr Arg Pro Ala Gly Asp Gly  
645 650 655

Thr Phe Gln Lys Trp Ala Ala Val Val Val Pro Leu Gly Lys Glu Gln  
660 665 670

Asn Tyr Thr Cys His Val His Lys Gly Leu Pro Glu Pro Leu Thr  
675 680 685

Leu Arg Trp Ala Ala Ala Gly Gly Pro Arg Gly Pro Thr Ile Lys Pro  
690 695 700

Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser  
705 710 715 720

Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu  
725 730 735

Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro  
740 745 750

Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala  
755 760 765

Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val  
770 775 780

Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe  
785 790 795 800

Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr  
805 810 815

Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu  
820 825 830

Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys  
835 840 845

Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn  
850 855 860

Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp  
865 870 875 880

Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys  
885 890 895

Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly  
900 905 910

Ter. Val. Ser. Thr. Glu. Asn. Tyr. Ser. Cys. Ser. Val. Val. His. Glu. Gly.

Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys  
 915 920 925

Gly Gly Ser His His His His His His His His  
 930 935

<210> 74

<211> 225

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic polypeptide

<400> 74

Met Ser Arg Ser Val Ala Leu Ala Val Leu Ala Leu Leu Ser Leu Ser  
 1 5 10 15

Gly Leu Glu Ala Ala Cys Pro Trp Ala Val Ser Gly Ala Arg Ala Ser  
 20 25 30

Pro Gly Ser Ala Ala Ser Pro Arg Leu Arg Glu Gly Pro Glu Leu Ser  
 35 40 45

Pro Asp Asp Pro Ala Gly Leu Leu Asp Leu Arg Gln Gly Met Phe Ala  
 50 55 60

Cys Leu Val Ala Gln Asn Val Leu Leu Ile Asp Gly Pro Leu Ser Trp  
 65 70 75 80

Tyr Ser Asp Pro Gly Leu Ala Gly Val Ser Leu Thr Gly Gly Leu Ser  
 85 90 95

Tyr Lys Glu Asp Thr Lys Glu Leu Val Val Ala Lys Ala Gly Val Tyr  
 100 105 110

Tyr Val Phe Phe Gln Leu Glu Leu Arg Arg Val Val Ala Gly Glu Gly  
 115 120 125

Ser Gly Ser Val Ser Leu Ala Leu His Leu Gln Pro Leu Arg Ser Ala  
 130 135 140

Ala Gly Ala Ala Ala Leu Ala Leu Thr Val Asp Leu Pro Pro Ala Ser  
 145 150 155 160

Ser Glu Ala Arg Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His  
 165 170 175

Leu Ser Ala Gly Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg  
 180 185 190

Ala Arg His Ala Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu  
 195 200 205

Phe Arg Val Thr Cys Glu Ile Pro Ala Gly Leu Pro Ser Pro Arg Ser  
 210 215 220

Glu  
225

<210> 75

<211> 939

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic polypeptide

<400> 75

Met Ser Arg Ser Val Ala Leu Ala Val Leu Ala Leu Leu Ser Leu Ser  
1 5 10 15

Gly Leu Glu Ala Val Tyr Leu Lys Thr Asn Val Phe Leu Gly Cys Gly  
20 25 30

Ala Ser Gly Gly Gly Ser Gly Gly Gly Ser Met Ile Gln Lys  
35 40 45

Thr Pro Gln Ile Gln Val Tyr Ser Arg His Pro Pro Glu Asn Gly Lys  
50 55 60

Pro Asn Ile Leu Asn Cys Tyr Val Thr Gln Phe His Pro Pro His Ile  
65 70 75 80

Glu Ile Gln Met Leu Lys Asn Gly Lys Lys Ile Pro Lys Val Glu Met  
85 90 95

Ser Asp Met Ser Phe Ser Lys Asp Trp Ser Phe Tyr Ile Leu Ala His  
100 105 110

Thr Glu Phe Thr Pro Thr Glu Thr Asp Thr Tyr Ala Cys Arg Val Lys  
115 120 125

His Ala Ser Met Ala Glu Pro Lys Thr Val Tyr Trp Asp Arg Asp Met  
130 135 140

Gly Gly Gly Ser Gly Gly Ser Gly Gly Gly Ser Gly  
145 150 155 160

Gly Gly Gly Ser Gly Gly Ser Ala Cys Pro Trp Ala Val Ser  
165 170 175

Gly Ala Arg Ala Ser Pro Gly Ser Ala Ala Ser Pro Arg Leu Arg Glu  
180 185 190

Gly Pro Glu Leu Ser Pro Asp Asp Pro Ala Gly Leu Leu Asp Leu Arg  
195 200 205

Cys Gly Met Phe Ala Gln Leu Val Ala Gln Asn Val Leu Leu Ile Asp  
210 215 220

Gly Pro Leu Ser Trp Tyr Ser Asp Pro Gly Cys Ala Gly Val Ser Leu  
 225 230 235 240

Thr Gly Gly Leu Ser Tyr Lys Glu Asp Thr Lys Glu Leu Val Val Ala  
 245 250 255

Lys Ala Gly Val Tyr Tyr Val Phe Phe Gln Leu Glu Leu Arg Arg Val  
 260 265 270

Val Ala Gly Glu Gly Ser Gly Ser Val Ser Leu Ala Leu His Leu Gln  
 275 280 285

Pro Leu Arg Ser Ala Ala Gly Ala Ala Ala Leu Ala Leu Thr Val Asp  
 290 295 300

Leu Pro Pro Ala Ser Ser Glu Ala Arg Asn Ser Ala Phe Gly Phe Gln  
 305 310 315 320

Gly Arg Leu Leu His Leu Ser Ala Gly Gln Arg Leu Gly Val His Leu  
 325 330 335

His Thr Glu Ala Arg Ala Arg His Ala Trp Gln Leu Thr Gln Gly Ala  
 340 345 350

Thr Val Leu Gly Leu Phe Arg Val Thr Pro Glu Ile Cys Ala Gly Leu  
 355 360 365

Pro Ser Pro Arg Ser Glu Ser Gly Ser Gly Ala Thr Asn Phe Ser Leu  
 370 375 380

Leu Lys Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Ser Arg  
 385 390 395 400

Ser Val Ala Leu Ala Val Leu Ala Leu Leu Ser Leu Ser Gly Leu Glu  
 405 410 415

Ala Gly Pro His Ser Leu Arg Tyr Phe Val Thr Ala Val Ser Arg Pro  
 420 425 430

Gly Leu Gly Glu Pro Arg Phe Ile Ala Val Gly Tyr Val Asp Asp Thr  
 435 440 445

Gln Phe Val Arg Phe Asp Ser Asp Ala Asp Asn Pro Arg Phe Glu Pro  
 450 455 460

Arg Ala Pro Trp Met Glu Gln Glu Gly Pro Glu Tyr Trp Glu Glu Gln  
 465 470 475 480

Thr Gln Arg Ala Lys Ser Asp Glu Gln Trp Phe Arg Val Ser Leu Arg  
 485 490 495

Thr Ala Gln Arg Cys Tyr Asn Gln Ser Lys Gly Gly Ser His Thr Phe  
 500 505 510

Gln Arg Met Phe Gly Cys Asp Val Gly Ser Asp Trp Arg Leu Leu Arg  
 515 520 525

Gly Tyr Gln Gln Phe Ala Tyr Asp Gly Arg Asp Tyr Ile Ala Leu Asn  
 530 535 540

Glu Asp Leu Lys Thr Trp Thr Ala Ala Asp Thr Ala Ala Leu Ile Thr  
 545 550 555 560

Arg Arg Lys Trp Glu Gln Ala Gly Asp Ala Glu Tyr Tyr Arg Ala Tyr  
 565 570 575

Leu Glu Gly Glu Cys Val Glu Trp Leu Arg Arg Tyr Leu Glu Leu Gly  
 580 585 590

Asn Glu Thr Leu Leu Arg Thr Asp Ser Pro Lys Ala His Val Thr Tyr  
 595 600 605

His Pro Arg Ser Gln Val Asp Val Thr Leu Arg Cys Trp Ala Leu Gly  
 610 615 620

Phe Tyr Pro Ala Asp Ile Thr Leu Thr Trp Gln Leu Asn Gly Glu Asp  
 625 630 635 640

Leu Thr Gln Asp Met Glu Leu Val Glu Thr Arg Pro Ala Gly Asp Gly  
 645 650 655

Thr Phe Gln Lys Trp Ala Ala Val Val Val Pro Leu Gly Lys Glu Gln  
 660 665 670

Asn Tyr Thr Cys His Val His Lys Gly Leu Pro Glu Pro Leu Thr  
 675 680 685

Leu Arg Trp Ala Ala Ala Gly Gly Pro Arg Gly Pro Thr Ile Lys Pro  
 690 695 700

Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser  
 705 710 715 720

Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu  
 725 730 735

Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro  
 740 745 750

Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala  
 755 760 765

Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val  
 770 775 780

Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe  
 785 790 795 800

Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr  
 805 810 815

Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu  
 820 825 830

Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys

835 840 845

Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn  
850 855 860

Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp  
865 870 875 880

Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys  
885 890 895

Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly  
900 905 910

Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys  
915 920 925

Gly Gly Ser His His His His His His His His  
930 935

<210> 76

<211> 225

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic polypeptide

<400> 76

Met Ser Arg Ser Val Ala Leu Ala Val Leu Ala Leu Leu Ser Leu Ser  
1 5 10 15

Gly Leu Glu Ala Ala Cys Pro Trp Ala Val Ser Gly Ala Arg Ala Ser  
20 25 30

Pro Gly Ser Ala Ala Ser Pro Arg Leu Arg Glu Gly Pro Glu Leu Ser  
35 40 45

Pro Asp Asp Pro Ala Gly Leu Leu Asp Leu Arg Cys Gly Met Phe Ala  
50 55 60

Gln Leu Val Ala Gln Asn Val Leu Leu Ile Asp Gly Pro Leu Ser Trp  
65 70 75 80

Tyr Ser Asp Pro Gly Cys Ala Gly Val Ser Leu Thr Gly Gly Leu Ser  
85 90 95

Tyr Lys Glu Asp Thr Lys Glu Leu Val Val Ala Lys Ala Gly Val Tyr  
100 105 110

Tyr Val Phe Phe Gln Leu Glu Leu Arg Arg Val Val Ala Gly Glu Gly  
115 120 125

Ser Gly Ser Val Ser Leu Ala Leu His Leu Gln Pro Leu Arg Ser Ala  
 130 135 140

Ala Gly Ala Ala Ala Leu Ala Leu Thr Val Asp Leu Pro Pro Ala Ser  
 145 150 155 160

Ser Glu Ala Arg Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His  
 165 170 175

Leu Ser Ala Gly Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg  
 180 185 190

Ala Arg His Ala Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu  
 195 200 205

Phe Arg Val Thr Pro Glu Ile Cys Ala Gly Leu Pro Ser Pro Arg Ser  
 210 215 220

Glu  
 225

<210> 77

<211> 1285

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic polypeptide

<400> 77

Met Ser Arg Ser Val Ala Leu Ala Val Leu Ala Leu Leu Ser Leu Ser  
 1 5 10 15

Gly Leu Glu Ala Val Tyr Leu Lys Thr Asn Val Phe Leu Gly Cys Gly  
 20 25 30

Ala Ser Gly Gly Gly Ser Gly Gly Gly Ser Met Ile Gln Lys  
 35 40 45

Thr Pro Gln Ile Gln Val Tyr Ser Arg His Pro Pro Glu Asn Gly Lys  
 50 55 60

Pro Asn Ile Leu Asn Cys Tyr Val Thr Gln Phe His Pro Pro His Ile  
 65 70 75 80

Glu Ile Gln Met Leu Lys Asn Gly Lys Lys Ile Pro Lys Val Glu Met  
 85 90 95

Ser Asp Met Ser Phe Ser Lys Asp Trp Ser Phe Tyr Ile Leu Ala His  
 100 105 110

Thr Glu Phe Thr Pro Thr Glu Thr Asp Thr Tyr Ala Cys Arg Val Lys  
 115 120 125

His Ala Ser Met Ala Glu Pro Lys Thr Val Tyr Trp Asp Arg Asp Met

130

135

140

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly  
145 150 155 160

Gly Gly Gly Ser Gly Gly Gly Ser Asp Pro Ala Gly Leu Leu Asp  
165 170 175

Leu Arg Gln Gly Met Phe Ala Gln Leu Val Ala Gln Asn Val Leu Leu  
180 185 190

Ile Asp Gly Pro Leu Ser Trp Tyr Ser Asp Pro Gly Leu Ala Gly Val  
195 200 205

Ser Leu Thr Gly Gly Leu Ser Tyr Lys Glu Asp Thr Lys Glu Leu Val  
210 215 220

Val Ala Lys Ala Gly Val Tyr Tyr Val Phe Phe Gln Leu Glu Leu Arg  
225 230 235 240

Arg Val Val Ala Gly Glu Gly Ser Gly Ser Val Ser Leu Ala Leu His  
245 250 255

Leu Gln Pro Leu Arg Ser Ala Ala Gly Ala Ala Ala Leu Ala Leu Thr  
260 265 270

Val Asp Leu Pro Pro Ala Ser Ser Glu Ala Arg Asn Ser Ala Phe Gly  
275 280 285

Phe Gln Gly Arg Leu Leu His Leu Ser Ala Gly Gln Arg Leu Gly Val  
290 295 300

His Leu His Thr Glu Ala Arg Ala Arg His Ala Trp Gln Leu Thr Gln  
305 310 315 320

Gly Ala Thr Val Leu Gly Leu Phe Arg Val Thr Pro Glu Ile Pro Ala  
325 330 335

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly  
340 345 350

Gly Gly Gly Ser Gly Gly Gly Ser Asp Pro Ala Gly Leu Leu Asp  
355 360 365

Leu Arg Gln Gly Met Phe Ala Gln Leu Val Ala Gln Asn Val Leu Leu  
370 375 380

Ile Asp Gly Pro Leu Ser Trp Tyr Ser Asp Pro Gly Leu Ala Gly Val  
385 390 395 400

Ser Leu Thr Gly Gly Leu Ser Tyr Lys Glu Asp Thr Lys Glu Leu Val  
405 410 415

Val Ala Lys Ala Gly Val Tyr Tyr Val Phe Phe Gln Leu Glu Leu Arg  
420 425 430

Arg Val Val Ala Gly Glu Gly Ser Gly Ser Val Ser Leu Ala Leu His

435

440

445

Leu Gln Pro Leu Arg Ser Ala Ala Gly Ala Ala Ala Leu Ala Leu Thr  
 450 455 460

Val Asp Leu Pro Pro Ala Ser Ser Glu Ala Arg Asn Ser Ala Phe Gly  
 465 470 475 480

Phe Gln Gly Arg Leu Leu His Leu Ser Ala Gly Gln Arg Leu Gly Val  
 485 490 495

His Leu His Thr Glu Ala Arg Ala Arg His Ala Trp Gln Leu Thr Gln  
 500 505 510

Gly Ala Thr Val Leu Gly Leu Phe Arg Val Thr Pro Glu Ile Pro Ala  
 515 520 525

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly  
 530 535 540

Gly Gly Gly Ser Gly Gly Ser Asp Pro Ala Gly Leu Leu Asp  
 545 550 555 560

Leu Arg Gln Gly Met Phe Ala Gln Leu Val Ala Gln Asn Val Leu Leu  
 565 570 575

Ile Asp Gly Pro Leu Ser Trp Tyr Ser Asp Pro Gly Leu Ala Gly Val  
 580 585 590

Ser Leu Thr Gly Gly Leu Ser Tyr Lys Glu Asp Thr Lys Glu Leu Val  
 595 600 605

Val Ala Lys Ala Gly Val Tyr Tyr Val Phe Phe Gln Leu Glu Leu Arg  
 610 615 620

Arg Val Val Ala Gly Glu Gly Ser Gly Ser Val Ser Leu Ala Leu His  
 625 630 635 640

Leu Gln Pro Leu Arg Ser Ala Ala Gly Ala Ala Leu Ala Leu Thr  
 645 650 655

Val Asp Leu Pro Pro Ala Ser Ser Glu Ala Arg Asn Ser Ala Phe Gly  
 660 665 670

Phe Gln Gly Arg Leu Leu His Leu Ser Ala Gly Gln Arg Leu Gly Val  
 675 680 685

His Leu His Thr Glu Ala Arg Ala Arg His Ala Trp Gln Leu Thr Gln  
 690 695 700

Gly Ala Thr Val Leu Gly Leu Phe Arg Val Thr Pro Glu Ile Pro Ala  
 705 710 715 720

Ser Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp  
 725 730 735

Val Glu Glu Asn Pro Gly Pro Met Ser Arg Ser Val Ala Leu Ala Val  
 740 745 750

Leu Ala Leu Leu Ser Leu Ser Gly Leu Glu Ala Gly Pro His Ser Leu  
 755 760 765

Arg Tyr Phe Val Thr Ala Val Ser Arg Pro Gly Ile Gly Glu Pro Arg  
 770 775 780

Phe Ile Ala Val Gly Tyr Val Asp Asp Thr Gln Phe Val Arg Phe Asp  
 785 790 795 800

Ser Asp Ala Asp Asn Pro Arg Phe Glu Pro Arg Ala Pro Trp Met Glu  
 805 810 815

Gln Glu Gly Pro Glu Tyr Trp Glu Glu Gln Thr Gln Arg Ala Lys Ser  
 820 825 830

Asp Glu Gln Trp Phe Arg Val Ser Leu Arg Thr Ala Gln Arg Cys Tyr  
 835 840 845

Asn Gln Ser Lys Gly Gly Ser His Thr Phe Gln Arg Met Phe Gly Cys  
 850 855 860

Asp Val Gly Ser Asp Trp Arg Leu Leu Arg Gly Tyr Gln Gln Phe Ala  
 865 870 875 880

