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(71) Applicant: **CUE BIOPHARMA, INC.** [US/US]; 675 West Kendall Street, Cambridge, Massachusetts 02142 (US).

(72) Inventors: **SEIDEL, III, Ronald D.**; 675 West Kendall Street, Cambridge, Massachusetts 02142 (US). **CHAPARRO, Rodolfo**; 675 West Kendall Street, Cambridge, Massachusetts 02142 (US).

(74) Agent: **BORDEN, Paula A.**; 201 Redwood Shores Parkway, Suite 200, Redwood City, California 94065 (US).

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(54) Title: T-CELL MODULATORY MULTIMERIC POLYPEPTIDES AND METHODS OF USE THEREOF

(57) Abstract: The present disclosure provides variant immunomodulatory polypeptides, and fusion polypeptides comprising the variant immunomodulatory peptides. The present disclosure provides T-cell modulatory multimeric polypeptides, and compositions comprising same, where the T-cell modulatory multimeric polypeptides comprise a variant immunomodulatory polypeptide of the present disclosure. The present disclosure provides nucleic acids comprising nucleotide sequences encoding the T-cell modulatory multimeric polypeptides, and host cells comprising the nucleic acids. The present disclosure provides methods of modulating the activity of a T cell; the methods comprise contacting the T cell with a T-cell modulatory multimeric polypeptide of the present disclosure.

T-CELL MODULATORY MULTIMERIC POLYPEPTIDES AND METHODS OF USE THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/302,654, filed March 2, 2016, which application is incorporated herein by reference in its entirety.

INTRODUCTION

[0002] An adaptive immune response involves the engagement of the T cell receptor (TCR), present on the surface of a T cell, with a small peptide antigen non-covalently presented on the surface of an antigen presenting cell (APC) by a major histocompatibility complex (MHC; also referred to in humans as a human leukocyte antigen (HLA) complex). This engagement represents the immune system's targeting mechanism and is a requisite molecular interaction for T cell modulation (activation or inhibition) and effector function. Following epitope-specific cell targeting, the targeted T cells are activated through engagement of costimulatory proteins found on the APC with counterpart costimulatory proteins the T cells. Both signals – epitope/TCR binding and engagement of APC costimulatory proteins with T cell costimulatory proteins – are required to drive T cell specificity and activation or inhibition. The TCR is specific for a given epitope; however, the costimulatory protein not epitope specific and instead is generally expressed on all T cells or on large T cell subsets.

SUMMARY

[0003] The present disclosure provides variant immunomodulatory polypeptides, and fusion polypeptides comprising the variant immunomodulatory peptides. The present disclosure provides T-cell modulatory multimeric polypeptides, and compositions comprising same, where the T-cell modulatory multimeric polypeptides comprise a variant immunomodulatory polypeptide of the present disclosure. The present disclosure provides nucleic acids comprising nucleotide sequences encoding the T-cell modulatory multimeric polypeptides, and host cells comprising the nucleic acids. The present disclosure provides methods of modulating the activity of a T cell; the methods comprise contacting the T cell with a T-cell modulatory multimeric polypeptide of the present disclosure.

[0004] The present disclosure provides a variant CD80 immunomodulatory polypeptide comprising an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the CD80 amino acid sequence depicted in FIG. 2A or to

the CD80 amino acid sequence set forth in SEQ ID NO:1, wherein the variant CD80 immunomodulatory polypeptide has one or more amino acid substitutions relative to the CD80 amino acid sequence depicted in FIG. 2A or to the CD80 amino acid sequence set forth in SEQ ID NO:1; and wherein the variant CD80 immunomodulatory polypeptide exhibits reduced binding affinity to a CD86 polypeptide having an amino acid sequence depicted in one of FIG. 3A-C, compared to the binding affinity of the CD80 amino acid sequence depicted in FIG. 2A, or compared to the binding affinity of the CD80 amino acid sequence as set forth in SEQ ID NO:1, for the CD86 polypeptide. In some cases, the polypeptide comprises a substitution of amino acid N19, N63, I67, K86, Q157, D158, L25, Y31, Q33, M38, V39, I49, Y53, D60, F108, or S156. In some cases, the polypeptide comprises a substitution of amino acid N19. In some cases, the polypeptide comprises a substitution of amino acid I67. In some cases, the polypeptide comprises a substitution of amino acid K86. In some cases, the polypeptide comprises a substitution of amino acid Q157. In some cases, the polypeptide comprises a substitution of amino acid D158. In some cases, the polypeptide comprises a substitution of amino acid L25. In some cases, the polypeptide comprises a substitution of amino acid Y31. In some cases, the polypeptide comprises a substitution of amino acid Q33. In some cases, the polypeptide comprises a substitution of amino acid M38. In some cases, the polypeptide comprises a substitution of amino acid V39. In some cases, the polypeptide comprises a substitution of amino acid I49. In some cases, the polypeptide comprises a substitution of amino acid Y53. In some cases, the polypeptide comprises a substitution of amino acid D60. In some cases, the polypeptide comprises a substitution of amino acid F108. In some cases, the polypeptide comprises a substitution of amino acid S156. In some cases, the variant immunomodulatory polypeptide exhibits from less than 10% to less than 50% of binding affinity exhibited by to the CD80 amino acid sequence depicted in FIG. 2A, or as set forth in SEQ ID NO:1, for the CD86 polypeptide.

[0005] 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 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, 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, wherein the immunomodulatory domain is a variant immunomodulatory polypeptide as described above, or

elsewhere herein; and wherein the multimeric polypeptide exhibits reduced binding affinity to a CD28 polypeptide having an amino acid sequence depicted in one of FIG. 3A-3C, compared to the binding affinity of a control multimeric polypeptide comprising an immunomodulatory domain comprising the CD80 amino acid sequence depicted in FIG. 2A, or compared to the binding affinity of a control multimeric polypeptide comprising an immunomodulatory domain comprising the CD80 amino acid sequence as set forth in SEQ ID NO:1, for the CD28 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 iv) 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 elastin-like polypeptide, a silk-like polypeptide, or a silk-elastin-like polypeptide. In some cases, the first MHC polypeptide is a β2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide. In some cases, the β2-microglobulin polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to one of the amino acid sequences set forth in FIG. 6. 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 one of FIG. 5A-5C. 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% amino acid sequence identity to an amino acid sequence depicted in FIG. 4A-4C. 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 variant CD80 immunomodulatory polypeptide comprises a substitution of amino acid N19, N63, I67, K86, Q157, D158, L25, Y31, Q33, M38, V39, I49, Y53, D60, F108, or S156. In some cases, the multimeric polypeptide comprises 2 or more immunomodulatory polypeptides. In some cases, the multimeric polypeptide comprises 3 immunomodulatory polypeptides. In some cases, the 2 or more (e.g., 3) 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.

[0006] 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 major histocompatibility complex (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; wherein the immunomodulatory polypeptide is a variant immunomodulatory

polypeptide as described above, or elsewhere herein; 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, wherein the immunomodulatory polypeptide is a variant immunomodulatory polypeptide as described above, or elsewhere herein. In some cases, the first MHC polypeptide is a β2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide. In some cases, the β2-microglobulin polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to one of the amino acid sequences set forth in FIG. 6. 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 any one of FIG. 5A-5C. 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 4A-4C. In some cases, the variant CD80 immunomodulatory polypeptide comprises a substitution of amino acid N19, N63, I67, K86, Q157, D158, L25, Y31, Q33, M38, V39, I49, Y53, D60, F108, or S156. In some cases, the multimeric polypeptide comprises a second immunomodulatory polypeptide 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:78); b) ENLYTQS (SEQ ID NO:79); c) a furin cleavage site; d) LVPR (SEQ ID NO:80); e) GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:81); f) GSGEGRGSLLTCGDVEENPGP (SEQ ID NO:82); g) GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO:83); and h) GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:84). 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 β2-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 a nucleic acid as described above or elsewhere herein. In some cases, the vector is a viral vector or a non-viral vector. The present disclosure provides a host cell genetically modified with a recombinant expression vector as described above or 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 β2-microglobulin polypeptide. In some cases, the host cell is a T lymphocyte.

[0007] 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, wherein the immunomodulatory domain is a variant immunomodulatory polypeptide as described above or elsewhere herein; 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. In some cases, the first and/or the second nucleic acid is present in a recombinant expression vector. 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, wherein the immunomodulatory domain is a variant immunomodulatory polypeptide as described above or elsewhere herein; 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 as described above or elsewhere herein.

[0008] The present disclosure provides a method of producing a multimeric polypeptide as described above or elsewhere herein, the method comprising: a) culturing a genetically modified 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.

[0009] 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 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*.

[0010] 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.

[0011] 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 a multimeric polypeptide as described above or elsewhere herein; or c) one or more mRNAs comprising nucleotide sequences encoding a multimeric polypeptide as described above or elsewhere herein, 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, said administering is subcutaneous. In some cases, said administering is intravenous. In some cases, said administering is intramuscular. In some cases, said administering is systemic. In some cases, said administering is distal to a

treatment site. In some cases, said administering is local. In some cases, said administering is at or near a treatment site.

[0012] The present disclosure provides a composition comprising: a) a multimeric polypeptide as described above or elsewhere herein; and b) a pharmaceutically acceptable excipient.

[0013] 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

[0014] FIG. 1A-1D schematically depict various embodiments of a T-cell modulatory multimeric polypeptide of the present disclosure. In these embodiments, disulfide bonds are formed between MHC (e.g., HLA) polypeptides present in separate polypeptides.

[0015] FIG. 2A-2II provide an amino acid sequence of a CD80 (FIG. 2A) and examples of variant CD80 polypeptides (FIG. 2B-2II).

[0016] FIG. 3A-3D provide amino acid sequences of CD28 (FIG. 3A-3C) and CTLA4 (FIG. 3D).

[0017] FIG. 4A-4C provide amino acid sequences of immunoglobulin Fc polypeptides.

[0018] FIG. 5A-5C provide amino acid sequences of human leukocyte antigen (HLA) Class I heavy chain polypeptides. Signal sequences are underlined.

[0019] FIG. 6 provides a multiple amino acid sequence alignment of beta-2 microglobulin (β2M) precursors (i.e., including the leader sequence) from *Homo sapiens* (NP_004039.1; SEQ ID NO:52), *Pan troglodytes* (NP_001009066.1; SEQ ID NO:53), *Macaca mulatta* (NP_001040602.1; SEQ ID NO:54), Bos Taurus (NP_776318.1; SEQ ID NO:55) and Mus musculus (NP_033865.2; SEQ ID NO:56). Amino acids 1-20 are a signal peptide.

[0020] FIG. 7A-7B provide amino acid sequences of PD-L1 polypeptides.

[0021] FIG. 8 provides an amino acid sequence of a 4-1BBL polypeptide.

[0022] FIG. 9 provides an amino acid sequence of an ICOS-L polypeptide.

[0023] FIG. 10 provides an amino acid sequence of an OX40L polypeptide.

[0024] FIG. 11 provides an amino acid sequence of a PD-L2 polypeptide.

[0025] FIG. 12 provides an amino acid sequence of a CD86 (B7-2) polypeptide.

[0026] FIG. 13 provides an amino acid sequence of a Fas ligand (FAS-L) polypeptide.

[0027] FIG. 14 depicts interferon-gamma (IFN-γ) secretion by target cells contacted with a synTac polypeptide according to an embodiment of the present disclosure.

[0028] FIG. 15 depicts interleukin-2 (IL-2) secretion by target cells contacted with a synTac polypeptide according to an embodiment of the present disclosure.

[0029] FIG. 16 depicts interleukin-6 (IL-6) secretion by target cells contacted with a synTac polypeptide according to an embodiment of the present disclosure.

[0030] FIG. 17 depicts tumor necrosis factor (TNF) secretion by target cells contacted with a synTac polypeptide according to an embodiment of the present disclosure.

[0031] FIG. 18 depicts proliferation of target cells contacted with a synTac polypeptide according to an embodiment of the present disclosure.

[0032] FIG. 19 depicts viability of target cells contacted with a synTac polypeptide according to an embodiment of the present disclosure.

[0033] FIG. 20 depicts the *in vivo* effect of a synTac polypeptide of the present disclosure on tumor volume.

DEFINITIONS

[0034] The terms “polynucleotide” and “nucleic acid,” used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases.

[0035] 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.

[0036] A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same, and in the same relative position, when comparing the two sequences. Sequence identity can be determined in a number of different ways. To determine sequence identity, sequences can be aligned using various convenient methods and computer programs (e.g., BLAST, T-COFFEE, MUSCLE, MAFFT, etc.), available over the world wide web at sites including ncbi.nlm.nili.gov/BLAST, ebi.ac.uk/Tools/msa/tcoffee/, ebi.ac.uk/Tools/msa/muscle/, mafft.cbrc.jp/alignment/software/. See, e.g., Altschul et al. (1990), J. Mol. Bioi. 215:403-10.

[0037] The term "conservative amino acid substitution" refers to the interchangeability in proteins of amino acid residues having similar side chains. For example, a group of amino acids having aliphatic side chains consists of glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains consists of serine and threonine; a group of

amino acids having amide containing side chains consisting of asparagine and glutamine; a group of amino acids having aromatic side chains consists of phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains consists of lysine, arginine, and histidine; a group of amino acids having acidic side chains consists of glutamate and aspartate; and a group of amino acids having sulfur containing side chains consists of cysteine and methionine. Exemplary conservative amino acid substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine-glycine, and asparagine-glutamine.