Tyr Asp Gly Arg Asp Tyr Ile Ala Leu Asn Glu Asp Leu Lys Thr Trp  
 885 890 895

Thr Ala Ala Asp Thr Ala Ala Leu Ile Thr Arg Arg Lys Trp Glu Gln  
 900 905 910

Ala Gly Asp Ala Glu Tyr Tyr Arg Ala Tyr Leu Glu Gly Glu Cys Val  
 915 920 925

Glu Trp Leu Arg Arg Tyr Leu Glu Leu Gly Asn Glu Thr Leu Leu Arg  
 930 935 940

Thr Asp Ser Pro Lys Ala His Val Thr Tyr His Pro Arg Ser Gln Val  
 945 950 955 960

Asp Val Thr Leu Arg Cys Trp Ala Leu Gly Phe Tyr Pro Ala Asp Ile  
 965 970 975

Thr Leu Thr Trp Gln Leu Asn Gly Glu Asp Leu Thr Gln Asp Met Glu  
 980 985 990

Leu Val Glu Thr Arg Pro Ala Gly Asp Gly Thr Phe Gln Lys Trp Ala  
 995 1000 1005

Ala Val Val Val Pro Leu Gly Lys Glu Gln Asn Tyr Thr Cys His  
 1010 1015 1020

Val His His Lys Gly Leu Pro Glu Pro Leu Thr Leu Arg Trp Ala  
 1025 1030 1035

Ala Ala Gly Gly Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro  
1040 1045 1050

Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe  
1055 1060 1065

Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser  
1070 1075 1080

Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro  
1085 1090 1095

Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr  
1100 1105 1110

Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg  
1115 1120 1125

Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly  
1130 1135 1140

Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro  
1145 1150 1155

Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro  
1160 1165 1170

Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys  
1175 1180 1185

Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp  
1190 1195 1200

Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr  
1205 1210 1215

Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met  
1220 1225 1230

Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn  
1235 1240 1245

Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His  
1250 1255 1260

Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys Gly Gly Ser His  
1265 1270 1275

His His His His His His  
1280 1285

<210> 78

<211> 119

<212> PRT

<213> Homo sapiens

<400> 78

Met Ser Arg Ser Val Ala Leu Ala Val Leu Ala Leu Leu Ser Leu Ser  
1 5 10 15

Gly Leu Glu Ala Ile Gln Arg Thr Pro Lys Ile Gln Val Tyr Ser Arg  
20 25 30

His Pro Ala Glu Asn Gly Lys Ser Asn Phe Leu Asn Cys Tyr Val Ser  
 35 40 45

Gly Phe His Pro Ser Asp Ile Glu Val Asp Leu Leu Lys Asn Gly Glu  
50 55 60

Arg Ile Glu Lys Val Glu His Ser Asp Leu Ser Phe Ser Lys Asp Trp  
65 70 75 80

Ser Phe Tyr Leu Leu Tyr Tyr Thr Glu Phe Thr Pro Thr Glu Lys Asp  
85 90 95

Glu Tyr Ala Cys Arg Val Asn His Val Thr Leu Ser Gln Pro Lys Ile  
100 105 110

Val Lys Trp Asp Arg Asp Met  
115

<210> 79

<211> 119

<212> PRT

<213> Pan troglodytes

<400> 79

Met Ser Asp

Gly Leu Glu Ala Ile C

Gly Leu Glu Ala Ile Gln Arg Thr Pro Lys Ile Gln Val Tyr Ser Arg  
20 25 30

His Pro Ala Glu Asn Gly Lys Ser Asn Phe Leu Asn Cys Tyr Val Ser  
35 40 45

Gly Phe His Pro Ser Asp Ile Glu Val Asp Leu Leu Lys Asn Gly Glu  
50 55 60

Arg Ile Glu Lys Val Glu His Ser Asp Leu Ser Phe Ser Lys Asp Trp  
65 70 75 80

Ser Phe Tyr Leu Leu Tyr Tyr Thr Glu Phe Thr Pro Thr Glu Lys Asp  
85 90 95

Glu Tyr Ala Cys Arg Val Asn His Val Thr Leu Ser Gln Pro Lys Ile  
100 105 110

Val Lys Trp Asp Arg Asp Met

115

&lt;210&gt; 80

&lt;211&gt; 119

&lt;212&gt; PRT

&lt;213&gt; Macaca mulatta

&lt;400&gt; 80

Met	Ser	Arg	Ser	Val	Ala	Leu	Ala	Val	Leu	Ala	Leu	Leu	Ser	Leu	Ser
1				5				10					15		

Gly	Leu	Glu	Ala	Ile	Gln	Arg	Thr	Pro	Lys	Ile	Gln	Val	Tyr	Ser	Arg
				20				25				30			

His	Pro	Pro	Glu	Asn	Gly	Lys	Pro	Asn	Phe	Leu	Asn	Cys	Tyr	Val	Ser
				35			40				45				

Gly	Phe	His	Pro	Ser	Asp	Ile	Glu	Val	Asp	Leu	Leu	Lys	Asn	Gly	Glu
				50			55				60				

Lys	Met	Gly	Lys	Val	Glu	His	Ser	Asp	Leu	Ser	Phe	Ser	Lys	Asp	Trp
65				70			75				80				

Ser	Phe	Tyr	Leu	Leu	Tyr	Tyr	Thr	Glu	Phe	Thr	Pro	Asn	Glu	Lys	Asp
				85			90				95				

Glu	Tyr	Ala	Cys	Arg	Val	Asn	His	Val	Thr	Leu	Ser	Gly	Pro	Arg	Thr
				100			105				110				

Val	Lys	Trp	Asp	Arg	Asp	Met									
		115													

&lt;210&gt; 81

&lt;211&gt; 118

&lt;212&gt; PRT

&lt;213&gt; Bos taurus

&lt;400&gt; 81

Met	Ala	Arg	Phe	Val	Ala	Leu	Val	Leu	Leu	Gly	Leu	Leu	Ser	Leu	Ser
1				5			10			15					

Gly	Leu	Asp	Ala	Ile	Gln	Arg	Pro	Pro	Lys	Ile	Gln	Val	Tyr	Ser	Arg
				20			25				30				

His	Pro	Pro	Glu	Asp	Gly	Lys	Pro	Asn	Tyr	Leu	Asn	Cys	Tyr	Val	Tyr
				35			40				45				

Gly	Phe	His	Pro	Pro	Gln	Ile	Glu	Ile	Asp	Leu	Leu	Lys	Asn	Gly	Glu
				50			55				60				

Lys	Ile	Lys	Ser	Glu	Gln	Ser	Asp	Leu	Ser	Phe	Ser	Lys	Asp	Trp	Ser
65				70			75			80					

Phe	Tyr	Leu	Leu	Ser	His	Ala	Glu	Phe	Thr	Pro	Asn	Ser	Lys	Asp	Gln
				85			90				95				

Tyr Ser Cys Arg Val Lys His Val Thr Leu Glu Gln Pro Arg Ile Val  
 100 105 110

Lys Trp Asp Arg Asp Leu  
 115

<210> 82

<211> 119

<212> PRT

<213> Mus musculus

<400> 82

Met Ala Arg Ser Val Thr Leu Val Phe Leu Val Leu Val Ser Leu Thr  
 1 5 10 15

Gly Leu Tyr Ala Ile Gln Lys Thr Pro Gln Ile Gln Val Tyr Ser Arg  
 20 25 30

His Pro Pro Glu Asn Gly Lys Pro Asn Ile Leu Asn Cys Tyr Val Thr  
 35 40 45

Gln Phe His Pro Pro His Ile Glu Ile Gln Met Leu Lys Asn Gly Lys  
 50 55 60

Lys Ile Pro Lys Val Glu Met Ser Asp Met Ser Phe Ser Lys Asp Trp  
 65 70 75 80

Ser Phe Tyr Ile Leu Ala His Thr Glu Phe Thr Pro Thr Glu Thr Asp  
 85 90 95

Thr Tyr Ala Cys Arg Val Lys His Ala Ser Met Ala Glu Pro Lys Thr  
 100 105 110

Val Tyr Trp Asp Arg Asp Met  
 115

<210> 83

<211> 227

<212> PRT

<213> Homo sapiens

<400> 83

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

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met  
 20 25 30

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His  
 35 40 45

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

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
 65 70 75 80

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly  
 85 90 95

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile  
 100 105 110

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

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

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

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
 165 170 175

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val  
 180 185 190

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
 195 200 205

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
 210 215 220

Pro Gly Lys  
 225

<210> 84

<211> 325

<212> PRT

<213> Homo sapiens

<400> 84

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser  
 1 5 10 15

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

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly  
 35 40 45

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu  
 50 55 60

Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr  
 65 70 75 80

Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr

85

90

95

Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro  
 100 105 110

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

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val  
 130 135 140

Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val  
 145 150 155 160

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser  
 165 170 175

Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu  
 180 185 190

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala  
 195 200 205

Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro  
 210 215 220

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln  
 225 230 235 240

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala  
 245 250 255

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr  
 260 265 270

Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu  
 275 280 285

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser  
 290 295 300

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser  
 305 310 315 320

Leu Ser Pro Gly Lys  
 325

<210> 85

<211> 246

<212> PRT

<213> Homo sapiens

<400> 85

His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Leu Lys Thr  
 1 5 10 15

Pro Leu Gly Asp Thr Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
 20 25 30

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 35 40 45

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
 50 55 60

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
 65 70 75 80

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
 85 90 95

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
 100 105 110

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
 115 120 125

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
 130 135 140

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn  
 145 150 155 160

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
 165 170 175

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
 180 185 190

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
 195 200 205

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
 210 215 220

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
 225 230 235 240

Ser Leu Ser Pro Gly Lys  
 245

<210> 86

<211> 383

<212> PRT

<213> Homo sapiens

<400> 86

Pro Thr Lys Ala Pro Asp Val Phe Pro Ile Ile Ser Gly Cys Arg His  
 1 5 10 15

Dna Tua Ben Ben Ser Dna Val Val Tua Ala Cys Tua Tla Thr Cys Thr

20 25 30

His Pro Thr Ser Val Thr Val Thr Trp Tyr Met Gly Thr Gln Ser Gln  
35 40 45

Pro Gln Arg Thr Phe Pro Glu Ile Gln Arg Arg Asp Ser Tyr Tyr Met  
50 55 60

Thr Ser Ser Gln Leu Ser Thr Pro Leu Gln Gln Trp Arg Gln Gly Glu  
65 70 75 80

Tyr Lys Cys Val Val Gln His Thr Ala Ser Lys Ser Lys Lys Glu Ile  
85 90 95

Phe Arg Trp Pro Glu Ser Pro Lys Ala Gln Ala Ser Ser Val Pro Thr  
100 105 110

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

Ala Thr Thr Arg Asn Thr Gly Arg Gly Glu Glu Lys Lys Lys Glu  
130 135 140

Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys  
145 150 155 160

Pro Ser His Thr Gln Pro Leu Gly Val Tyr Leu Leu Thr Pro Ala Val  
165 170 175

Gln Asp Leu Trp Leu Arg Asp Lys Ala Thr Phe Thr Cys Phe Val Val  
180 185 190

Gly Ser Asp Leu Lys Asp Ala His Leu Thr Trp Glu Val Ala Gly Lys  
195 200 205

Val Pro Thr Gly Gly Val Glu Glu Gly Leu Leu Glu Arg His Ser Asn  
210 215 220

Gly Ser Gln Ser Gln His Ser Arg Leu Thr Leu Pro Arg Ser Leu Trp  
225 230 235 240

Asn Ala Gly Thr Ser Val Thr Cys Thr Leu Asn His Pro Ser Leu Pro  
245 250 255

Pro Gln Arg Leu Met Ala Leu Arg Glu Pro Ala Ala Gln Ala Pro Val  
260 265 270

Lys Leu Ser Leu Asn Leu Leu Ala Ser Ser Asp Pro Pro Glu Ala Ala  
275 280 285

Ser Trp Leu Leu Cys Glu Val Ser Gly Phe Ser Pro Pro Asn Ile Leu  
290 295 300

Leu Met Trp Leu Glu Asp Gln Arg Glu Val Asn Thr Ser Gly Phe Ala  
305 310 315 320

Pro Ala Arg Pro Pro Pro Gln Pro Arg Ser Thr Thr Phe Trp Ala Trp

325	330	335
Ser Val Leu Arg Val Pro Ala Pro Pro Ser Pro Gln Pro Ala Thr Tyr		
340	345	350
Thr Cys Val Val Ser His Glu Asp Ser Arg Thr Leu Leu Asn Ala Ser		
355	360	365
Arg Ser Leu Glu Val Ser Tyr Val Thr Asp His Gly Pro Met Lys		
370	375	380
<210> 87		
<211> 276		
<212> PRT		
<213> Homo sapiens		
<400> 87		
Val Thr Ser Thr Leu Thr Ile Lys Glx Ser Asp Trp Leu Gly Glu Ser		
1	5	10
15		
Met Phe Thr Cys Arg Val Asp His Arg Gly Leu Thr Phe Gln Gln Asn		
20	25	30
Ala Ser Ser Met Cys Val Pro Asp Gln Asp Thr Ala Ile Arg Val Phe		
35	40	45
Ala Ile Pro Pro Ser Phe Ala Ser Ile Phe Leu Thr Lys Ser Thr Lys		
50	55	60
Leu Thr Cys Leu Val Thr Asp Leu Thr Thr Tyr Asx Ser Val Thr Ile		
65	70	75
80		
Ser Trp Thr Arg Glu Glu Asn Gly Ala Val Lys Thr His Thr Asn Ile		
85	90	95
Ser Glu Ser His Pro Asn Ala Thr Phe Ser Ala Val Gly Glu Ala Ser		
100	105	110
Ile Cys Glu Asp Asx Asp Trp Ser Gly Glu Arg Phe Thr Cys Thr Val		
115	120	125
Thr His Thr Asp Leu Pro Ser Pro Leu Lys Gln Thr Ile Ser Arg Pro		
130	135	140
Lys Gly Val Ala Leu His Arg Pro Asx Val Tyr Leu Leu Pro Pro Ala		
145	150	155
160		
Arg Glx Glx Leu Asn Leu Arg Glu Ser Ala Thr Ile Thr Cys Leu Val		
165	170	175
Thr Gly Phe Ser Pro Ala Asp Val Phe Val Glu Trp Met Gln Arg Gly		
180	185	190
Glu Pro Leu Ser Pro Gln Lys Tyr Val Thr Ser Ala Pro Met Pro Glu		
195	200	205

Pro Gln Ala Pro Gly Arg Tyr Phe Ala His Ser Ile Leu Thr Val Ser  
 210 215 220

Glu Glu Glu Trp Asn Thr Gly Gly Thr Tyr Thr Cys Val Val Ala His  
 225 230 235 240

Glu Ala Leu Pro Asn Arg Val Thr Glu Arg Thr Val Asp Lys Ser Thr  
 245 250 255

Gly Lys Pro Thr Leu Tyr Asn Val Ser Leu Val Met Ser Asp Thr Ala  
 260 265 270

Gly Thr Cys Tyr  
 275

<210> 88

<211> 353

<212> PRT

<213> Homo sapiens

<400> 88

Ala Ser Pro Thr Ser Pro Lys Val Phe Pro Leu Ser Leu Cys Ser Thr  
 1 5 10 15

Gln Pro Asp Gly Asn Val Val Ile Ala Cys Leu Val Gln Gly Phe Phe  
 20 25 30

Pro Gln Glu Pro Leu Ser Val Thr Trp Ser Glu Ser Gly Gln Gly Val  
 35 40 45

Thr Ala Arg Asn Phe Pro Pro Ser Gln Asp Ala Ser Gly Asp Leu Tyr  
 50 55 60

Thr Thr Ser Ser Gln Leu Thr Leu Pro Ala Thr Gln Cys Leu Ala Gly  
 65 70 75 80

Lys Ser Val Thr Cys His Val Lys His Tyr Thr Asn Pro Ser Gln Asp  
 85 90 95

Val Thr Val Pro Cys Pro Val Pro Ser Thr Pro Pro Thr Pro Ser Pro  
 100 105 110

Ser Thr Pro Pro Thr Pro Ser Pro Ser Cys Cys His Pro Arg Leu Ser  
 115 120 125

Leu His Arg Pro Ala Leu Glu Asp Leu Leu Leu Gly Ser Glu Ala Asn  
 130 135 140

Leu Thr Cys Thr Leu Thr Gly Leu Arg Asp Ala Ser Gly Val Thr Phe  
 145 150 155 160