[0038] "Binding" as used herein (e.g. with reference to binding of a T-cell modulatory multimeric polypeptide of the present disclosure to a polypeptide (e.g., a T-cell receptor) on a T cell) refers to a non-covalent interaction between. Binding interactions are generally characterized by a dissociation constant (K_D) of less than 10^{-6} M, less than 10^{-7} M, less than 10^{-8} M, less than 10^{-9} M, less than 10^{-10} M, less than 10^{-11} M, less than 10^{-12} M, less than 10^{-13} M, less than 10^{-14} M, or less than 10^{-15} M. "Affinity" refers to the strength of binding, increased binding affinity being correlated with a lower K_D.

[0039] 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; the disclosure of which is incorporated herein by reference in its entirety.

[0040] "T cell" includes all types of immune cells expressing CD3, including T-helper cells ($CD4^+$ cells), cytotoxic T-cells ($CD8^+$ cells), T-regulatory cells (Treg), and NK-T cells.

[0041] "Co-stimulatory polypeptide," as the term is used herein, includes a polypeptide on an antigen presenting cell (APC) (e.g., a dendritic cell, a B cell, and the like) that specifically binds a cognate co-stimulatory polypeptide 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 a major histocompatibility complex (MHC) polypeptide 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, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds to CD83.

[0042] A "modulatory domain" of a T-cell modulatory multimeric polypeptide of the present disclosure comprises a co-stimulatory polypeptide.

[0043] "Heterologous," as used herein, means a nucleotide or polypeptide that is not found in the native nucleic acid or protein, respectively.

[0044] "Recombinant," as used herein, means that a particular nucleic acid (DNA or RNA) is the product of various combinations of cloning, restriction, polymerase chain reaction (PCR) and/or ligation steps resulting in a construct having a structural coding or non-coding sequence distinguishable from endogenous nucleic acids found in natural systems. DNA sequences encoding polypeptides can be assembled from cDNA fragments or from a series of synthetic oligonucleotides, to provide a synthetic nucleic acid which is capable of being expressed from a recombinant transcriptional unit contained in a cell or in a cell-free transcription and translation system.

[0045] The terms "recombinant expression vector," or "DNA construct" are used interchangeably herein to refer to a DNA molecule comprising a vector and one insert. Recombinant expression vectors are usually generated for the purpose of expressing and/or propagating the insert(s), or for the construction of other recombinant nucleotide sequences. The insert(s) may or may not be operably linked to a promoter sequence and may or may not be operably linked to DNA regulatory sequences.

[0046] A cell has been "genetically modified" or "transformed" or "transfected" by exogenous DNA, e.g. a recombinant expression vector, when such DNA has been introduced inside the cell. The presence of the exogenous DNA results in permanent or transient genetic change. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell. In prokaryotes, yeast, and mammalian cells, for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication.

[0047] 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.

[0048] The terms "treatment", "treating" and the like are used herein to generally mean 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 or symptom in a mammal, and includes: (a) preventing the disease or symptom from occurring in a subject which may be predisposed to acquiring the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease or symptom, i.e., arresting its development; or (c) relieving the disease, i.e., causing regression of the disease. The therapeutic agent may be administered before, during or after the onset of disease or injury. The treatment of ongoing disease, where the treatment stabilizes or reduces the undesirable clinical symptoms of the patient, is of particular interest. Such treatment is desirably performed prior to complete loss of function in the affected tissues. The subject therapy will desirably be administered during the symptomatic stage of the disease, and in some cases after the symptomatic stage of the disease.

[0049] The terms "individual," "subject," "host," and "patient," are used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired. Mammals include, e.g., humans, non-human primates, rodents (e.g., rats; mice), lagomorphs (e.g., rabbits), ungulates (e.g., cows, sheep, pigs, horses, goats, and the like), etc.

[0050] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. 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.

[0051] 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.

[0052] 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. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0053] 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 modulatory domain" includes a plurality of such modulatory domains and reference to "the HLA polypeptide" includes reference to one or more HLA 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.

[0054] 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.

[0055] 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

[0056] The present disclosure provides variant immunomodulatory polypeptides, and fusion polypeptides comprising the variant immunomodulatory peptides. The present disclosure provides T-cell modulatory multimeric polypeptides, and compositions comprising same, where the T-cell modulatory multimeric polypeptides comprise a variant immunomodulatory polypeptide of the present disclosure. The present disclosure provides nucleic acids comprising nucleotide sequences encoding the T-cell modulatory multimeric polypeptides, and host cells comprising the nucleic acids. The present disclosure provides methods of modulating the activity of a T cell; the methods comprise contacting the T cell with a T-cell modulatory multimeric polypeptide of the present disclosure.

[0057] A T-cell modulatory multimeric polypeptide of the present disclosure is also referred to as a "synTac polypeptide." A synTac polypeptide of the present disclosure comprises a variant modulatory domain, where the variant modulatory domain exhibits reduced binding affinity to an immunomodulatory polypeptide, compared to the affinity of a wild-type modulatory domain for the immunomodulatory polypeptide. A synTac polypeptide of the present disclosure can modulate the activity of a target T-cell. A synTac polypeptide of the present disclosure provides for enhanced target cell specificity.

VARIANT IMMUNOMODULATORY POLYPEPTIDES

[0058] The present disclosure provides variant CD80 modulatory polypeptides. A wild-type amino acid sequence of human CD80 is provided in FIG. 2A. The ectodomain of human CD80 comprises amino acids 1-208 of the amino acid sequence depicted in FIG. 2A. Thus, a wild-type amino acid sequence of the ectodomain of human CD80 can be as follows:

[0059] VIHVTK EVKEVATLSC GHNVSVEELA QTRIYWQKEK KMVLTMMSGD MNIWPEYKNR TIFDITNNLS IVILALRPSD EGTYECVVLK YEKDAFKREH LAEVTLSVKA DFPTPSISDF EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DN (SEQ ID NO:1).

[0060] Wild-type CD80 binds to CD28 and to CTLA4. Amino acid sequences of CD28 are provided in FIG. 3A-3C. An amino acid sequence of CTLA4 is provided in FIG. 3D. A variant CD80

polypeptide of the present disclosure binds to CD80 and/or CTLA4 with reduced affinity compared to binding of wild-type CD80 to CD28 or to CTLA4.

[0061] In some cases, a variant CD80 polypeptide of the present disclosure exhibits reduced binding affinity to CD28, compared to the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A for CD28. For example, in some cases, a variant CD80 polypeptide of the present disclosure binds CD28 with a binding affinity that is less than the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A for a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C. For example, in some cases, a variant CD80 polypeptide of the present disclosure binds CD28 with a binding affinity that is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C).

[0062] In some cases, a variant CD80 polypeptide of the present disclosure exhibits reduced binding affinity to CD28, compared to the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for CD28. For example, in some cases, a variant CD80 polypeptide of the present disclosure binds CD28 with a binding affinity that is less than the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C. For example, in some cases, a variant CD80 polypeptide of the present disclosure binds CD28 with a binding affinity that is at least 10% less, at least 15% less, at least 20% less, at least 25%, at least 30% less, at least 35% less, at least 40% less, at least 45% less, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C).

[0063] In some cases, a variant CD80 polypeptide of the present disclosure has a binding affinity to CD28 that is from 100 nM to 100 μM. As another example, in some cases, a variant CD80 polypeptide of the present disclosure has a binding affinity for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C) that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to

about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0064] In some cases, a variant CD80 polypeptide of the present disclosure exhibits reduced binding affinity to CTLA4, compared to the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A. For example, in some cases, a variant CD80 polypeptide of the present disclosure binds CTLA4 with a binding affinity that less than the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A for a CTLA4 polypeptide comprising the amino acid sequence depicted in FIG. 3D. For example, in some cases, a variant CD80 polypeptide of the present disclosure binds CTLA4 with a binding affinity that is at least 10% less, at least 15% less, at least 20% less, at least 25%, at least 30% less, at least 35% less, at least 40% less, at least 45% less, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A for CTLA4 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in FIG. 3D).

[0065] A variant CD80 polypeptide of the present disclosure can have a single amino acid substitution relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide of the present disclosure has from 2 to 10 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide of the present disclosure has 2 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide of the present disclosure has 3 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide of the present disclosure has 4 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide of the present disclosure has 5 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A

or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide of the present disclosure has 6 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide of the present disclosure has 7 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide of the present disclosure has 8 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide of the present disclosure has 9 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide of the present disclosure has 10 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1).

[0066] In some cases, a variant CD80 polypeptide of the present disclosure has from 11 to 50 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). For example, in some cases, a variant CD80 polypeptide of the present disclosure has from 11 to 15, from 15 to 20, from 20 to 25, from 25 to 30, from 30 to 35, from 35 to 40, from 40 to 45, or from 45 to 50, amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1).

[0067] A variant CD80 polypeptide of the present disclosure can have a length of from 150 to 254 amino acids. For example, in some cases, a variant CD80 polypeptide of the present disclosure has a length of from 150 amino acids to 175 amino acids, from 175 amino acids to 200 amino acids, from 200 amino acids to 225 amino acids, or from 225 amino acids to 254 amino acids. In some cases, a variant CD80 polypeptide of the present disclosure has a length of from 200 amino acids to 225 amino acids. In some cases, a variant CD80 polypeptide of the present disclosure has a length of from 200 amino acids to 210 amino acids. In some cases, a variant CD80 polypeptide of the present disclosure has a length of from 205 amino acids to 210 amino acids. In some cases, a variant CD80 polypeptide of the present disclosure has a length of 208 amino acids.

N19 substitution

[0068] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence

identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is an amino acid other than an asparagine, e.g., where amino acid 19 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0069] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at N19. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at N19. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B,

where "x" is any amino acid other than asparagine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where "x" is Ile. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2C. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

N63 Substitution

[0070] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is an amino acid other than an asparagine, e.g., where amino acid 63 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid

sequence depicted in FIG. 2D, where amino acid 63 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0071] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at N63. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at N63. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is any amino acid other than asparagine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is Ile.

For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

I67 Substitution

[0072] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is an amino acid other than an isoleucine, e.g., where amino acid 67 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is Ala, Gly, Val, or Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is Leu. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM,

from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0073] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at I67. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at I67. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at I67. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is any amino acid other than asparagine; for example, "x" can be Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is Ala, Gly, Val, or Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is Gly. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

K86 Substitution

[0074] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence

identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is an amino acid other than a lysine, e.g., where amino acid 86 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0075] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at K86. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at K86. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H,

where "x" is any amino acid other than lysine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where "x" is Ile. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Q157 Substitution

[0076] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is an amino acid other than a glutamine, e.g., where amino acid 157 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid

sequence depicted in FIG. 2J, where amino acid 157 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0077] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Q157. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Q157. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is any amino acid other than glutamine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Ile. For

example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

D158 Substitution

[0078] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is an amino acid other than an aspartic acid, e.g., where amino acid 158 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about

150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0079] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at D158. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at D158. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is any amino acid other than aspartic acid; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Ile. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

L25 substitution

[0080] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is an amino acid other than a leucine, e.g., where amino acid 25 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is Ala, Gly, Val, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0081] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at L25. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L25. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N,

where "x" is any amino acid other than leucine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where "x" is Ala, Gly, Val, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where "x" is Ile. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Y31 substitution

[0082] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is an amino acid other than a tyrosine, e.g., where amino acid 31 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid

sequence depicted in FIG. 2P, where amino acid 31 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0083] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Y31. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Y31. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is any amino acid other than tyrosine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is Ile.

For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Q33 substitution

[0084] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is an amino acid other than a glutamine, e.g., where amino acid 33 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about

150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0085] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Q33. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Q33. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is any amino acid other than glutamine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Ile. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

M38 substitution

[0086] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is an amino acid other than a methionine, e.g., where amino acid 38 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0087] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at M38. In some cases, a variant

CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at M38. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is any amino acid other than methionine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is Ile. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

V39 substitution

[0088] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is an amino acid other than a valine, e.g., where amino acid 39 is Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is Ala, Gly, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid

sequence depicted in FIG. 2V, where amino acid 39 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0089] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at V39. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V39. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is any amino acid other than valine; for example, "x" can be Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is Ala, Gly, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is Ile. For

example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

I49 substitution

[0090] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is an amino acid other than an isoleucine, e.g., where amino acid 49 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is Ala, Gly, Val, or Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is Leu. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM,

from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0091] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at I49. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at I49. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where "x" is any amino acid other than isoleucine; for example, "x" can be Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where "x" is Ala, Gly, Val, or Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where "x" is Ile. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Y53 substitution

[0092] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is an amino acid

other than a tyrosine, e.g., where amino acid 53 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0093] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Y53. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Y53. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is any amino acid other than tyrosine; for example, "x" can be Gly, Ala, Val, Leu, Ile,

Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Ile. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

D60 substitution

[0094] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is an amino acid other than an aspartic acid, e.g., where amino acid 60 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is Gly. In some cases, a variant CD80

polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0095] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at D60. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at D60. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is any amino acid other than aspartic acid; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Ile. For example, in some cases, a variant CD80 polypeptide of the present

disclosure comprises the amino acid sequence set forth in FIG. 2CC. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

F108 substitution

[0096] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is an amino acid other than a phenylalanine, e.g., where amino acid 108 is Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about

150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0097] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at F108. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at F108. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is any amino acid other than phenylalanine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Ile. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

S156 substitution

[0098] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is an amino acid other than a serine, e.g., where amino acid 156 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0099] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at S156. In some cases, a variant

CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S156. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is any amino acid other than serine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Ile. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

P111 substitution

[00100] In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is an amino acid other than a proline, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence

having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is Val. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00101] In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at P111. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at P111. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is any amino acid other than proline; for example, "x" can be Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is Ala. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is Val. In some cases, a variant CD80

polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is Leu. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is Gly. In some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is Ile. For example, in some cases, a variant CD80 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

FUSION POLYPEPTIDES

[00102] The present disclosure provides CD80 fusion polypeptides. A fusion polypeptide of the present disclosure comprises: a) a variant CD80 polypeptide of the present disclosure; and b) a heterologous fusion partner. In some cases, the heterologous fusion partner is fused to the N-terminus of the variant CD80 polypeptide. In some cases, the heterologous fusion partner is fused to the C-terminus of the variant CD80 polypeptide. In some cases, a CD80 fusion polypeptide of the present disclosure comprises a first heterologous fusion partner fused to the N-terminus of the variant CD80 polypeptide, and a second heterologous fusion partner fused to the C-terminus of the variant CD80 polypeptide.