Thr Trp Thr Pro Ser Ser Gly Lys Ser Ala Val Gln Gly Pro Pro Glu  
 165 170 175

Arg Asp Leu Cys Gly Cys Tyr Ser Val Ser Ser Val Leu Pro Gly Cys

180	185	190
Ala Glu Pro Trp Asn His Gly Lys Thr Phe Thr Cys Thr Ala Ala Tyr		
195	200	205
Pro Glu Ser Lys Thr Pro Leu Thr Ala Thr Leu Ser Lys Ser Gly Asn		
210	215	220
Thr Phe Arg Pro Glu Val His Leu Leu Pro Pro Pro Ser Glu Glu Leu		
225	230	235
Ala Leu Asn Glu Leu Val Thr Leu Thr Cys Leu Ala Arg Gly Phe Ser		
245	250	255
Pro Lys Asp Val Leu Val Arg Trp Leu Gln Gly Ser Gln Glu Leu Pro		
260	265	270
Arg Glu Lys Tyr Leu Thr Trp Ala Ser Arg Gln Glu Pro Ser Gln Gly		
275	280	285
Thr Thr Thr Phe Ala Val Thr Ser Ile Leu Arg Val Ala Ala Glu Asp		
290	295	300
Trp Lys Lys Gly Asp Thr Phe Ser Cys Met Val Gly His Glu Ala Leu		
305	310	315
Pro Leu Ala Phe Thr Gln Lys Thr Ile Asp Arg Leu Ala Gly Lys Pro		
325	330	335
Thr His Val Asn Val Ser Val Val Met Ala Glu Val Asp Gly Thr Cys		
340	345	350
Tyr		
<210> 89		
<211> 222		
<212> PRT		
<213> Homo sapiens		
<400> 89		
Ala Asp Pro Cys Asp Ser Asn Pro Arg Gly Val Ser Ala Tyr Leu Ser		
1	5	10
15		
Arg Pro Ser Pro Phe Asp Leu Phe Ile Arg Lys Ser Pro Thr Ile Thr		
20	25	30
Cys Leu Val Val Asp Leu Ala Pro Ser Lys Gly Thr Val Asn Leu Thr		
35	40	45
Trp Ser Arg Ala Ser Gly Lys Pro Val Asn His Ser Thr Arg Lys Glu		
50	55	60
Glu Lys Gln Arg Asn Gly Thr Leu Thr Val Thr Ser Thr Leu Pro Val		
65	70	75
80		

Gly Thr Arg Asp Trp Ile Glu Gly Glu Thr Tyr Gln Cys Arg Val Thr  
85 90 95

His	Pro	His	Leu	Pro	Arg	Ala	Leu	Met	Arg	Ser	Thr	Thr	Lys	Thr	Ser
100								105					110		

Gly Pro Arg Ala Ala Pro Glu Val Tyr Ala Phe Ala Thr Pro Glu Trp  
115 120 125

Pro Gly Ser Arg Asp Lys Arg Thr Leu Ala Cys Leu Ile Gln Asn Phe  
130 135 140

Met	Pro	Glu	Asp	Ile	Ser	Val	Gln	Trp	Leu	His	Asn	Glu	Val	Gln	Leu
145					150				155					160	

Pro Asp Ala Arg His Ser Thr Thr Gln Pro Arg Lys Thr Lys Gly Ser  
165 170 175

Gly Phe Phe Val Phe Ser Arg Leu Glu Val Thr Arg Ala Glu Trp Glu  
 180 185 190

Gln Lys Asp Glu Phe Ile Cys Arg Ala Val His Glu Ala Ala Ser Pro  
195 200 205

Ser Gln Thr Val Gln Arg Ala Val Ser Val Asn Pro Gly Lys  
210 215 220

<210> 90

<211> 327

<212> PRT

<213> Homo sapiens

<400> 90

Ala Ser Thr

Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys  
 115 120 125

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val  
 130 135 140

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp  
 145 150 155 160

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe  
 165 170 175

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp  
 180 185 190

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu  
 195 200 205

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg  
 210 215 220

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys  
 225 230 235 240

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp  
 245 250 255

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys  
 260 265 270

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser  
 275 280 285

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser  
 290 295 300

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser  
 305 310 315 320

Leu Ser Leu Ser Leu Gly Lys  
 325

<210> 91

<211> 365

<212> PRT

<213> Homo sapiens

<400> 91

Met Ala Val Met Ala Pro Arg Thr Leu Leu Leu Leu Ser Gly Ala  
 1 5 10 15

Leu Ala Leu Thr Gln Thr Trp Ala Gly Ser His Ser Met Arg Tyr Phe  
 20 25 30

Phe Thr Ser Val Ser Arg Pro Gly Arg Gly Glu Pro Arg Phe Ile Ala  
 35 40 45

Val Gly Tyr Val Asp Asp Thr Gln Phe Val Arg Phe Asp Ser Asp Ala  
 50 55 60

Ala Ser Gln Lys Met Glu Pro Arg Ala Pro Trp Ile Glu Gln Glu Gly  
 65 70 75 80

Pro Glu Tyr Trp Asp Gln Glu Thr Arg Asn Met Lys Ala His Ser Gln  
 85 90 95

Thr Asp Arg Ala Asn Leu Gly Thr Leu Arg Gly Tyr Tyr Asn Gln Ser  
 100 105 110

Glu Asp Gly Ser His Thr Ile Gln Ile Met Tyr Gly Cys Asp Val Gly  
 115 120 125

Pro Asp Gly Arg Phe Leu Arg Gly Tyr Arg Gln Asp Ala Tyr Asp Gly  
 130 135 140

Lys Asp Tyr Ile Ala Leu Asn Glu Asp Leu Arg Ser Trp Thr Ala Ala  
 145 150 155 160

Asp Met Ala Ala Gln Ile Thr Lys Arg Lys Trp Glu Ala Val His Ala  
 165 170 175

Ala Glu Gln Arg Arg Val Tyr Leu Glu Gly Arg Cys Val Asp Gly Leu  
 180 185 190

Arg Arg Tyr Leu Glu Asn Gly Lys Glu Thr Leu Gln Arg Thr Asp Pro  
 195 200 205

Pro Lys Thr His Met Thr His His Pro Ile Ser Asp His Glu Ala Thr  
 210 215 220

Leu Arg Cys Trp Ala Leu Gly Phe Tyr Pro Ala Glu Ile Thr Leu Thr  
 225 230 235 240

Trp Gln Arg Asp Gly Glu Asp Gln Thr Gln Asp Thr Glu Leu Val Glu  
 245 250 255

Thr Arg Pro Ala Gly Asp Gly Thr Phe Gln Lys Trp Ala Ala Val Val  
 260 265 270

Val Pro Ser Gly Glu Glu Gln Arg Tyr Thr Cys His Val Gln His Glu  
 275 280 285

Gly Leu Pro Lys Pro Leu Thr Leu Arg Trp Glu Leu Ser Ser Gln Pro  
 290 295 300

Thr Ile Pro Ile Val Gly Ile Ala Gly Leu Val Leu Leu Gly Ala  
 305 310 315 320

Val Ile Thr Gly Ala Val Val Ala Ala Val Met Trp Arg Arg Lys Ser  
 325 330 335

Ser Asp Arg Lys Gly Gly Ser Tyr Thr Gln Ala Ala Ser Ser Asp Ser

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345

350

Ala Gln Gly Ser Asp Val Ser Ileu Thr Ala Cys Lys Val  
355 360 365

<210> 92

<211> 362

<212> PRT

<213> Homo sapiens

<400> 92

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Leu Ala Leu Thr Glu Thr Trp Ala Gly Ser His Ser Met Arg Tyr Phe  
 20 25 30

Tyr Thr Ser Val Ser Arg Pro Gly Arg Gly Glu Pro Arg Phe Ile Ser  
 35 40 45

Val Gly Tyr Val Asp Asp Thr Gln Phe Val Arg Phe Asp Ser Asp Ala  
50 55 60

Ala Ser Pro Arg Glu Glu Pro Arg Ala Pro Trp Ile Glu Gln Glu Gly  
65 70 75 80

Pro Glu Tyr Trp Asp Arg Asn Thr Gln Ile Tyr Lys Ala Gln Ala Gln  
 85 90 95

Glu Ala Gly Ser His Thr Leu Gln Ser Met Tyr Gly Cys Asp Val Gly  
115 120 125

Pro Asp Gly Arg Leu Leu Arg Gly His Asp Gln Tyr Ala Tyr Asp Gly  
130 135 140

Lys Asp Tyr Ile Ala Leu Asn Glu Asp Leu Arg Ser Trp Thr Ala Ala  
 145 150 155 160

Asp Thr Ala Ala Gln Ile Thr Gln Arg Lys Trp Glu Ala Ala Arg Glu  
165 170 175

Ala Glu Gln Arg Arg Ala Tyr Leu Glu Gly Glu Cys Val Glu Trp Leu  
 180 185 190

Arg Arg Tyr Leu Glu Asn Gly Lys Asp Lys Leu Glu Arg Ala Asp Pro

195

200

205

Pro Lys Thr His Val Thr His His Pro Ile Ser Asp His Glu Ala Thr  
210 215 220

Leu Arg Cys Trp Ala Leu Gly Phe Tyr Pro Ala Glu Ile Thr Leu Thr  
 225 230 235 240

Trp Gln Arg Asp Gly Glu Asp Gln Thr Gln Asp Thr Glu Leu Val Glu  
 245 250 255

Thr Arg Pro Ala Gly Asp Arg Thr Phe Gln Lys Trp Ala Ala Val Val  
 260 265 270

Val Pro Ser Gly Glu Glu Gln Arg Tyr Thr Cys His Val Gln His Glu  
 275 280 285

Gly Leu Pro Lys Pro Leu Thr Leu Arg Trp Glu Pro Ser Ser Gln Ser  
 290 295 300

Thr Val Pro Ile Val Gly Ile Val Ala Gly Leu Ala Val Leu Ala Val  
 305 310 315 320

Val Val Ile Gly Ala Val Val Ala Ala Val Met Cys Arg Arg Lys Ser  
 325 330 335

Ser Gly Gly Lys Gly Ser Tyr Ser Gln Ala Ala Cys Ser Asp Ser  
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Ala Gln Gly Ser Asp Val Ser Leu Thr Ala  
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<210> 93

<211> 366

<212> PRT

<213> Homo sapiens

<400> 93

Met Arg Val Met Ala Pro Arg Ala Leu Leu Leu Leu Ser Gly Gly  
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Leu Ala Leu Thr Glu Thr Trp Ala Cys Ser His Ser Met Arg Tyr Phe  
 20 25 30

Asp Thr Ala Val Ser Arg Pro Gly Arg Gly Glu Pro Arg Phe Ile Ser  
 35 40 45

Val Gly Tyr Val Asp Asp Thr Gln Phe Val Arg Phe Asp Ser Asp Ala

50 55 60

Ala Ser Pro Arg Gly Glu Pro Arg Ala Pro Trp Val Glu Gln Glu Gly  
 65 70 75 80

Pro Glu Tyr Trp Asp Arg Glu Thr Gln Asn Tyr Lys Arg Gln Ala Gln  
 85 90 95

Ala Asp Arg Val Ser Leu Arg Asn Leu Arg Gly Tyr Tyr Asn Gln Ser  
 100 105 110

Glu Asp Gly Ser His Thr Leu Gln Arg Met Tyr Gly Cys Asp Leu Gly  
 115 120 125

Pro Asp Gly Arg Leu Leu Arg Gly Tyr Asp Gln Ser Ala Tyr Asp Gly  
 130 135 140

Lys Asp Tyr Ile Ala Leu Asn Glu Asp Leu Arg Ser Trp Thr Ala Ala  
 145 150 155 160

Asp Thr Ala Ala Gln Ile Thr Gln Arg Lys Leu Glu Ala Ala Arg Ala  
 165 170 175

Ala Glu Gln Leu Arg Ala Tyr Leu Glu Gly Thr Cys Val Glu Trp Leu  
 180 185 190

Arg Arg Tyr Leu Glu Asn Gly Lys Glu Thr Leu Gln Arg Ala Glu Pro  
 195 200 205

Pro Lys Thr His Val Thr His His Pro Leu Ser Asp His Glu Ala Thr  
 210 215 220

Leu Arg Cys Trp Ala Leu Gly Phe Tyr Pro Ala Glu Ile Thr Leu Thr  
 225 230 235 240

Trp Gln Arg Asp Gly Glu Asp Gln Thr Gln Asp Thr Glu Leu Val Glu  
 245 250 255

Thr Arg Pro Ala Gly Asp Gly Thr Phe Gln Lys Trp Ala Ala Val Val  
 260 265 270

Val Pro Ser Gly Gln Glu Gln Arg Tyr Thr Cys His Met Gln His Glu  
 275 280 285

Gly Leu Gln Glu Pro Leu Thr Leu Ser Trp Glu Pro Ser Ser Gln Pro  
 290 295 300

Thr Ile Pro Ile Met Gly Ile Val Ala Gly Leu Ala Val Leu Val Val  
 305 310 315 320

Leu Ala Val Leu Gly Ala Val Val Thr Ala Met Met Cys Arg Arg Lys  
 325 330 335

Ser Ser Gly Gly Lys Gly Ser Cys Ser Gln Ala Ala Cys Ser Asn  
 340 345 350

Ser Ala Gln Gly Ser Asp Glu Ser Leu Ile Thr Cys Lys Ala  
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<210> 94

<211> 290

<212> PRT

<213> Homo sapiens

<400> 94

Met Arg Ile Phe Ala Glu Ile Ile Phe Thr Ala Cys Cys His Leu Leu

1 5 10 15

Arg Ala Phe Thr Ile Thr Ala Pro Lys Asp Leu Tyr Val Val Glu Tyr  
20 25 30

Gly Ser Asn Val Thr Met Glu Cys Arg Phe Pro Val Glu Arg Glu Leu  
35 40 45

Asp Leu Leu Ala Leu Val Val Tyr Trp Glu Lys Glu Asp Glu Gln Val  
50 55 60

Ile Gln Phe Val Ala Gly Glu Glu Asp Leu Lys Pro Gln His Ser Asn  
65 70 75 80

Phe Arg Gly Arg Ala Ser Leu Pro Lys Asp Gln Leu Leu Lys Gly Asn  
85 90 95

Ala Ala Leu Gln Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr  
100 105 110

Cys Cys Ile Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Leu  
115 120 125

Lys Val Asn Ala Pro Tyr Arg Lys Ile Asn Gln Arg Ile Ser Val Asp  
130 135 140

Pro Ala Thr Ser Glu His Glu Leu Ile Cys Gln Ala Glu Gly Tyr Pro  
145 150 155 160

Glu Ala Glu Val Ile Trp Thr Asn Ser Asp His Gln Pro Val Ser Gly  
165 170 175

Lys Arg Ser Val Thr Thr Ser Arg Thr Glu Gly Met Leu Leu Asn Val  
180 185 190

Thr Ser Ser Leu Arg Val Asn Ala Thr Ala Asn Asp Val Phe Tyr Cys  
195 200 205

Thr Phe Trp Arg Ser Gln Pro Gly Gln Asn His Thr Ala Glu Leu Ile  
210 215 220

Ile Pro Glu Leu Pro Ala Thr His Pro Pro Gln Asn Arg Thr His Trp  
225 230 235 240

Val Leu Leu Gly Ser Ile Leu Leu Phe Leu Ile Val Val Ser Thr Val  
245 250 255

Leu Leu Phe Leu Arg Lys Gln Val Arg Met Leu Asp Val Glu Lys Cys  
260 265 270

Gly Val Glu Asp Thr Ser Ser Lys Asn Arg Asn Asp Thr Gln Phe Glu  
275 280 285

Glu Thr  
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<210> 95  
<211> 290  
<212> PRT  
<213> *Homo sapiens*

<400> 95  
Met Arg Ile Phe Ala Val Phe Ile Phe Met Thr Tyr Trp His Leu Leu  
1 5 10 15

Asn Ala Phe Thr Val Thr Val Pro Lys Asp Leu Tyr Val Val Glu Tyr  
                  20                 25                 30

Gly Ser Asn Met Thr Ile Glu Cys Lys Phe Pro Val Glu Lys Gln Leu  
35 40 45

Asp Leu Ala Ala Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Asn Ile  
 50 55 60

Ile	Gln	Phe	Val	His	Gly	Glu	Glu	Asp	Leu	Lys	Val	Gln	His	Ser	Ser
65					70					75					80

Tyr Arg Gln Arg Ala Arg Leu Leu Lys Asp Gln Leu Ser Leu Gly Asn  
85 90 95

Ala Ala Leu Gln Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr  
100 105 110

Arg Cys Met Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Val  
115 120 125

Lys Val Asn Ala Pro Tyr Asn Lys Ile Asn Gln Arg Ile Leu Val Val  
130 135 140

Asp	Pro	Val	Thr	Ser	Glu	His	Glu	Leu	Thr	Cys	Gln	Ala	Glu	Gly	Tyr
145				150						155					160

Pro Lys Ala Glu Val Ile Trp Thr Ser Ser Asp His Gln Val Leu Ser  
165 170 175

Gly Lys Thr Thr Thr Asn Ser Lys Arg Glu Glu Lys Leu Phe Asn  
 180 185 190

Val Thr Ser Thr Leu Arg Ile Asn Thr Thr Thr Ash Glu Ile Phe Tyr  
195 200 205

Cys Thr Phe Arg Arg Leu Asp Phe Glu Glu Asn His Thr Ala Glu Leu  
210 215 220

Val Ile Phe Glu Leu Phe Leu Ala His Phe Phe Ash Glu Arg Thr His  
225 230 235 240

Leu Val Ile Leu Gly Ala Ile Leu Leu Cys Leu Gly Val Ala Leu Thr  
245 250 255

Phe Ile Phe Arg Leu Arg Lys Gly Arg Met Met Asp Val Lys Lys Cys  
 260 265 270

Gly Ile Gln Asp Thr Asn Ser Lys Lys Gln Ser Asp Thr His Leu Glu  
 275 280 285

Glu Thr  
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<210> 96

<211> 254

<212> PRT

<213> Homo sapiens

<400> 96

Met Glu Tyr Ala Ser Asp Ala Ser Leu Asp Pro Glu Ala Pro Trp Pro  
 1 5 10 15

Pro Ala Pro Arg Ala Arg Ala Cys Arg Val Leu Pro Trp Ala Leu Val  
 20 25 30

Ala Gly Leu Leu Leu Leu Leu Leu Ala Ala Ala Cys Ala Val Phe  
 35 40 45

Leu Ala Cys Pro Trp Ala Val Ser Gly Ala Arg Ala Ser Pro Gly Ser  
 50 55 60

Ala Ala Ser Pro Arg Leu Arg Glu Gly Pro Glu Leu Ser Pro Asp Asp  
 65 70 75 80

Pro Ala Gly Leu Leu Asp Leu Arg Gln Gly Met Phe Ala Gln Leu Val  
 85 90 95

Ala Gln Asn Val Leu Leu Ile Asp Gly Pro Leu Ser Trp Tyr Ser Asp  
 100 105 110

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

Asp Thr Lys Glu Leu Val Val Ala Lys Ala Gly Val Tyr Tyr Val Phe  
 130 135 140

Phe Gln Leu Glu Leu Arg Arg Val Val Ala Gly Glu Gly Ser Gly Ser  
 145 150 155 160