[00103] The total length of a CD80 fusion polypeptide of the present disclosure can range from 215 amino acids to 2000 amino acids. For example, a CD80 fusion polypeptide of the present disclosure can range from 215 amino acids to 225 amino acids, from 225 amino acids to 250 amino acids, from 250 amino acids to 275 amino acids, from 275 amino acids to 300 amino acids, from 300 amino acids to 350 amino acids, from 350 amino acids, from 350 amino acids to 400 amino acids, from 400 amino acids, from 400 amino acids to 450 amino acids, from 450 amino acids to 500 amino acids, from 500 amino acids to 600 amino acids, from 600 amino acids to 700 amino acids, from 700 amino acids to 800 amino acids, from 800 amino acids to 900 amino acids, from 900 amino acids to 1000 amino acids, from 1000 amino acids to 1250 amino acids, from 1250 amino acids to 1500 amino acids, from 1500 amino acids to 1750 amino acids, or from 1750 amino acids to 2000 amino acids.

[00104] Suitable fusion partners include, but are not limited to, a transmembrane domain; an antibody Fc region; an antigen-binding region of an antibody; a cytokine; an immunomodulatory domain; an intracellular signaling domain; and the like.

T-CELL MODULATORY MULTIMERIC POLYPEPTIDES

[00105] The present disclosure provides multimeric (e.g., heterodimeric, heterotrimeric) polypeptides. The multimeric polypeptides are T cell modulatory polypeptides, and are also referred to herein as "T-cell modulatory multimeric polypeptides," or "synTac" (for "immunological synapse for T cell activation"). FIG. 1A-1D provide schematic depictions of T-cell modulatory multimeric polypeptides of the present disclosure. A T-cell modulatory multimeric polypeptide of the present disclosure is also referred to as a "synTac polypeptide" or a "multimeric polypeptide."

[00106] A synTac polypeptide of the present disclosure comprises a variant immunomodulatory polypeptide of the present disclosure. As noted above, a variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure exhibits reduced binding affinity to CD86, compared to the binding affinity of wild-type CD80 to CD86. A multimeric polypeptide of the present disclosure that comprises a variant CD80 polypeptide of the present disclosure also exhibits reduced binding affinity to CD86, compared to a control multimeric polypeptide comprising a wild-type CD80 (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A).

[00107] In some cases, a synTac polypeptide of the present disclosure exhibits reduced binding affinity to CD28, compared to the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A for CD28. For example, in some cases, a synTac polypeptide of the present disclosure binds CD28 with a binding affinity that is less than the binding affinity of a control synTac polypeptide comprising a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A for a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C. For example, in some cases, a synTac polypeptide of the present disclosure binds CD28 with a binding affinity that is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of a control synTac polypeptide comprising a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C).

[00108] In some cases, a synTac polypeptide of the present disclosure exhibits reduced binding affinity to CD28, compared to the binding affinity of a control synTac polypeptide comprising a CD80 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for CD28. For example, in some cases, a synTac polypeptide of the present disclosure binds CD28 with a binding affinity that is less than the binding affinity of a control synTac polypeptide comprises a CD80 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C. For example, in some cases, a synTac polypeptide of the present disclosure binds CD28 with a binding affinity that is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of a control synTac polypeptide comprising a CD80 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C).

[00109] In some cases, a synTac polypeptide of the present disclosure has a binding affinity for CD28 that is from 100 nm to about 100 μM. In some cases, a synTac polypeptide of the present disclosure has a binding affinity for CD28 that is from about 100 nM to 500 nM. For example, in some cases, a synTac polypeptide of the present disclosure has a binding affinity for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C) that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 450 nM, or from about 450 nM to about 500 nM. In some cases, a synTac polypeptide of the present disclosure has a binding affinity for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C) that is from about 500 nM to 1μM. For example, in some cases, a synTac polypeptide of the present disclosure has a binding affinity for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C) that is from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, or from about 900 nM to about 1 μM. In some cases, a synTac polypeptide of the present disclosure has a binding affinity for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C) that is from about 1 μM to 10 μM. For example, in some cases, a synTac polypeptide of the present disclosure has a binding affinity for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C) that is from about 1 μM to 2 μM, from about 2 μM to

about 3 μM, from about 3 μM to about 4 μM, from about 4 μM to about 5 μM, from about 5 μM to about 6 μM, from about 6 μM to about 7 μM, from about 7 μM to about 8 μM, from about 8 μM to about 9 μM, or from about 9 μM to about 10 μM. In some cases, a synTac polypeptide of the present disclosure has a binding affinity for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C) that is from about 10 μM to 100 μM. For example, in some cases, a synTac polypeptide of the present disclosure has a binding affinity for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C) that is from about 10 μM to about 20 μM, from about 20 μM to about 30 μM, from about 30 μM to about 40 μM, from about 40 μM to about 50 μM, from about 50 μM to about 60 μM, from about 60 μM to about 70 μM, from about 70 μM to about 80 μM, from about 80 μM to about 90 μM, or from about 90 μM to about 100 μM.

[00110] A variant CD80 polypeptide present in a synTac polypeptide of the present disclosure can have a single amino acid substitution relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide present in a synTac polypeptide of the present disclosure has from 2 to 10 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide present in a synTac polypeptide of the present disclosure has 2 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide present in a synTac polypeptide of the present disclosure has 3 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide present in a synTac polypeptide of the present disclosure has 4 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide present in a synTac polypeptide of the present disclosure has 5 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide present in a synTac polypeptide of the present disclosure has 6 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide present in a synTac polypeptide of the present disclosure has 7 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A

or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide present in a synTac polypeptide of the present disclosure has 8 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide present in a synTac polypeptide of the present disclosure has 9 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant CD80 polypeptide present in a synTac polypeptide of the present disclosure has 10 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1).

[00111] In some cases, a variant CD80 polypeptide present in a synTac polypeptide of the present disclosure has from 11 to 50 amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). For example, in some cases, a variant CD80 polypeptide present in a synTac polypeptide of the present disclosure has from 11 to 15, from 15 to 20, from 20 to 25, from 25 to 30, from 30 to 35, from 35 to 40, from 40 to 45, or from 45 to 50, amino acid substitutions relative to a wild-type CD80 polypeptide (e.g., a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1).

[00112] 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 (e.g., a variant CD80 polypeptide of the present disclosure); 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 (e.g., a variant CD80 polypeptide of the present disclosure); 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 β2-microglobulin (B2M or β2M) polypeptide, and the second MHC polypeptide is an MHC Class I heavy chain (H chain); or the first MHC polypeptide is an MHC Class I H chain, and the second MHC polypeptide is an MHC Class I β2M polypeptide). 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 α-chain polypeptide, and the second MHC polypeptide is an MHC Class II β-chain polypeptide. In other cases, the first polypeptide is an MHC Class II β-chain polypeptide, and the second MHC polypeptide is an MHC Class II α-chain polypeptide. In some cases, the multimeric polypeptide includes two or more immunomodulatory polypeptides, where at least one of the immunomodulatory polypeptides is a variant CD80 immunomodulatory polypeptide of the present disclosure. 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.

[00113] 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 (e.g., a variant CD80 polypeptide of the present disclosure). 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 (e.g., a variant CD80 polypeptide of the present disclosure). 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 (e.g., a variant CD80 polypeptide of the present disclosure); 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 (e.g., a variant CD80 polypeptide of the present disclosure); 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.

[00114] In some cases, a multimeric polypeptide of the present disclosure is monovalent. In some cases, a multimeric polypeptide of the present disclosure is multivalent. In some cases, a multivalent multimeric polypeptide of the present disclosure comprises an immunoglobulin Fc polypeptide on one of the first or the second polypeptide. 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.

[00115] 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 β2M polypeptide; and iii) a variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide of the present disclosure; ii) a Class I MHC heavy chain; and iii) an Fc 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, the first, second, and third variant CD80 polypeptides have the same amino acid sequence. In some cases, the first, second, and third variant CD80 polypeptides differ from one another in amino acid sequence. 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide.

In some cases, the first, second, and third variant CD80 polypeptides have the same amino acid sequence. In some cases, the first, second, and third variant CD80 polypeptides differ from one another in amino acid sequence.

Linkers

[00116] 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; between an MHC polypeptide and an Ig Fc polypeptide; between a first variant CD80 polypeptide and a second variant CD80 polypeptide; or a between a second variant CD80 polypeptide and a third variant CD80 polypeptide.

[00117] 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 to 25 amino acids, from 3 amino acids 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. A suitable linker can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids in length.

[00118] Exemplary linkers include glycine polymers $(G)_n$, glycine-serine polymers (including, for example, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO:65) and $(GGGS)_n$ (SEQ ID NO:66), 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:67), GGSGG (SEQ ID NO:68), GSGSG (SEQ ID NO:69), GSGGG (SEQ ID NO:70), GGGSG (SEQ ID NO:71), GSSSG (SEQ ID NO:72), and the like. Exemplary linkers can include, e.g., $Gly(Ser_4)n$, where n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some cases, a linker comprises the amino acid sequence (GSSSS)n (SEQ ID NO:73), where n is 4. In some cases, a linker comprises the amino acid sequence (GSSSS)n (SEQ ID NO:73), where n is 5. In some cases, a linker comprises the amino acid sequence (GGGGS)n (SEQ ID NO:74), where n is 4. In some cases, a linker comprises the amino acid sequence (GGGGS)n (SEQ ID NO:74), where n is 5.

[00119] 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 GCGASGGGGSGGGGS (SEQ ID NO:75).

Epitopes

[00120] 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.

[00121] 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.

[00122] Suitable epitopes include, but are not limited to, epitopes present in a cancer-associated antigen. Cancer-associated antigens include, but are not limited to, α-folate receptor; carbonic anhydrase IX (CAIX); CD19; CD20; CD22; CD30; CD33; CD44v7/8; carcinoembryonic antigen (CEA); epithelial glycoprotein-2 (EGP-2); epithelial glycoprotein-40 (EGP-40); folate binding protein (FBP); fetal acetylcholine receptor; ganglioside antigen GD2; Her2/neu; IL-13R-a2; kappa light chain; LeY; L1 cell adhesion molecule; melanoma-associated antigen (MAGE); MAGE-A1; mesothelin; MUC1; NKG2D ligands; oncofetal antigen (h5T4); prostate stem cell antigen (PSCA); prostate-specific membrane antigen (PSMA); tumor-associate glycoprotein-72 (TAG-72); and vascular endothelial growth factor receptor-2 (VEGF-R2). See, e.g., Vigneron et al. (2013) *Cancer Immunity* 13:15; and Vigneron (2015) *BioMed Res. Int'l* Article ID 948501.

MHC polypeptides

[00123] 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., β-2 microglobulin and MHC class I heavy chain) and MHC Class II polypeptides (e.g., MHC Class II α polypeptide and MHC Class II β polypeptide).

[00124] 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 β2-microglobulin (β2M) 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 α-chain polypeptide, and the second MHC polypeptide is an MHC Class II β-chain polypeptide. In other cases, the first polypeptide is an MHC Class II β-chain polypeptide, and the second MHC polypeptide is an MHC Class II α-chain polypeptide.

[00125] 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 β2-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 α chain or a Class II HLA β chain. MHC Class II polypeptides include MCH Class II DP α and β polypeptides, DM α and β polypeptides, DOA α and β polypeptides, DOB α and β polypeptides, DQ α and β polypeptides, and DR α and β polypeptides.

[00126] 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 5A.

[00127] 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:
GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEY
WDGETRKVKAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQ
YAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRY
LENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTE
LVETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID
NO:76).

[00128] 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 5B.

[00129] 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 5C.

[00130] 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:

[00131] GPHSLRYFVTAVSRPGLGEPRFIAVGYVDDTQFVRFDSDADNPRFEPRAPWMEQ
EGPEYWEEQTQRAKSDEQWFRVSLRTAQRYYNQSKGGSHTFQRMFGCDVGSDWRLLR
GYQQFAYDGRDYIALNEDLKTWTAADTAALITRRKWEQAGDAEYYRAYLEGECVEWL
RRYLELGNETLLRTDSPKAHVTYHPRSQVDVTLRCWALGFYPADITLTWQLNGEDLTQ
DMELVETRPAGDGTFQKWAAVVVPLGKEQNYTCHVHHKGLPEPLTLRW (SEQ ID
NO:77).

[00132] A β2-microglobulin (β2M) polypeptide of a multimeric polypeptide of the present disclosure can be a human β2M polypeptide, a non-human primate β2M polypeptide, a murine β2M polypeptide, and the like. In some instances, a β2M 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 β2M amino acid sequence depicted in FIG. 6. In some instances, a β2M 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 amino acids 21 to 119 of a β2M amino acid sequence depicted in FIG. 6.