Val Ser Leu Ala Leu His Leu Gln Pro Leu Arg Ser Ala Ala Gly Ala  
 165 170 175

Ala Ala Leu Ala Leu Thr Val Asp Leu Pro Pro Ala Ser Ser Glu Ala  
 180 185 190

Arg Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His Leu Ser Ala  
 195 200 205

Gly Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg Ala Arg His  
 210 215 220

Ala Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu Phe Arg Val  
 225 230 235 240

Thr Pro Glu Ile Pro Ala Gly Leu Pro Ser Pro Arg Ser Glu  
 245 250

<210> 97

<211> 302

<212> PRT

<213> Homo sapiens

<400> 97

Met Arg Leu Gly Ser Pro Gly Leu Leu Phe Leu Leu Phe Ser Ser Leu  
 1 5 10 15

Arg Ala Asp Thr Gln Glu Lys Glu Val Arg Ala Met Val Gly Ser Asp  
 20 25 30

Val Glu Leu Ser Cys Ala Cys Pro Glu Gly Ser Arg Phe Asp Leu Asn  
 35 40 45

Asp Val Tyr Val Tyr Trp Gln Thr Ser Glu Ser Lys Thr Val Val Thr  
 50 55 60

Tyr His Ile Pro Gln Asn Ser Ser Leu Glu Asn Val Asp Ser Arg Tyr  
 65 70 75 80

Arg Asn Arg Ala Leu Met Ser Pro Ala Gly Met Leu Arg Gly Asp Phe  
 85 90 95

Ser Leu Arg Leu Phe Asn Val Thr Pro Gln Asp Glu Gln Lys Phe His  
 100 105 110

Cys Leu Val Leu Ser Gln Ser Leu Gly Phe Gln Glu Val Leu Ser Val  
 115 120 125

Glu Val Thr Leu His Val Ala Ala Asn Phe Ser Val Pro Val Val Ser  
 130 135 140

Ala Pro His Ser Pro Ser Gln Asp Glu Leu Thr Phe Thr Cys Thr Ser  
 145 150 155 160