[00133] 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.

[00134] 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.

[00135] For example, in some cases, one of following pairs of residues in an HLA β2-microglobulin and an HLA Class I heavy chain is substituted with cysteines (where residue numbers are those of the mature polypeptide): 1) β2M residue 12, HLA Class I heavy chain residue 236; 2) β2M residue 12, HLA Class I heavy chain residue 237; 3) β2M residue 8, HLA Class I heavy chain residue 234; 4) β2M residue 10, HLA Class I heavy chain residue 235; 5) β2M residue 24, HLA Class I heavy chain residue 236; 6) β2M residue 28, HLA Class I heavy chain residue 232; 7) β2M residue 98, HLA Class I heavy chain residue 192; 8) β2M residue 99, HLA Class I heavy chain residue 234; 9) β2M residue 3, HLA Class I heavy chain residue 120; 10) β2M residue 31, HLA Class I heavy chain residue 96; 11) β2M residue 53, HLA Class I heavy chain residue 35; 12) β2M residue 60, HLA Class I heavy chain residue 96; 13) β2M residue 60, HLA Class I heavy chain residue 122; 14) β2M residue 63, HLA Class I heavy chain residue 27; 15) β2M residue Arg3, HLA Class I heavy chain residue Gly120; 16) β2M residue His31, HLA Class I heavy chain residue Gln96; 17) β2M residue Asp53, HLA Class I heavy chain residue Arg35; 18) β2M residue Trp60, HLA Class I heavy chain residue Gln96; 19) β2M residue Trp60, HLA Class I heavy chain residue Asp122; 20) β2M residue Tyr63, HLA Class I heavy chain residue Tyr27; 21) β2M residue Lys6, HLA Class I heavy chain residue Glu232; 22) β2M residue Gln8, HLA Class I heavy chain residue Arg234; 23) β2M residue Tyr10, HLA Class I heavy chain residue Pro235; 24) β2M residue Ser11, HLA Class I heavy chain residue

Gln242; 25) β2M residue Asn24, HLA Class I heavy chain residue Ala236; 26) β2M residue Ser28, HLA Class I heavy chain residue Glu232; 27) β2M residue Asp98, HLA Class I heavy chain residue His192; and 28) β2M 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 5A, which includes a signal peptide, Gly120 is Gly144; Gln96 is Gln120; etc. In some cases, the β2M polypeptide comprises an R12C substitution, and the HLA Class I heavy chain comprises an A236C substitution; in such cases, a disulfide bond forms between Cys-12 of the β2M polypeptide and Cys-236 of the HLA Class I heavy chain. For example, in some cases, residue 236 of the mature HLA-A amino acid sequence (i.e., residue 260 of the amino acid sequence depicted in FIG. 5A) is substituted with a Cys. In some cases, residue 236 of the mature HLA-B amino acid sequence (i.e., residue 260 of the amino acid sequence depicted in FIG. 5B) is substituted with a Cys. In some cases, residue 236 of the mature HLA-C amino acid sequence (i.e., residue 260 of the amino acid sequence depicted in FIG. 5C) is substituted with a Cys. In some cases, residue 32 (corresponding to Arg-12 of mature β2M) of an amino acid sequence depicted in FIG. 6 is substituted with a Cys.

[00136] In some cases, a β2M polypeptide comprises the amino acid sequence: IQRTPKIQVY S**R**HPAENGKS NFLNCYVSGF HPSDIEVDLLKNGERIEKVE HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSQP KIVKWDRDM (EQ ID NO:101). In some cases, a β2M polypeptide comprises the amino acid sequence: IQRTPKIQVY S**C**HPAENGKS NFLNCYVSGF HPSDIEVDLLKNGERIEKVE HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSQP KIVKWDRDM (SEQ ID NO:87).

[00137] In some cases, an HLA Class I heavy chain polypeptide comprises the amino acid sequence:

GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGET RKVKAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKE DLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHH AVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRP**A**GDGTFQKWAAVVVPSGQEQR YTCHVQHEGLPKPLTLRWEP (SEQ ID NO:85).

[00138] In some cases, an HLA Class I heavy chain polypeptide comprises the amino acid sequence:

[00139] GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEY WDGETRKVKAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDY IALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKT

HMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRP**C**GDGTFQKWAAVVVPS GQEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID NO:86).

[00140] In some cases, the β2M polypeptide comprises the following amino acid sequence:

[00141] IQRTPKIQVY S**C**HPAENGKS NFLNCYVSGF HPSDIEVDLLKNGERIEKVE HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSQP KIVKWDRDM (SEQ ID NO:87); and the HLA ClassI heavy chain polypeptide of a multimeric polypeptide of the present disclosure comprises the following amino acid sequence:

[00142] GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEY WDGETRKVKAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDY IALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKT HMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRP**C**GDGTFQKWAAVVVPS GQEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID NO:88), where the Cys residues that are underlined and in bold form a disulfide bond with one another in the multimeric polypeptide.

Immunomodulatory polypeptides

[00143] A multimeric polypeptide of the present disclosure comprises a variant immunomodulatory polypeptide, as described above. Thus, a multimeric polypeptide of the present disclosure comprises the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure.

[00144] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure exhibits reduced binding affinity to CD28, compared to the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A for CD28. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure binds CD28 with a binding affinity that is less than the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A for a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure binds CD28 with a binding affinity that is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C).

[00145] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure exhibits reduced binding affinity to CD28, compared to the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for CD28. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure binds CD28 with a binding affinity that is less than the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure binds CD28 with a binding affinity that is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C).

[00146] In some cases, a variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure has a binding affinity to CD28 that is from 100 nM to 100 μM. As another example, in some cases, a variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure has a binding affinity for CD28 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in one of FIG. 3A-3C) that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00147] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure exhibits reduced binding affinity to CTLA4, compared to the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure binds CTLA4 with a binding affinity that less than the binding affinity a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A for a CTLA4 polypeptide comprising the amino acid sequence depicted in FIG. 3D. For example, in some cases, the

variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure binds CTLA4 with a binding affinity that is at least at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of a CD80 polypeptide comprising the amino acid sequence depicted in FIG. 2A for CTLA4 (e.g., a CD28 polypeptide comprising the amino acid sequence depicted in FIG. 3D).