Ile Asn Gly Tyr Pro Arg Pro Asn Val Tyr Trp Ile Asn Lys Thr Asp  
 165 170 175

Asn Ser Leu Leu Asp Gln Ala Leu Gln Asn Asp Thr Val Phe Leu Asn  
 180 185 190

Met Arg Gly Leu Tyr Asp Val Val Ser Val Leu Arg Ile Ala Arg Thr  
 195 200 205

Pro Ser Val Asn Ile Gly Cys Cys Ile Glu Asn Val Leu Leu Gln Gln  
 210 215 220

Asn Leu Thr Val Gly Ser Gln Thr Gly Asn Asp Ile Gly Glu Arg Asp  
 ~~~ ~~~ ~~~

225

230

235

240

Lys Ile Thr Glu Asn Pro Val Ser Thr Gly Glu Lys Asn Ala Ala Thr  
 245 250 255

Trp Ser Ile Leu Ala Val Leu Cys Leu Leu Val Val Val Ala Val Ala  
 260 265 270

Ile Gly Trp Val Cys Arg Asp Arg Cys Leu Gln His Ser Tyr Ala Gly  
 275 280 285

Ala Trp Ala Val Ser Pro Glu Thr Glu Leu Thr Gly His Val  
 290 295 300

&lt;210&gt; 98

&lt;211&gt; 183

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 98

Met Glu Arg Val Gln Pro Leu Glu Glu Asn Val Gly Asn Ala Ala Arg  
 1 5 10 15

Pro Arg Phe Glu Arg Asn Lys Leu Leu Leu Val Ala Ser Val Ile Gln  
 20 25 30

Gly Leu Gly Leu Leu Leu Cys Phe Thr Tyr Ile Cys Leu His Phe Ser  
 35 40 45

Ala Leu Gln Val Ser His Arg Tyr Pro Arg Ile Gln Ser Ile Lys Val  
 50 55 60

Gln Phe Thr Glu Tyr Lys Lys Glu Lys Gly Phe Ile Leu Thr Ser Gln  
 65 70 75 80

Lys Glu Asp Glu Ile Met Lys Val Gln Asn Asn Ser Val Ile Ile Asn  
 85 90 95

Cys Asp Gly Phe Tyr Leu Ile Ser Leu Lys Gly Tyr Phe Ser Gln Glu  
 100 105 110

Val Asn Ile Ser Leu His Tyr Gln Lys Asp Glu Glu Pro Leu Phe Gln  
 115 120 125

Leu Lys Lys Val Arg Ser Val Asn Ser Leu Met Val Ala Ser Leu Thr  
 130 135 140

Tyr Lys Asp Lys Val Tyr Leu Asn Val Thr Thr Asp Asn Thr Ser Leu  
 145 150 155 160

Asp Asp Phe His Val Asn Gly Gly Glu Leu Ile Leu Ile His Gln Asn  
 165 170 175

Pro Gly Glu Phe Cys Val Leu  
 180

<210> 99

<211> 273

<212> PRT

<213> Homo sapiens

<400> 99

Met Ile Phe Leu Leu Leu Met Leu Ser Leu Glu Leu Gln Leu His Gln  
1 5 10 15

Ile Ala Ala Leu Phe Thr Val Thr Val Pro Lys Glu Leu Tyr Ile Ile  
20 25 30

Glu His Gly Ser Asn Val Thr Leu Glu Cys Asn Phe Asp Thr Gly Ser  
35 40 45

His Val Asn Leu Gly Ala Ile Thr Ala Ser Leu Gln Lys Val Glu Asn  
50 55 60

Asp Thr Ser Pro His Arg Glu Arg Ala Thr Leu Leu Glu Glu Gln Leu  
65 70 75 80

Pro Leu Gly Lys Ala Ser Phe His Ile Pro Gln Val Gln Val Arg Asp  
85 90 95

Glu Gly Gln Tyr Gln Cys Ile Ile Ile Tyr Gly Val Ala Trp Asp Tyr  
100 105 110

Lys Tyr Leu Thr Leu Lys Val Lys Ala Ser Tyr Arg Lys Ile Asn Thr  
115 120 125

His Ile Leu Lys Val Pro Glu Thr Asp Glu Val Glu Leu Thr Cys Gln  
130 135 140

Ala Thr Gly Tyr Pro Leu Ala Glu Val Ser Trp Pro Asn Val Ser Val  
145 150 155 160

Pro Ala Asn Thr Ser His Ser Arg Thr Pro Glu Gly Leu Tyr Gln Val  
165 170 175

Thr Ser Val Leu Arg Leu Lys Pro Pro Pro Gly Arg Asn Phe Ser Cys  
180 185 190

Val Phe Trp Asn Thr His Val Arg Glu Leu Thr Leu Ala Ser Ile Asp  
195 200 205

Leu Gln Ser Gln Met Glu Pro Arg Thr His Pro Thr Trp Leu Leu His  
210 215 220

Ile Phe Ile Pro Phe Cys Ile Ile Ala Phe Ile Phe Ile Ala Thr Val  
225 230 235 240

Ile Ala Leu Arg Lys Gln Leu Cys Gln Lys Leu Tyr Ser Ser Lys Asp  
245 250 255

Thr Thr Lys Arg Pro Val Thr Thr Lys Arg Glu Val Asn Ser Ala  
 260 265 270

Ile

<210> 100

<211> 288

<212> PRT

<213> Homo sapiens

<400> 100

Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro Ser Lys Cys Pro Tyr  
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Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly Leu Ser His Phe Cys  
 20 25 30

Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu  
 35 40 45

Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile  
 50 55 60

Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp  
 65 70 75 80

Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr  
 85 90 95

Asn Asn Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly  
 100 105 110

Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg  
 115 120 125

Glu His Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr  
 130 135 140

Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile  
 145 150 155 160

Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu  
 165 170 175

Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp  
 180 185 190

Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met  
 195 200 205

Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg  
 210 215 220

Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro  
 225 230 235 240

Asp Asn Leu Leu Pro Ser Trp Ala Ile Thr Leu Ile Ser Val Asn Gly  
 245 250 255

Ile Phe Val Ile Cys Cys Leu Thr Tyr Cys Phe Ala Pro Arg Cys Arg  
 260 265 270

Glu Arg Arg Arg Asn Glu Arg Leu Arg Arg Glu Ser Val Arg Pro Val  
 275 280 285

<210> 101

<211> 329

<212> PRT

<213> Homo sapiens

<400> 101

Met Asp Pro Gln Cys Thr Met Gly Leu Ser Asn Ile Leu Phe Val Met  
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Ala Phe Leu Leu Ser Gly Ala Ala Pro Leu Lys Ile Gln Ala Tyr Phe  
 20 25 30

Asn Glu Thr Ala Asp Leu Pro Cys Gln Phe Ala Asn Ser Gln Asn Gln  
 35 40 45

Ser Leu Ser Glu Leu Val Val Phe Trp Gln Asp Gln Glu Asn Leu Val  
 50 55 60

Leu Asn Glu Val Tyr Leu Gly Lys Glu Lys Phe Asp Ser Val His Ser  
 65 70 75 80

Lys Tyr Met Gly Arg Thr Ser Phe Asp Ser Asp Ser Trp Thr Leu Arg  
 85 90 95

Leu His Asn Leu Gln Ile Lys Asp Lys Gly Leu Tyr Gln Cys Ile Ile  
 100 105 110

His His Lys Pro Thr Gly Met Ile Arg Ile His Gln Met Asn Ser  
 115 120 125

Glu Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Val Pro Ile  
 130 135 140

Ser Asn Ile Thr Glu Asn Val Tyr Ile Asn Leu Thr Cys Ser Ser Ile  
 145 150 155 160

His Gly Tyr Pro Glu Pro Lys Lys Met Ser Val Leu Leu Arg Thr Lys  
 165 170 175

Asn Ser Thr Ile Glu Tyr Asp Gly Ile Met Gln Lys Ser Gln Asp Asn  
 180 185 190

Val Thr Glu Leu Tyr Asp Val Ser Ile Ser Leu Ser Val Ser Phe Pro  
 195 200 205

Asp Val Thr Ser Asn Met Thr Ile Phe Cys Ile Leu Glu Thr Asp Lys  
 210 215 220

210

215

220

Thr Arg Leu Leu Ser Ser Pro Phe Ser Ile Glu Leu Glu Asp Pro Gln  
 225 230 235 240

Pro Pro Pro Asp His Ile Pro Trp Ile Thr Ala Val Leu Pro Thr Val  
 245 250 255

Ile Ile Cys Val Met Val Phe Cys Leu Ile Leu Trp Lys Trp Lys Lys  
 260 265 270

Lys Lys Arg Pro Arg Asn Ser Tyr Lys Cys Gly Thr Asn Thr Met Glu  
 275 280 285

Arg Glu Glu Ser Glu Gln Thr Lys Lys Arg Glu Lys Ile His Ile Pro  
 290 295 300

Glu Arg Ser Asp Glu Ala Gln Arg Val Phe Lys Ser Ser Lys Thr Ser  
 305 310 315 320

Ser Cys Asp Lys Ser Asp Thr Cys Phe  
 325

<210> 102

<211> 281

<212> PRT

<213> Homo sapiens

<400> 102

Met Gln Gln Pro Phe Asn Tyr Pro Tyr Pro Gln Ile Tyr Trp Val Asp  
 1 5 10 15

Ser Ser Ala Ser Ser Pro Trp Ala Pro Pro Gly Thr Val Leu Pro Cys  
 20 25 30

Pro Thr Ser Val Pro Arg Arg Pro Gly Gln Arg Arg Pro Pro Pro Pro  
 35 40 45

Pro Pro Pro Pro Pro Leu Pro Pro Pro Pro Pro Pro Pro Leu Pro  
 50 55 60

Pro Leu Pro Leu Pro Pro Leu Lys Lys Arg Gly Asn His Ser Thr Gly  
 65 70 75 80

Leu Cys Leu Leu Val Met Phe Phe Met Val Leu Val Ala Leu Val Gly  
 85 90 95

Leu Gly Leu Gly Met Phe Gln Leu Phe His Leu Gln Lys Glu Leu Ala  
 100 105 110

Glu Leu Arg Glu Ser Thr Ser Gln Met His Thr Ala Ser Ser Leu Glu  
 115 120 125

Lys Gln Ile Gly His Pro Ser Pro Pro Glu Lys Lys Glu Leu Arg  
 130 135 140

Lys Val Ala His Leu Thr Gly Lys Ser Asn Ser Arg Ser Met Pro Leu  
 145 150 155 160

Glu Trp Glu Asp Thr Tyr Gly Ile Val Leu Leu Ser Gly Val Lys Tyr  
 165 170 175

Lys Lys Gly Gly Leu Val Ile Asn Glu Thr Gly Leu Tyr Phe Val Tyr  
 180 185 190

Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys Asn Asn Leu Pro Leu Ser  
 195 200 205

His Lys Val Tyr Met Arg Asn Ser Lys Tyr Pro Gln Asp Leu Val Met  
 210 215 220

Met Glu Gly Lys Met Met Ser Tyr Cys Thr Thr Gly Gln Met Trp Ala  
 225 230 235 240

Arg Ser Ser Tyr Leu Gly Ala Val Phe Asn Leu Thr Ser Ala Asp His  
 245 250 255

Leu Tyr Val Asn Val Ser Glu Leu Ser Leu Val Asn Phe Glu Glu Ser  
 260 265 270

Gln Thr Phe Phe Gly Leu Tyr Lys Leu  
 275 280

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**P a t e n t k r a v****1. Multimerisk polypeptid omfattende:**

et heterodimerisk polypeptid omfattende

a) et første polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

5 i) et peptidepitop; og

ii) et første major histokompatibilitetskompleks-(MHC)-polypeptid;

b) et andet polypeptid omfattende et andet MHC-polypeptid, og

c) mindst et immunomodulatorisk polypeptid,

hvor det første og/eller det andet polypeptid omfatter det mindst ene immunomodulatoriske polypeptid,

10 hvor det første MHC-polypeptid er et  $\beta$ 2-mikroglobulin-polypeptid, og hvor det andet MHC-polypeptid er et MHC-klasse I-tungkæde-polypeptid,

hvor peptidepitopet er et cancer-associeret epitop, og

15 eventuelt hvor det multimeriske polypeptid omfatter et immunoglobulin-(Ig)-Fc-polypeptid eller et ikke-Ig-scaffold.

**2. Multimerisk polypeptid ifølge krav 1, hvor det multimeriske polypeptid omfatter**

a1) et første polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

20 i) et peptidepitop; og

ii) et første MHC-polypeptid; og

b1) et andet polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

25 i) mindst et immunomodulatorisk polypeptid,

ii) et andet MHC-polypeptid; og

iii) et immunoglobulin-(Ig)-Fc-polypeptid; eller

a2) et første polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

30 i) et peptidepitop;

ii) et første MHC-polypeptid; og

iii) mindst et immunomodulatorisk polypeptid; og

b2) et andet polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

i) et andet MHC-polypeptid; og

ii) et Ig-Fc-polypeptid; eller

a3) et første polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

- i) et peptidepitop; og
- ii) et første MHC-polypeptid; og

5 b3) et andet polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

- i) et andet MHC-polypeptid; og
- ii) et Ig-Fc-polypeptid; og
- iii) mindst et immunomodulatorisk polypeptid; eller

a4) et første polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

10 b4) et andet polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

- i) et andet MHC-polypeptid; og
- ii) mindst et immunomodulatorisk polypeptid; eller

a5) et første polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

15 b5) et andet polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

- i) mindst et immunomodulatorisk polypeptid; og
- ii) et andet MHC-polypeptid; eller

a6) et første polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

20 b6) et andet polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

- i) et peptidepitop;
- ii) et første MHC-polypeptid; og
- iii) mindst et immunomodulatorisk polypeptid; og

25 i) et andet MHC-polypeptid.

30 **3. Multimerisk polypeptid ifølge krav 1, hvor ikke-Ig-scaffold'et er et XTEN-polypeptid, et transferrin-polypeptid, et Fc-receptor-polypeptid, et elastin-lignende polypeptid, et silkelignende polypeptid eller et silke-elastin-lignende polypeptid.**

35

4. Multimerisk polypeptid ifølge et af kravene 1-3, hvor det mindst ene immunomodulatoriske polypeptid er udvalgt blandt et 4-1BBL-polypeptid, et B7-1-polypeptid; et B7-2-polypeptid, et ICOS-L-polypeptid, et OX-40L-polypeptid, et CD80-polypeptid, et CD86-polypeptid, et PD-L1-polypeptid, et FasL-polypeptid, et cytokin og et PD-L2-polypeptid.

5 5. Multimerisk polypeptid ifølge krav 4, hvor det mindst ene immunomodulatoriske polypeptid er et cytokin.

10 6. Multimerisk polypeptid ifølge krav 4, hvor det mindst ene immunomodulatoriske polypeptid er udvalgt blandt et 4-1BBL-polypeptid og et CD80-polypeptid.

7. Multimerisk polypeptid ifølge et af kravene 1-6, omfattende 2 eller flere immunomodulatoriske polypeptider.

15 8. Multimerisk polypeptid ifølge krav 7, hvor de 2 eller flere immunomodulatoriske polypeptider er i tandem.

9. Multimerisk polypeptid ifølge et af kravene 1-8, hvor det multimeriske polypeptid omfatter:

20 a1) et første polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:  
i) et peptidepitop; og  
ii) et første MHC-polypeptid; og

25 b1) et andet polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:  
i) mindst et immunomodulatorisk polypeptid,  
ii) et andet MHC-polypeptid; og  
iii) et immunoglobulin-(Ig)-Fc-polypeptid.

30 10. Multimerisk polypeptid ifølge et af kravene 1-8, hvor det multimeriske polypeptid omfatter:

35 a3) et første polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:  
i) et peptidepitop; og  
ii) et første MHC-polypeptid; og

b3) et andet polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:

- i) et andet MHC-polypeptid; og
- ii) et Ig-Fc-polypeptid; og
- iii) mindst et immunomodulatorisk polypeptid.

5      **11.** Multimerisk polypeptid ifølge et af kravene 1-8, hvor det multimeriske polypeptid omfatter:  
a4) et første polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:  
i) et peptidepitop; og  
ii) et første MHC-polypeptid; og  
b4) et andet polypeptid, der i rækkefølge fra N-terminus til C-terminus omfatter:  
i) et andet MHC-polypeptid; og  
ii) mindst et immunomodulatorisk polypeptid.

10     **12.** Multimerisk polypeptid ifølge et af kravene 1-11, hvor det første polypeptid og det andet polypeptid er forbundet til hinanden med en disulfidbinding.

15     **13.** Multimerisk polypeptid ifølge krav 12, hvor  $\beta$ 2M-polypeptidet og MHC-tungkædepolypeptidet er forbundet af en disulfidbinding, der forbinder en Cys-rest i  $\beta$ 2M-polypeptidet og en Cys-rest i MHC-tungkædepolypeptidet.

20     **14.** Multimerisk polypeptid ifølge krav 13, hvor en Cys ved aminosyrerest 12 af  $\beta$ 2M-polypeptidet har en disulfidbinding til en Cys ved aminosyrerest 236 af MHC-tungkædepolypeptidet.

25     **15.** Multimerisk polypeptid ifølge krav 13, hvor den første polypeptidkæde omfatter en linker mellem peptidepitopet og  $\beta$ 2M-polypeptidet, og hvor disulfidbindingen forbinder en Cys, der er til stede i linkeren med en Cys i MHC-tungkædepolypeptidet.

30     **16.** Multimerisk polypeptid ifølge krav 15, hvor den første polypeptidkæde omfatter en linker mellem peptidepitopet og  $\beta$ 2M-polypeptidet, og hvor disulfidbindingen forbinder en Cys, der er substitueret for Gly2 i linkeren med en Cys, der er substitueret for Tyr84 i MHC-tungkædepolypeptidet.

35

17. Multimerisk polypeptid ifølge et af kravene 1-16, hvor det immunomodulatoriske polypeptid et et aktiverende polypeptid.

5        18. Protein omfattende to af de multimeriske polypeptider ifølge et af kravene 1 til 17, hvor hvert af de to multimeriske polypeptider omfatter et immunoglobulin-(Ig)-Fc-polypeptid, og hvor de to multimeriske polypeptider er forbundet af en eller flere disulfidbindinger mellem de respektive Ig-Fc-polypeptider.

10        19. Nukleinsyre omfattende en nukleotidsekvens, der koder for et første og/eller andet polypeptid ifølge et af kravene 1-17, hvor det første eller det andet polypeptid omfatter mindst et immunomodulatorisk polypeptid.

20        20. Ekspressionsvektor omfattende nukleinsyren ifølge krav 19.

15        21. Værtscelle, der er genmodificeret med ekspressionsvektoren ifølge krav 20.

20        22. Fremgangsmåde til selektiv modulering af aktiviteten i en T-celle, som binder til en cancer-associeret epitop, hvor fremgangsmåden omfatter at bringe kontakt til T-cellen *in vitro* med det multimeriske polypeptid ifølge et af kravene 1-17 eller protein ifølge krav 18, hvor kontaktoptagelsen selektivt modulerer T-cellens aktivitet.

25        23. Multimerisk polypeptid ifølge et af kravene 1-17 eller protein ifølge krav 18 eller nukleinsyre ifølge krav 19 eller ekspressionsvektor ifølge krav 20 til anvendelse i en fremgangsmåde til selektiv modulering af aktiviteten af en T-celle, som binder til en cancer-associeret epitop hos et individ, hvor det multimeriske polypeptid eller proteinet eller nukleinsyren eller ekspressionsvektoren indgives for selektivt at modulere T-cellens aktivitet hos individet.

30        24. Multimerisk polypeptid ifølge et af kravene 1-17 eller protein ifølge krav 18 eller nukleinsyre ifølge krav 19 eller rekombinant ekspressionsvektor ifølge krav 20 eller et eller flere mRNA'er omfattende nukleotidsekvenser, der koder for det multimeriske polypeptid ifølge et af kravene 1-17, eller proteinet ifølge krav 18, til anvendelse i en fremgangsmåde til behandling af cancer hos et

individ.

**25.** Sammensætning omfattende:

a) multimerisk polypeptid ifølge et af kravene 1-17 eller protein ifølge krav 18;  
5 og  
b) en farmaceutisk acceptabel excipiens.

**26.** Multimerisk polypeptid ifølge et af kravene 1-17 eller protein ifølge krav 18  
eller nukleinsyre ifølge krav 19 eller ekspressionsvektor ifølge krav 20 til an-  
10 vendelse ifølge krav 22 eller 23, hvor T-cellen er en CD8<sup>+</sup> T-celle, en CD4<sup>+</sup> T-  
celle eller en T-regulatorisk celle.

## DRAWINGS

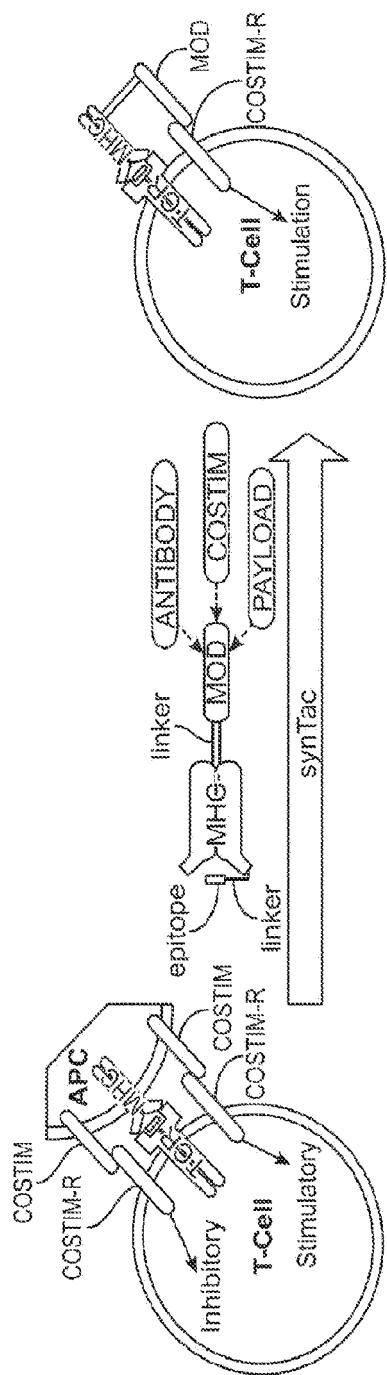


FIG. 1

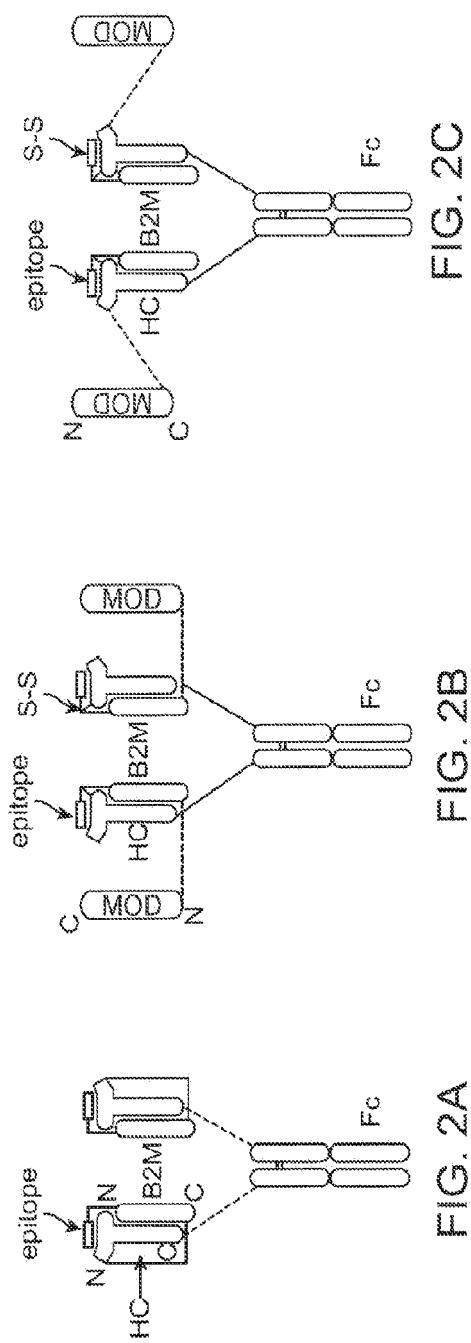


FIG. 2A

FIG. 2B

FIG. 2C

Light Chain (LC)



FIG. 3A

Heavy Chain (LC)



FIG. 3B

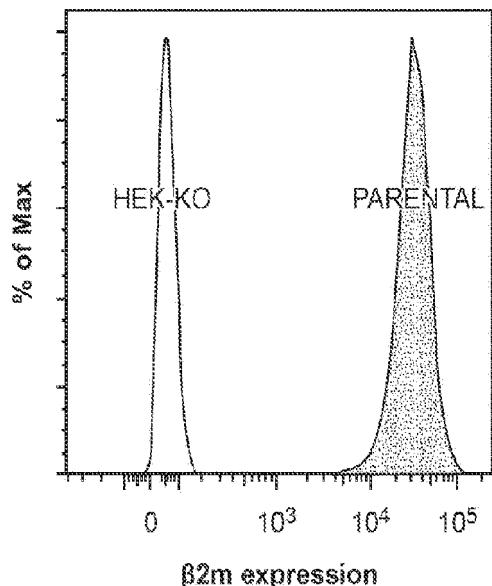


FIG. 4

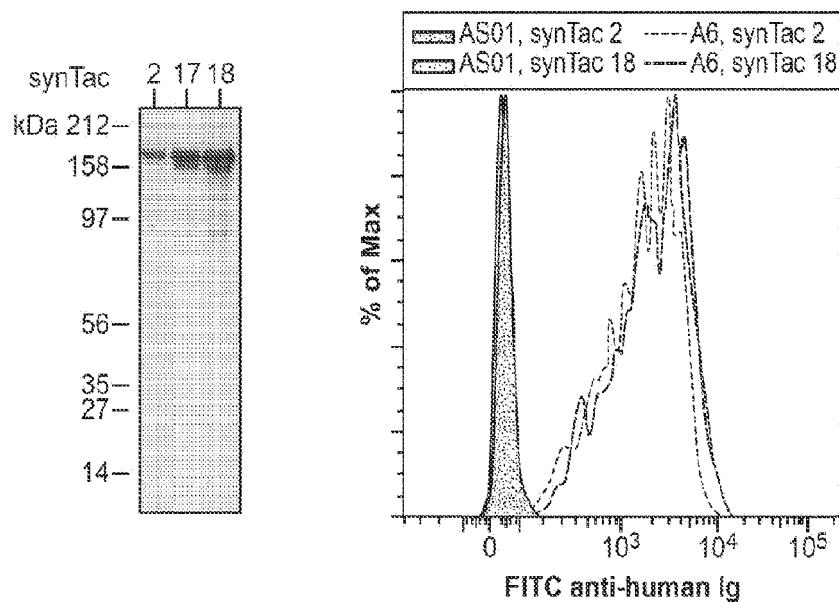


FIG. 5A

FIG. 5B

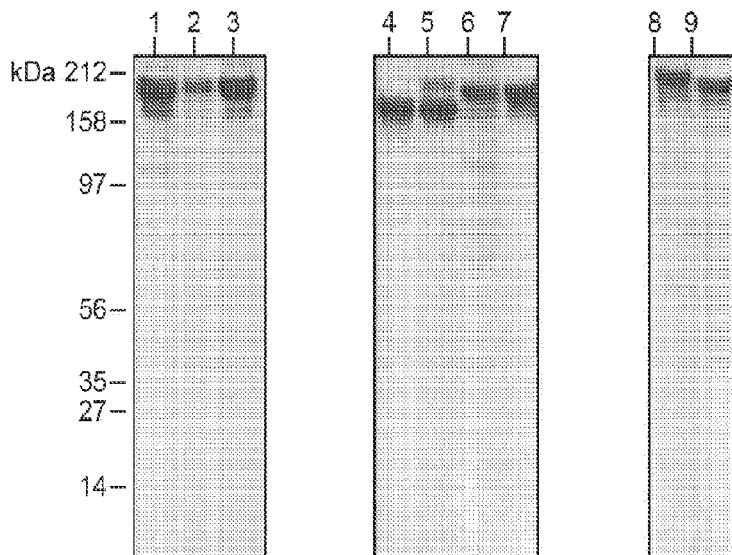


FIG. 6A

FIG. 6B

FIG. 6C

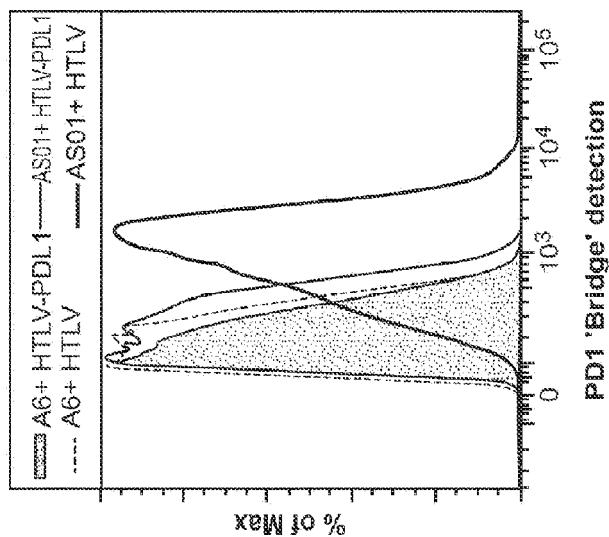


FIG. 7B

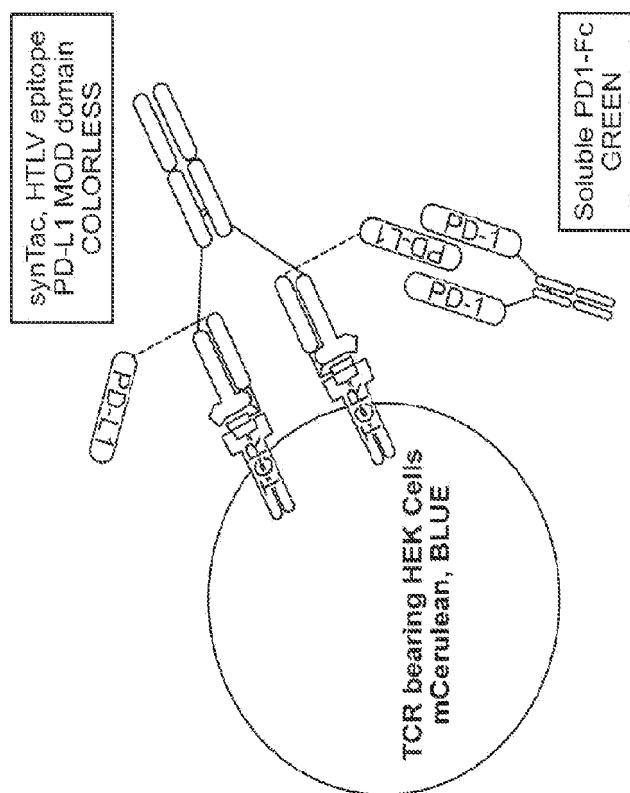


FIG. 7A

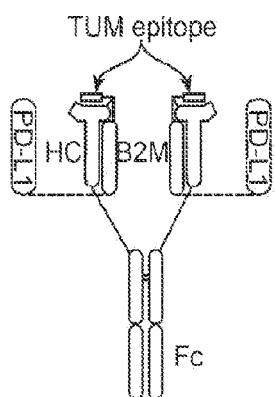


FIG. 8A

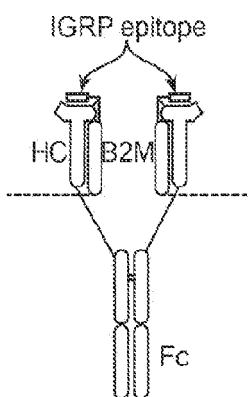


FIG. 8B

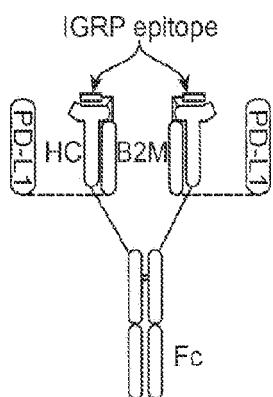


FIG. 8C

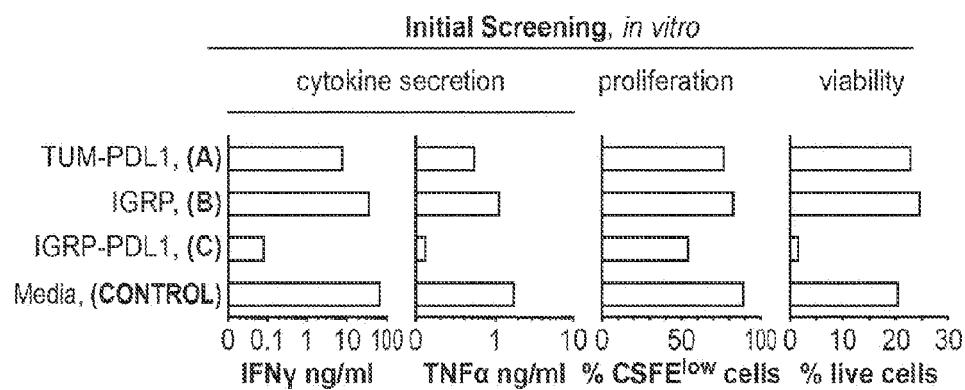


FIG. 8D

synTrac 51 - Free BBL (SEQ ID NO:69)

MSRSVALVALLISIGLEAACPWAWSGARASPGSAASPRRLREGPELSPDDPAGLLDRQGMFAQLVQNVLILDGPLSWYSDPGLAGVSLTGGL  
SYKEDTKELVAKAGVYVFFQLELRRVWAGEGSGSVSLAHLHQPLRSAAGAAALTVDLPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEA  
KARHAWQLTQGATVVLGLFRVTEPAGLSPRSE

**BOLD:** Beta-2-microglobulin leader peptide

**BOLD ITALIC:** human 4-1BBL, residues 50-254 (full ectodomain)

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syntac 40 : Dimeric BBL (SEQ ID NO:70)

B01/D: Beta-2-microglobulin leader peptide

UNIDEB LINE: tarantella asitosa (CGRB) THM control nonide is KVOAVTTI (SEQ ID NO: 18)

ONE-TO-ONE: targeting specific receptors.

WALL: murine Beta-2-microglobulin

**BOLD ITALIC:** human 4-1BBL residues.

**ITALIC UNDERLINE**: Viral p2A sequence

## **BOLD UNDERLINE** **MURINE** **III**. **A** **Heavy** **Chain** **(H-2** **K<sup>a</sup>)**

BOLD ITALIC UNDERLINE: *curious* *Loc222* *Ec* followed by *88 HIS tag*

EVIDENCE OF HUMAN SUBCULTURE

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**DK/EP 3157552 T3**

69 -Disulfide bridge formed between residues Q94C and P245C (SEQ ID NO:71)

**BOLD:** Beta-2 -microglobulin leader peptide  
**UNDERLINE:** targeting epitope (GKRP), TUM control peptide is **KYQAVVITTL** (SEQ ID NO:18)  
*ITALIC:* murine Beta-2-microglobulin  
**BOLD ITALIC:** Disulfide Locked (DL) -human 4-1BBL residues 50-254 (full ectodomain), Q94C-P245C double mutant – mutations shown lowercase  
**ITALIC UNDERLINE:** Viral P2A sequence  
**BOLD UNDERLINE:** Murine HLA Heavy Chain (H-2 K<sup>c</sup>)  
**BOLD ITALIC UNDERLINE:** murine IgG2a Fc, followed by 8x HIS tag  
Y84C mutation (lowercase)

Disulfide locked (DL) trimers formed by coexpression of Free-DL-4-1BBL with synTac harboring same mutations (SEQ ID NO:72)

MRSRVALAVLALLSGLEA4CPWAVGARASPGSAAASPRLEQGPSPDPAQLLDRQGMFACLVAQNVLILIDPLSWYSDPCLAGVSLTG  
SYKEDIKELVVAKAGVYVHOLELRRVVAEGSGSVSLALHQLPLRSAAGAAALATIVDUPPASEARNSAHGFQGRILHLSAQQLGVHLH  
RARAHAWVOLTOCATAVGLERVTPEICAGLSPRSI

6

synTac 70 -Disulfide bridge formed between residues Q94C and P242C (SEQ ID NO:73)

**BOLD:** Beta-2 -microglobulin leader peptide  
**UNDERLINE:** targeting epitope (IGRP). TUM control peptide is **KYQAVTTTL** (SEQ ID NO:18)  
*ITALIC:* murine Beta-2-microglobulin  
**BOLD ITALIC:** Disulfide Locked (DL) - human 4-1BBL, residues 50-254 [full ectodomain], Q94C-P242C double mutant - mutations shown lowercase  
**UNDERLINE ITALIC:** Viral P2A sequence  
**BOLD UNDERLINE ITALIC:** Murine HLA Heavy Chain (H-2 K<sup>d</sup>)  
**BOLD UNDERLINE ITALIC:** murine IgG2a Fc, followed by 8x HIS tag  
Y84C mutation (flowercase)

Disulfide locked (DL) trimers formed by coexpression of Free-DL-4-1BBL with synTac harboring same mutations (SEQ ID NO:74)

MRSVALAVALLISGLEAACPWAVGARASP3AASPRLREGPELSPDDPAGLIDLRQGMFACLVAQNVLLIDGPLSWYSDPGLAGVSLTGGL  
SIVEDIKELVAKAGVYVFFQLELRVVAGEGGSVSLALHLQPLRSAAGAAALALIVDLPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEA  
RARHAWOITOGATVGLFRVTCPEAGLPSPRSE

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**synTac 71** - Disulfide bridge formed between residues Q89C and L115C (SEQ ID NO:75)

BOLD: Beta-2-microglobulin leader peptide

HTA11C murino Bata-2 monocloablin

**BOLD ITALIC:** Disulfide Locked (DL) - human 4-1BBL, residues 50-254 (full ectodomain), Q89C-L115C double mutant - mutations in HALAC, mutine Deltaz-2-mucoglycan

shown in lowercase

*ITALIC UNDERLINE*: Viral P2A sequence  
**BOLD UNDERLINE**: Murine HLA Heavy Chain (H-2 K<sup>a</sup>)

**BOLD UNDERLINE ITALIC:**

Disulfide-linked (DI) trimers formed by coexpression of Free-DI and 4-1-BBL with synPar harboring same mutations (see ID NO:Z6)

MSRSVALAVLALLSGLFEAACPWAWSGARAASPGSAASPRIUREGPELSPDDPAIGLDIRGCMFAQLVAQNVLIDGPLSWYSDPGAGVSLTGGL  
SYKEDTKELVAKAGVYVTFQLELRRVAGEGSVSLAHLQPLRSAAGAAAALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEA  
RARAHAWOLTOGATVGLFRVTPCIGLSPSRE

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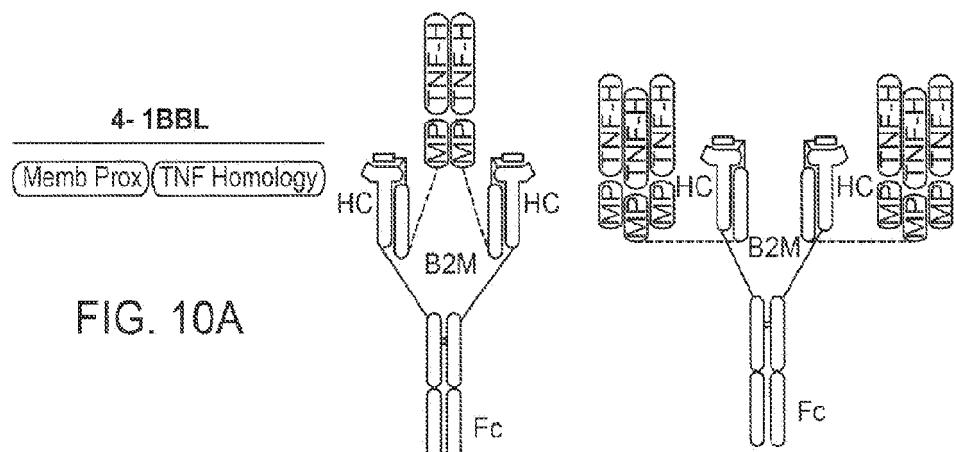


FIG. 10A

FIG. 10B

FIG. 10C

|          |                          |
|----------|--------------------------|
| Syntac # | molecular weight (kDa±%) |
| 40+51    | 236.2<br>(±4.9%)         |
| 40+51    | 257.9<br>(±0.5%)         |

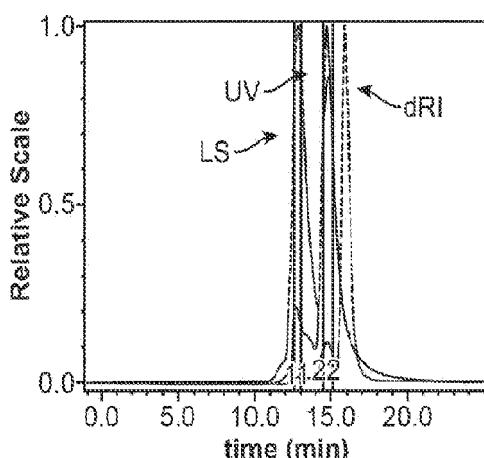


FIG. 11A

FIG. 11B

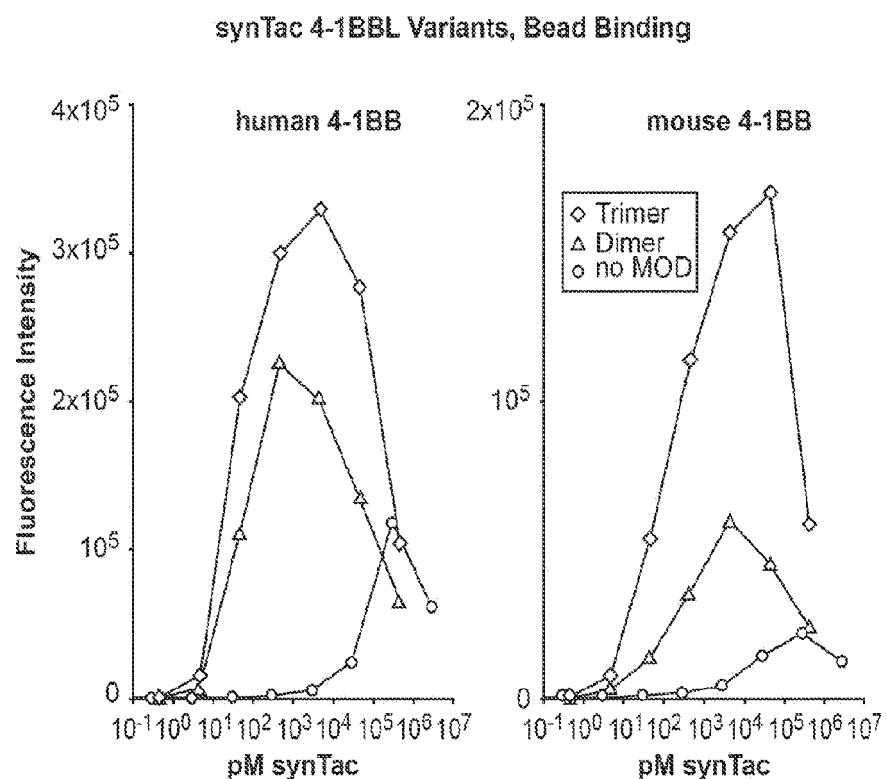


FIG. 12

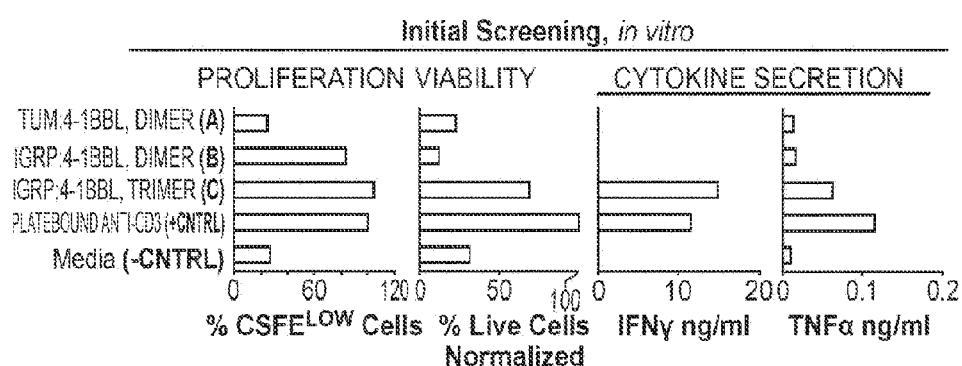
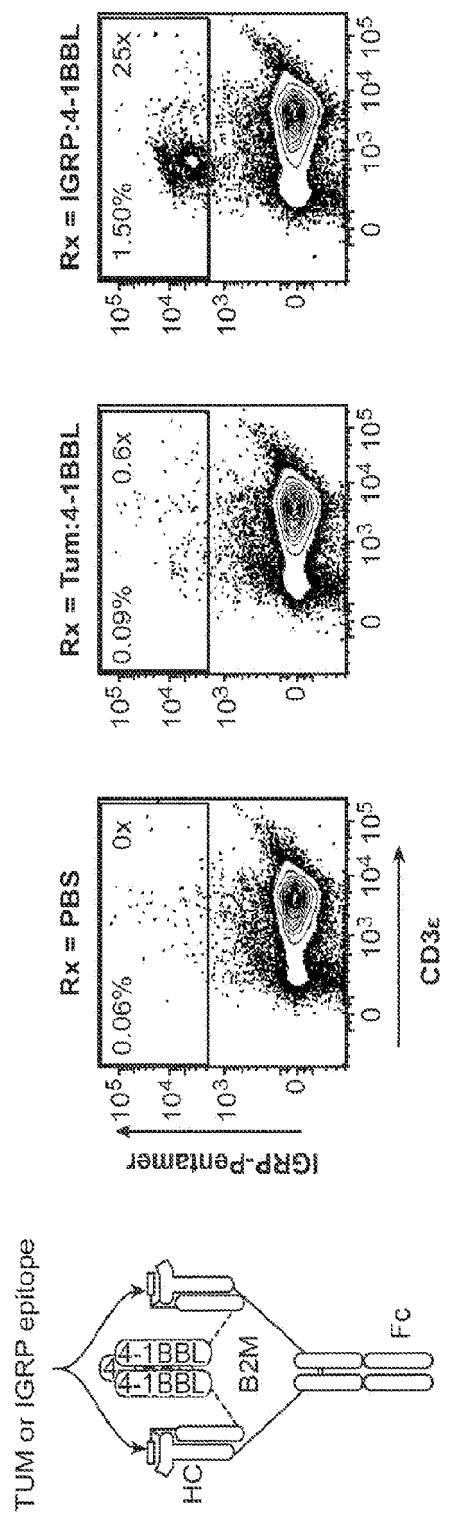


FIG. 13



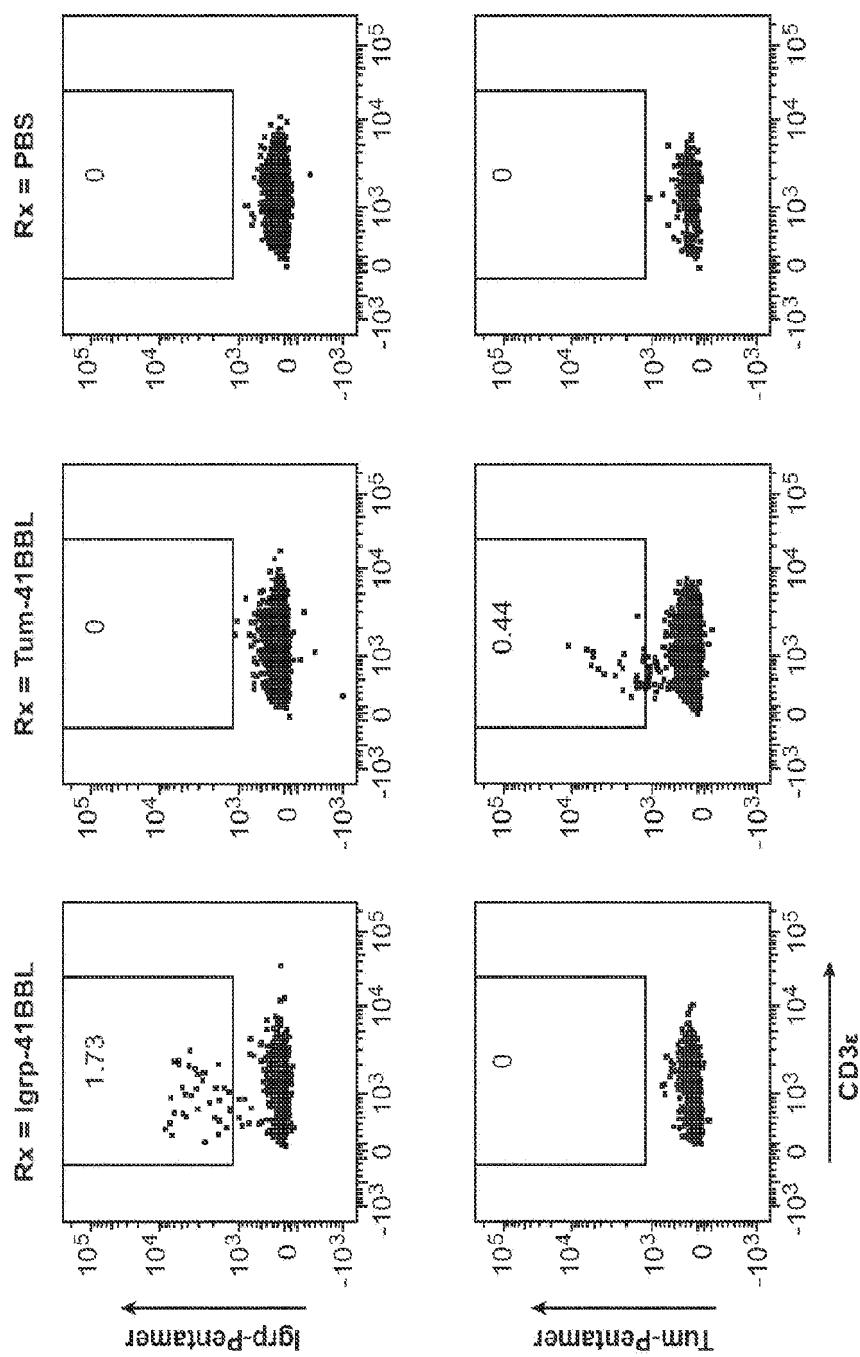


FIG. 15

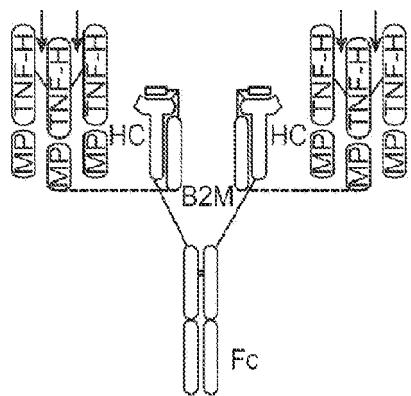


FIG. 16A

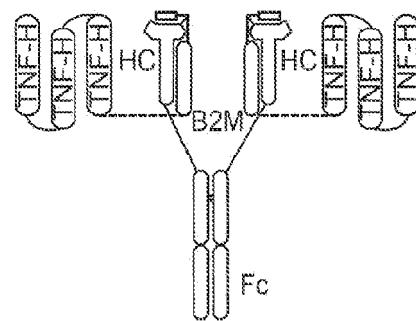


FIG. 16B

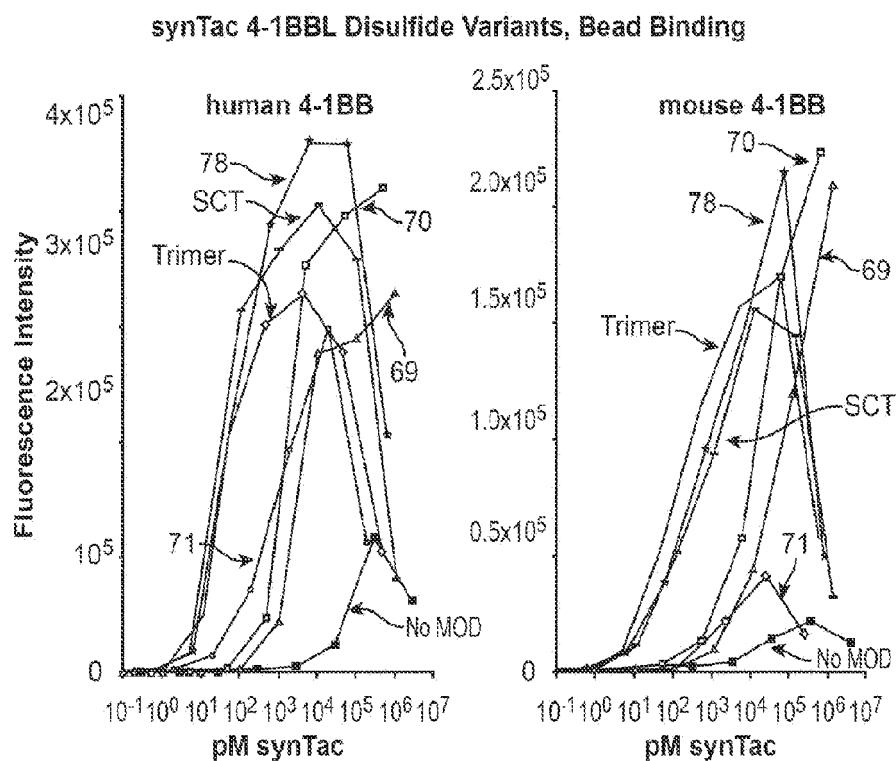


FIG. 17

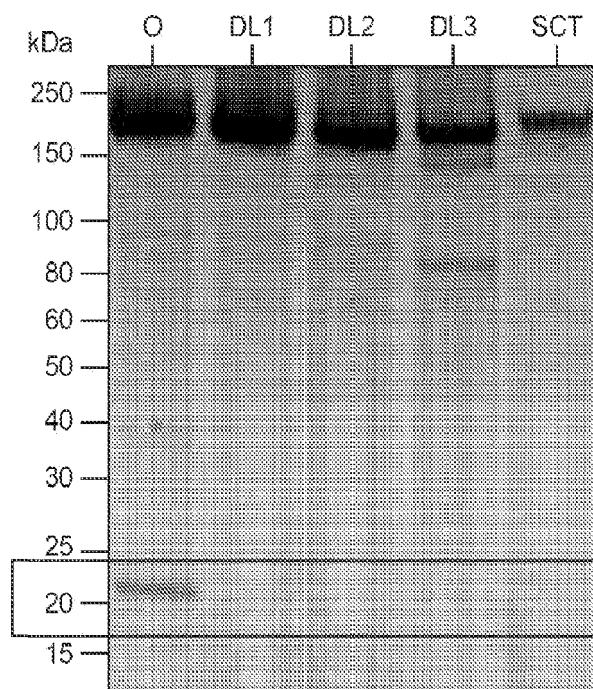


FIG. 18



FIG. 19A

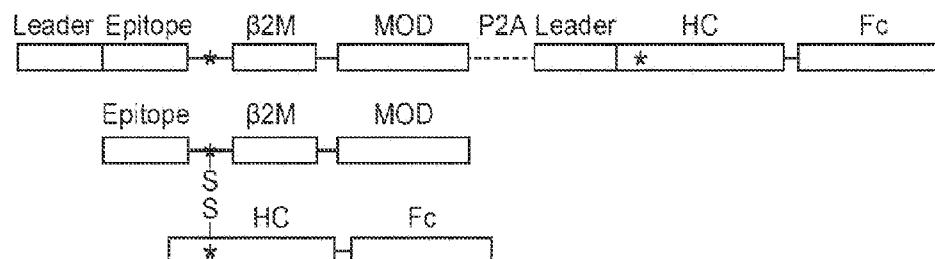


FIG. 19B

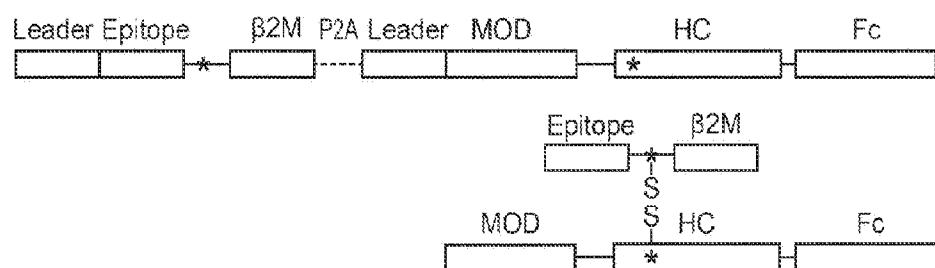


FIG. 19C

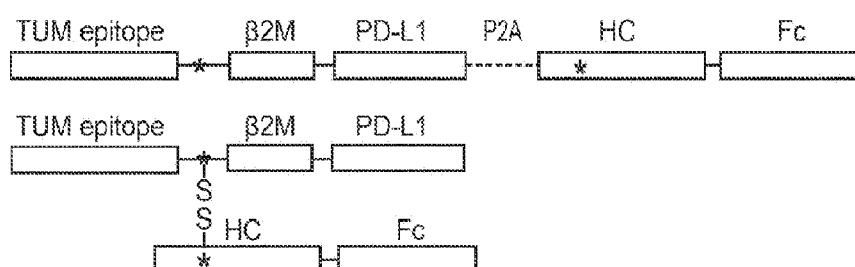


FIG. 19D

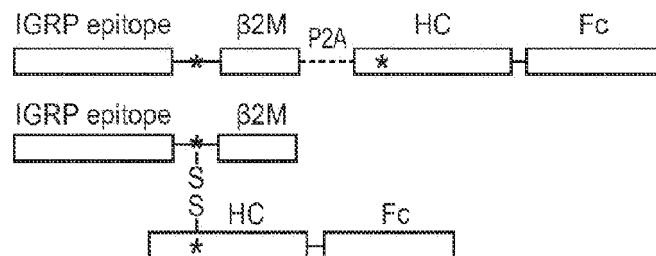


FIG. 19E

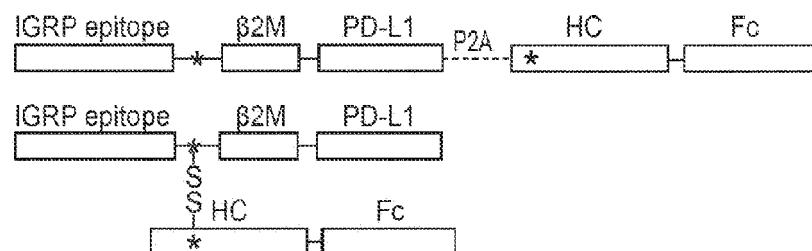


FIG. 19F

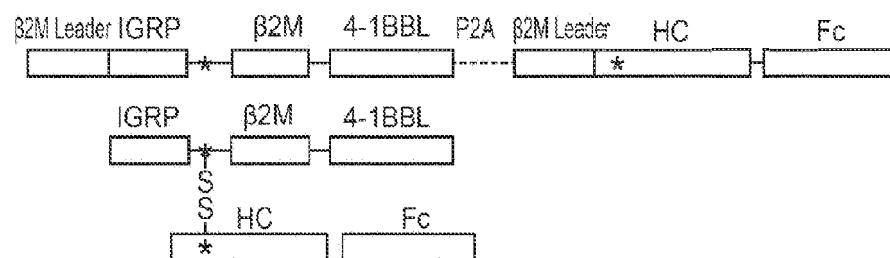


FIG. 19G

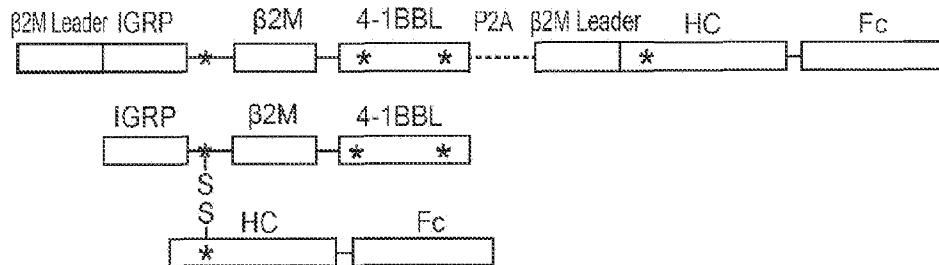


FIG. 19H

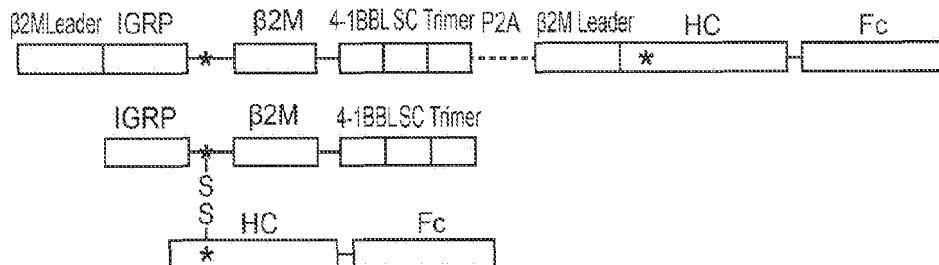


FIG. 191

FIG. 20

Domain Structure of the recombinant polypeptide construct of SEQ ID NO:6

## **DOUBLE UNDERLINE BOLD:** HTLV-1 epitope

UNDERLINE: Linker (cysteine modification in lowercase)

ITALIC: B2M domain

**BOLD ITALIC:** Mouse PD-L1 domain

## UNDERLINE IT ALL: P2A self-cleaving peptide

## **BOLD UNDERLINE**: B2M leader sequence

BOLD: Human heavy chain (cysteine-rich)

## **BOLD ITALIC UNDERLINE**: human Fc

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## Domain Structure of the recombinant polypeptide construct of SEQ ID NO:2

## **DOUBLE UNDERLINE BOLD: HTLV-1 epitope**

UNDERLINE: Linker (cysteine modification in lowercase)

*ITALIC: B2M domain*

## **BOLD ITALIC:** Mouse PD-L1 domain

UNDERLINE ITALIC: P2A self-cleaving peptide

**BOLD UNDERLINE**: B2M leader sequence

BOLD: Human heavy chain (cysteine)

## **BOLD ITALIC UNDERLINE**: human Fc

## **DOUBLE UNDERLINE: His Tag**

G 22

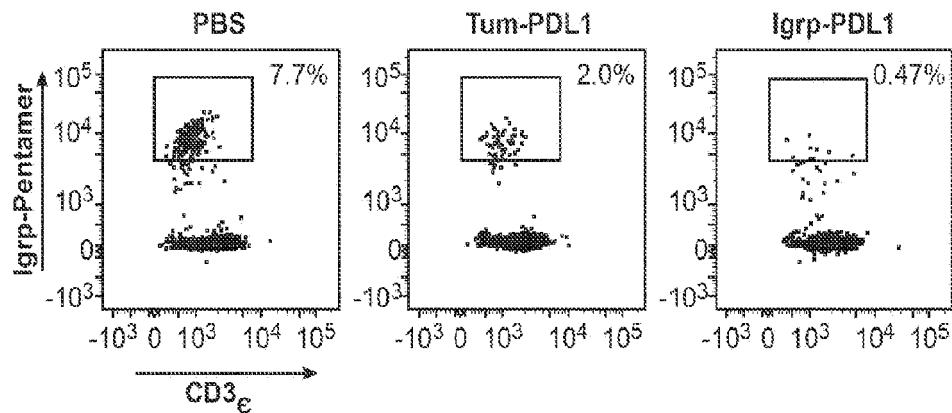


FIG. 23

GenBank 3S7G\_A  
*Homo sapiens IgG1 Fc* (SEQ ID NO:83)  
 227 aa

```

1 dkthtcppcp apellggpsv flfppkpkdt lmisrtpevt cvvvdvshed pevkfnwyvd
61 gvevhnaktk preeqynsty rvvsvltvlh qdwlngkeyk ckvsnkalpa piektiskak
121 gqppepqvyt lppsrdeltk nqvslltclvk gfypsdiaive wesngqpenn ykttppvlds
181 dgsfflyskl tvdksrwqgg nvfscsvmhe alhnhytqks lsłspgk

```

GenBank AAN76044  
*Homo sapiens IgG2 Fc* (amino acids 99-325) (SEQ ID NO:84)  
 227 aa

```

1 stkgpsvfpl apcsrstsae taalgclvkd yfpepvtvsw nsgaltsgvh tfpavlqssg
61 lyslssvvttv pssnfgtqty tcnvdhikpsn tkvdktverk ccvecppcpa ppvagpsvfl
121 fppkpkdtkl isrtpevtcv vvdvshedpe vqfnwyvdgv evhnaktkpr eeqfnstirv
181 vsvltvvhqd wlingkeykck vsnkglpapi ektisktkqq prepqvytlip psreemtknq
241 vslltclvkgf ypsdiavewe sngqpennyk tppmildsg sfflyskltv dksrwqggv
301 fscsvmheal hnhytqksls lsłpgk

```

GenBank AAW65947  
*Homo sapiens IgG3 Fc* (amino acids 19-246) (SEQ ID NO:85)  
 238 aa

```

1 hkpsntkvdk rvelktpi gd tthtcppcpa pellggpsvf lfppkpkdtl misrtpevtc
61 vvvvdvshedp evkfnwyvdg vevhnaktkp reeqynstyr vvsvltvlhq dwlngkeykc
121 kvsnkalpap iektiskakg qprepqvytl ppsrdeltkn qvslltclvk fypsdiaivew
181 esngqpenny ktppvlds qsflysklt vdksrwqgg vfcscsvmhea lhnhytqksl
241 slspgk

```

FIG. 24A

GenBank AAA52770

*Homo sapiens* IgD Fc (amino acids 162-383) (SEQ ID NO:86)  
222 aa

```

1 ptkapdvpipi isgcrhpkdn spvvlaclit gyhptevtvty wymgtqscpq rtfpeiqrid
61 syymtssqls tlpqqwraqgs ykcvvqhtas kskkeifrwp espkacassv ptaqpgaeqs
121 lakattapat tnttgrggee kkkekakeeq eeretktpec pshtqplgvy lltptavqdlw
181 lrkakatfcf wvgdlsdkdah ltwevagkqf tggveeglie rhangsqsqh srlltprelw
241 naqtsvtctl nhpslppqkl malrepaaqa pkvlslnlla ssdppeaaww licevsgfsp
301 pnillmwied grevntsgfa parpppqprs ttfwawsylr vpappspcpa tytcvvshed
361 srtllnasrs levsyvtcdh pmk

```

GenBank Q308221A

*Homo sapiens* IgM Fc (SEQ ID NO:87)  
276 aa

```

1 vtstltikzs dwlgesmftc rvdhrgltfq qnassmcvdpd qdtairvfai ppsfasiflt
61 kstktltclvt dittybsvii swtreengav kthtnisesh pnatfsavge asicadbdws
121 gerftctvth tdlpsplkgt isrpkgvalh rpbvylppa rzzlnresa titclvtqfs
181 padvfviewmq rgeplspgky vtsapmppeq apgryfahsi ltvseeewnt ggttytcvvah
241 ealpnryter tdkstgkpt lynvslvmsd tagtcty

```

**FIG. 24B**

GenBank P01876

*Homo sapiens* IgA Fc (amino acids 120-353) (SEQ ID NO:88)  
234 aa

```

1 asptspkvpf lslcstcpdq nvviaclvlgg ffpqeqpks vwsasggvta rnfppsqdas
61 qdlyttsqql tipatqplaq ksvtchvkhv tnpqdvtvp cpvpstppptp spstppptpsp
121 ecchprlslh rpaedtctit girdasqvtf twtpssgksa vyypperdic
181 qcyssvssvlp qcaeprwnhgk tftctaaope sktpltqas ksntfrpev hilpppseel
241 alneltvtlc largfspkdv lvrwlqgsq eprekyltis srqepsgttt tfavvisilrv
301 aeedwkkqdt fscmvghael plaftqktid rlaqkpthvn vsvvmaevdg tcy

```

GenBank 1F6A\_B

*Homo sapiens* IgE Fc (amino acids 6-222) (SEQ ID NO:89)  
212 aa

```

1 adpcdsnprg vsaylrspsp fdifirkspit itclvvdiap skgtvnltws rasgkpvnhs
61 trkeekqrgn tilvtstlpv qtrdwieget yqcrvthphl pralmrstdk tsgpraapev
121 yafatpewpg srdkrtlacl iqnmfpedis vqwlhnevql pdarhsttqp zktkgsgffv
181 fsrlevtrae weqkdaefior avheasapsq tvqravsvnp gk

```

GenBank P01861

*Homo sapiens* IgG4 Fc (amino acids 100-327) (SEQ ID NO:90)  
228 aa

```

1 astkgpsvfp lapcsrstse staaalgcivk dyfpepvtvs wasgaltsqv htfpavlgss
61 glyslssvvt vpssslgtkt ytcnvdhkpns ntkvdkrves kyppccpscp apeflggpsv
121 flpppkpkdt lmisrtpevt cvvvdvsqed pevqfnwyvd gvevhnaktk preeqinstk
181 rvvavltvih qdwlnqkeyk ckvsnkglps siektiskak ggprepgvvt lqpsqeeemtk
241 nqvslltclyk gfypsdiave wesngqpen ykttppvlds dgsfflysrl tvdksrwqeg
301 nvscsvwhe alhnhytqks lslslgk

```

**FIG. 24C**

*Homo sapiens*  
GenBank NP\_001229687  
HLA-A  
Amino acids 25-365 (SEQ ID NO:91)

```

1 mavmaprtll lllsgalalt qtwagshsmr yfftsvsrpg rgeprfiavg yvddtqfvrf
61 dsdaasqkma prapwieqeg peywdqetrn mkahsqtdra nlgtlrgyyn qsedgshtiq
121 imygedvgpd grflrgyrqd aydgkdyial nedlrshtaa dmaaqitkrk weavhaaeqr
181 rvylegrcvd glrrylengk etlqrtddpk thmthhpisd heatlrcwal gfypaeitlt
241 wqrdgedqtq dtelvetrpa gdgtfqkwa vvvpseeeqr ytchvqhegl pkpltlrwel
301 ssqptipivg iiaglvlga vitgavvaav mwrrkssdrk ggsytqaass dsaqgsdvsl
361 tackv

```

FIG. 25A

*Homo sapiens*  
GenBank NP\_005505  
HLA-B  
Amino acids 25-362 (SEQ ID NO:92)

```

1 mlvmaprtvl lllsaalalt etwagshsmr yfytsvsrpg rgeprfisvg yvddtqfvrf
61 dsdaaspree prapwieqeg peywdrntqi ykaqaqtdre sirnlrgyyn qseagshtlq
121 smygedvgpd grllrghdqy aydgkdyial nedlrshtaa dtaaqitqrk weaareeeqr
181 raylegcve wlrrylengk dkleradppk thvthhpisd heatlrcwal gfypaeitit
241 wqrdgedqtq dtelvetrpa gdrtfqkwa vvvpseeeqr ytchvqhegl pkpltlrwep
301 ssqstvpivg ivaglavlav vvigavvaav mcccrrkssggk ggsysqaacs dsaqgsdvsl
361 ta

```

FIG. 25B

*Homo sapiens*  
GenBank NP\_001229971  
HLA-C  
Amino acids 25-366 (SEQ ID NO:93)

```

1 mrvmaprall lllsgglalt etwacshsmr yfdtavsrpg rgeprfisvg yvddtqfvrf
61 dsdaaspzge prapwveqeg peywdretqn ykrqaqadrv sirnlrgyyn qsedgshtiq
121 rmygcdlgpd grllrgydzs aydgkdyial nedlrshtaa dtaaqitqrk leaaraaeql
181 raylegtcve wlrrylengk etlgraeppk thvthhpisd heatlrcwal gfypaeitlt
241 wqrdgedqtq dtelvetrpa gdgtfqkwa vvvpseeeqr ytchmchegl qepltlswep
301 ssqptipimg ivaglavlvv lavlgavvta mmccrrkssgg kggscsqaac snsaggsdes
361 litcka

```

FIG. 25C

PD-L1

*Mus musculus*

NP\_068693

Amino acids 19-290 (SEQ ID NO:94)

1 mrifagiiift acchlraft itapkdllyvv eygsnvtmec rfpvereldl lalvvyweke  
 61 deqviqfvag eedlkipqhsn frgraslpkd qlkgnaalq itdvlqdag vyciisygg  
 121 adykrilky napyrkingr isvdpatseh elicqaegyp eaeviwtnd hcpvsgxrsv  
 181 ttsrtegmlt nvtsslrnna tandvfyctf wrsqpgcnht aelielpa thppqrnrthw  
 241 vllgsillfl ivvstvllfl rkqvrmidve kcgvedtssk nrndtqfeet

**FIG. 26A**

PD-L1

*Homo sapiens*

NP\_054852

Amino acids 19-290 (SEQ ID NO:95)

1 mrifavfifm tywhllnaft vtvpkdlyvv eygsnmtie kfpvekqlldl aalivyweme  
 61 dkniqfvhg eedikvghss yrqrarilkd qlsignaqlq itdvlqdag vyrcmisygg  
 121 adykritykv napynkingr ilvvdpvtse heltcqaegy pkaeviwtss dhqvlsgktt  
 181 ttnskreekl fnvtstlrin tttneifyct frrldpeen taelvipelp lahppnerth  
 241 lvilgaille lgvaltfifr lrggrmmvdvk kcgqdttsk kqsdthleet

**FIG. 26B**

4-1-BBL

*Homo sapiens*

GenBank NP\_003802

Amino acids 50-254 = ectodomain (SEQ ID NO:96)

1 meyasdasld peapwppapr aracrvlpwa lvagllllll laaacavfla cpwavsgara  
 61 spgsaasprl regpelspdd pagllcrlrgg mfaqlvaqnv llidgplswy sdpglagvsi  
 121 tgglysykedt kelvvakagv yyvffqlelr rvvagegssgs vslalhlqpl rsaagaala  
 181 ltvdlpass earnsaqfq grlhlsagg rlgvhltea rarhawqltq gatvlgfrv  
 241 tpeipaglps prse

**FIG. 27***Homo sapiens*

ICOS-L

GenBank NP\_056074

Amino acids 19-302 (SEQ ID NO:97)

1 mrlgspgllf llfsslradt qekavramvg sdvelscacp egsrfdlndv yvywqtseks  
 61 tvvtyhipqn sslenvdsry rnralmspag mlrgdfslrl fnvtpqdeqk fhclvlsqsl  
 121 qfqevlsvev tlhvaanfsv pvvaphspq qdeltftcts ingyprpnvy winktdnsll  
 181 dgalgndtvf lnmrqlydvv svlriartps vnigccienv llqqnltvgs qtgndigerd  
 241 kitenvpstg eknaatwsil avlcllvvva vaigwvcrdr clqhsyagaw avspeteltg  
 301 hv

**FIG. 28**

*Homo sapiens*  
GenBank NP\_003317  
OX4L (SEQ ID NO:98)

1 mervqpleen vgnaarprfe rnklilvasv igglgllicf tyiclhfsal qvshrypriq  
61 sikvqfteyk kekgfiltsq kedeimkvqn nsviincdgf ylislkgyfs qevnislhqyq  
121 kdeoplqlk krvsvnslmv asltykdkvq lnvtdnts1 ddfhvnggel ilihqnpgef  
181 cv1

FIG. 29

*Homo sapiens*  
GenBank NP\_079515  
PD-L2  
Amino acids 20-273 (SEQ ID NO:99)

1 miflllmlsl elqlhqiaal ftvtvpkely iiehgsnvt1 ecnfdtgshv nlgaitaslg  
61 kvendtsphr eratlleeq1 plgkasfhip qvqvrdeqgy qciiiygvaw dykyltlkvk  
121 asyrkinti lkvpetdeve ltcqatgypl aevswpnvs1 pantshsrt1 egiyqvt1v  
181 r1kppgrnf scv1wnthvr eltlasidiq sqmeprthpt w1lhifipfc iiafifiatv  
241 ialrkqlcqk lysskdttkr pvt1tkre1n sai

FIG. 30

*Homo sapiens*  
GenBank NP\_005182  
CD80 (B7-1)  
Amino acids 35-288 (SEQ ID NO:100)

1 mghtrrqgts pskc1ylnff q11vlag1sh fcsqvi1v1tk evkevat1sc ghnvsveela  
61 qtriywqkek kmvl1tmnsgd mniwpeyknr t1fditnn1s i1v1alr1psd eg1yecvv1k  
121 yekdakfreh laev11svka d1f1t1ps1s1df e1ptshirri ic1stsggfpe ph1s1w1l1nge  
181 elnain1tt1vs qd1petelyav ssk1df1n1mtt m1s1fm1c1ky ghlrvnq1t1n w1tt1kqeh1p  
241 dn1l1ps1wait 11s1vng1f1vi cc1t1yc1f1apr cr1err1ner1 r1res1vr1v

FIG. 31

*Homo sapiens*  
GenBank NP\_787058  
CD86 (B7-2)  
Amino acids 31-329 (SEQ ID NO:101)

1 mdpqctmg1s n1lfvma111 sgaaplki1q1 yfnetad1pc qf1ansqnc1 selvvfwqdq  
61 en1vlnev1l gkekfd1svhs kymgr1tsf1ds d1sw1l1h1n1 q1kdkg1yq1c i1hhkkptgm  
121 ir1hqmn1sel svlanf1sqpe ivp1sn1ten vyin1t1c1ssi hg1y1pe1kk1ms v1l1rt1kn1sti  
181 eyd1q1m1q1k1sq dn1tely1d1vs is1s1vs1fp1dv t1sn1mt1f1c1l1 etd1k1r1l1ss p1f1s1e1d1p1q  
241 pppd1h1p1w1t1 av1l1pt1vi1c1v1 m1vf1cl1l1w1k1 k1kk1r1p1r1n1sy k1cg1t1n1m1re1 e1s1eq1t1kk1rek  
301 ih1p1ers1de1a q1rv1f1k1ss1k1ts sc1dk1s1d1t1cf

FIG. 32

Fas ligand (FasL)  
*Homo sapiens*  
 GenBank NP\_000630  
 Amino acids 1-281 (SEQ ID NO:102)

```

  1 mqqpfnypyp qiyvdssas spwappgtvl pcptsvprrp gqrrpppppp ppplpoooo
  61 ppipplipp lkkrgnhstg lcllvaffmv lvalvgigig mfqlfhlgkc lacirostsq
  121 mhtasslekg ighpspppek kelrkvahgksnsrsmpl ewedtygivl lsgvkykkgg
  181 lvinetglyf vyskvyfrqg scnnlplishk vymrnskypq dlvmmegkmm sycttgqmwa
  241 rssylgavfn ltsadhllyn vselslvnfe esqtfglyk 1
  
```

FIG. 33

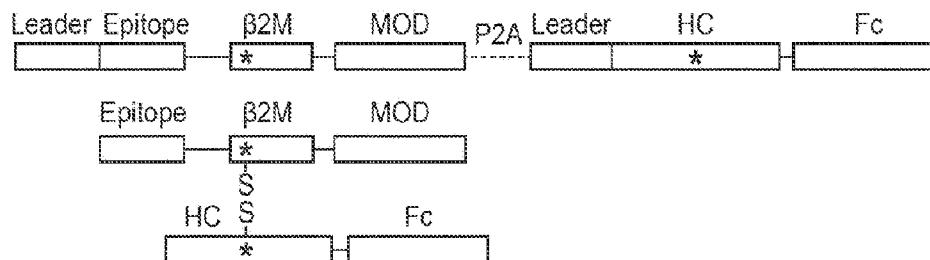


FIG. 34A

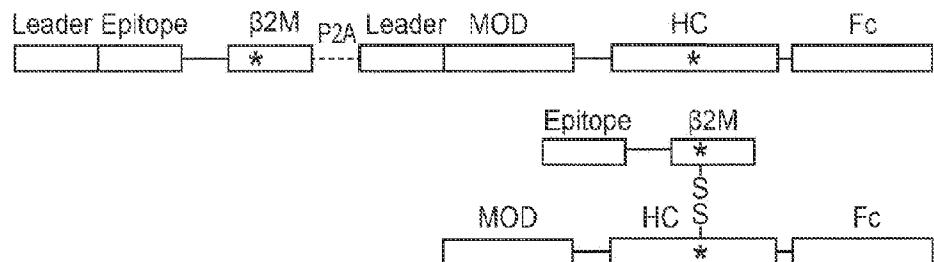


FIG. 34B

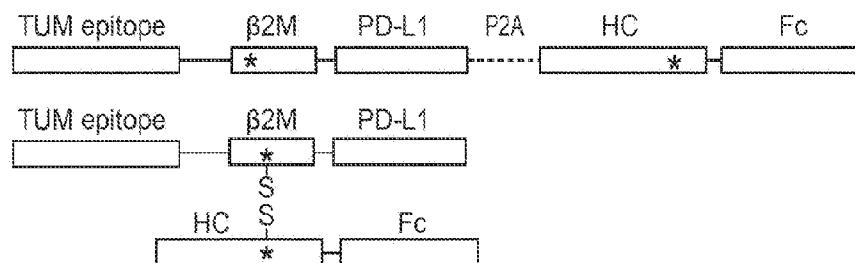


FIG. 34C

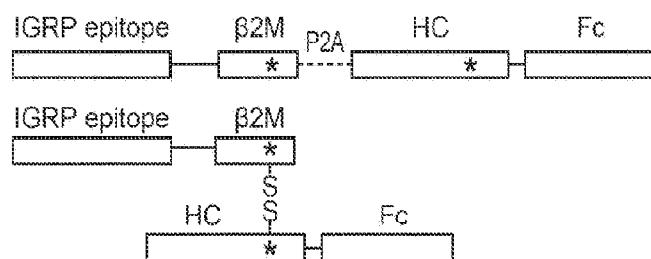


FIG. 34D

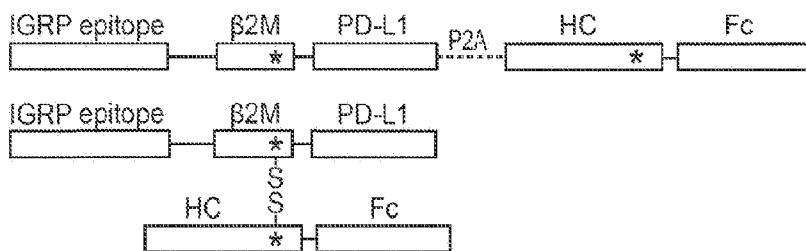


FIG. 34E

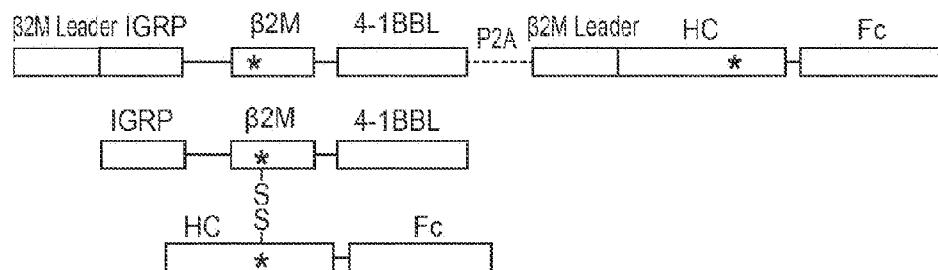


FIG. 34F

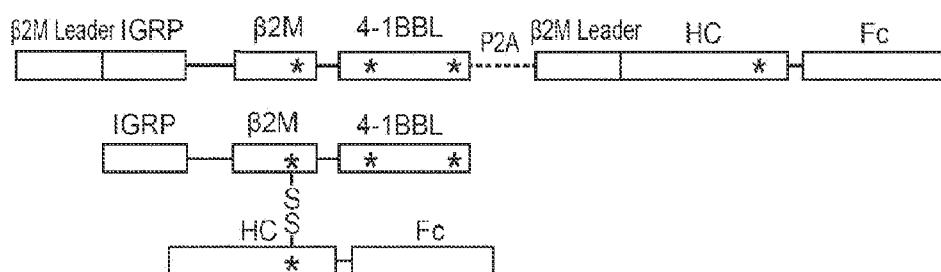


FIG. 34G

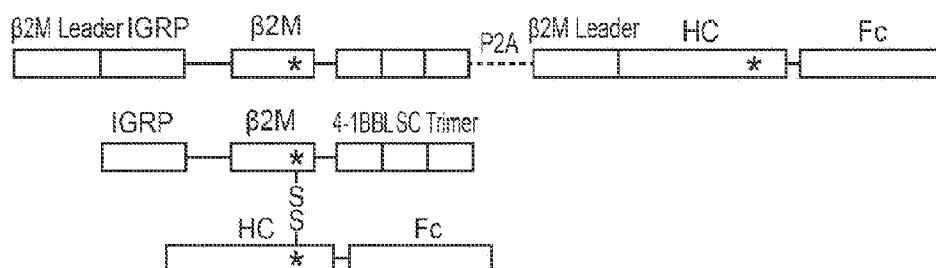


FIG. 34H