N19 substitution

[00148] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is an amino acid other than an asparagine, e.g., where amino acid 19 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from

about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00149] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at N19. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at N19. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where "x" is any amino acid other than asparagine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2C. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5

μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

N63 Substitution

[00150] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is an amino acid other than an asparagine, e.g., where amino acid 63 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about

5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00151] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at N63. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at N63. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is any amino acid other than asparagine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

I67 Substitution

[00152] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is an amino acid other than an isoleucine, e.g., where amino acid 67 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is Ala, Gly, Val, or Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is Leu. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00153] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at I67. In some cases, the variant CD80 polypeptide present in a multimeric

polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at I67. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is any amino acid other than asparagine; for example, "x" can be Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is Ala, Gly, Val, or Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is Gly. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

K86 Substitution

[00154] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is an amino acid other than a lysine, e.g., where amino acid 86 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H,

where amino acid 86 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00155] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at K86. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at K86. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where "x" is any amino acid other than lysine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of

the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Q157 Substitution

[00156] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is an amino acid other than a glutamine, e.g., where amino acid 157 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is Ala. In some cases, the

variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00157] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Q157. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Q157. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is any amino acid other than glutamine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric

polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

D158 Substitution

[00158] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is an amino acid other than an aspartic acid, e.g., where amino acid 158 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is Gly. In some cases, the variant CD80 polypeptide present in a

multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00159] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at D158. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at D158. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is any amino acid other than aspartic acid; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric

polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

L25 substitution

[00160] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is an amino acid other than a leucine, e.g., where amino acid 25 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is Ala, Gly, Val, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino

acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00161] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at L25. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L25. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where "x" is any amino acid other than leucine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where "x" is Ala, Gly, Val, or Ile.In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to

about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Y31 substitution

[00162] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is an amino acid other than a tyrosine, e.g., where amino acid 31 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from

about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00163] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Y31. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Y31. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is any amino acid other than tyrosine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. . In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5

μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Q33 substitution

[00164] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is an amino acid other than a glutamine, e.g., where amino acid 33 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5

μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00165] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Q33. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Q33. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is any amino acid other than glutamine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

M38 substitution

[00166] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is an amino acid other than a methionine, e.g., where amino acid 38 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00167] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at M38. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at M38. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is any amino acid other than methionine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

V39 substitution

[00168] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG.

2V, where amino acid 39 is an amino acid other than a valine, e.g., where amino acid 39 is Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is Ala, Gly, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00169] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at V39. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V39.For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is any amino acid other than valine; for example,

"x" can be Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is Ala, Gly, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

I49 substitution

[00170] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is an amino acid other than an isoleucine, e.g., where amino acid 49 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is Ala, Gly, Val, or Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence

having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is Leu. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00171] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at I49. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at I49. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where "x" is any amino acid other than isoleucine; for example, "x" can be Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where "x" is Ala, Gly, Val, or Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X,

where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Y53 substitution

[00172] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is an amino acid other than a tyrosine, e.g., where amino acid 53 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at

least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00173] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Y53. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Y53.For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is any amino acid other than tyrosine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z,

where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

D60 substitution

[00174] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is an amino acid other than an aspartic acid, e.g., where amino acid 60 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid

sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00175] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at D60. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at D60. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is any amino acid other than aspartic acid; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth

in FIG. 2CC. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

F108 substitution

[00176] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is an amino acid other than a phenylalanine, e.g., where amino acid 108 is Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG.

2DD, where amino acid 108 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00177] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at F108. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at F108. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is any amino acid other than phenylalanine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to

about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

S156 substitution

[00178] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is an amino acid other than a serine, e.g., where amino acid 108 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM,

from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00179] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at S156. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S156. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is any amino acid other than serine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to

about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

P111 substitution

[00180] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is an amino acid other than a proline, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is Ile. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM,

from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00181] In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at P111. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at P111. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is any amino acid other than proline; for example, "x" can be Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is Ala, Gly, Val, Leu, or Ile. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is Ala. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is Val. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is Leu. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is Gly. In some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where "x" is Ile. For example, in some cases, the variant CD80 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II. In some cases, the variant CD80 polypeptide has a binding affinity to CD28 that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Additional immunomodulatory polypeptides

[00182] 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.

[00183] 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.

[00184] 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.

Multiple immunomodulatory domains

[00185] As noted above, in some cases, a multimeric polypeptide of the present disclosure comprises two or more immunomodulatory polypeptides, where at least one of the two or more immunomodulatory polypeptide is a variant CD80 polypeptide of the present disclosure.

[00186] In some cases, a multimeric polypeptide of the present disclosure comprises two or more copies of a variant CD80 polypeptide of the present disclosure. In some cases, the two or more variant CD80 polypeptides are on the same polypeptide chain of a multimeric polypeptide of the present disclosure. In some cases, the two or more variant CD80 polypeptides are on separate polypeptide chains of a multimeric polypeptide of the present disclosure.

[00187] In some cases, a multimeric polypeptide of the present disclosure comprises a first immunomodulatory polypeptide, and at least a second immunomodulatory polypeptide, where the first immunomodulatory polypeptide is a variant CD80 polypeptide of the present disclosure, and the second immunomodulatory polypeptide is not a CD80 polypeptide. For example, in some cases, the second immunomodulatory polypeptide is a member of the tumor necrosis factor (TNF) superfamily; e.g., a FasL polypeptide, a 4-1BBL polypeptide, a CD40 polypeptide, an OX40L polypeptide, a CD30L polypeptide, a CD70 polypeptide, etc. In some cases, the second 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. In some cases, the second immunomodulatory polypeptide is 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, or HVEM.

[00188] Further T cell modulatory domains (MODs) that can be included in a multimeric polypeptide of the present disclosure 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.

Scaffold polypeptides

[00189] A T-cell modulatory multimeric polypeptide of the present disclosure comprises an Fc polypeptide, or another suitable scaffold polypeptide.

[00190] Suitable scaffold polypeptides include antibody-based scaffold polypeptides and non-antibody-based scaffolds. Non-antibody-based scaffolds include, e.g., albumin, an XTEN (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 iany 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 XTEN 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.

[00191] 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.

Fc polypeptides

[00192] In some cases, the first and/or the second polypeptide chain of a multimeric polypeptide of the present disclosure comprises an Fc polypeptide. 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 4A-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 4A. 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 4A; and comprises a substitution of N77; e.g., the Fc polypeptide comprises an N77A substitution. 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 4A; 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 4A. 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 4A; 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 4A. 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 IgM Fc polypeptide depicted in FIG. 4B; 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. 4B. 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 4C; 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. 4C.

Additional polypeptides

[00193] 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

[00194] Suitable epitope tags include, but are not limited to, hemagglutinin (HA; e.g., YPYDVPDYA (SEQ ID NO:89); FLAG (e.g., DYKDDDDK (SEQ ID NO:90); c-myc (e.g., EQKLISEEDL; SEQ ID NO:91), and the like.

Affinity domain

[00195] 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:92), HisX6 (HHHHHH) (SEQ ID NO:93), C-myc (EQKLISEEDL) (SEQ ID NO:94), Flag (DYKDDDDK) (SEQ ID NO:95), StrepTag (WSHPQFEK) (SEQ ID NO:96), hemagglutinin, e.g., HA Tag (YPYDVPDYA) (SEQ ID NO:97), glutathione-S-transferase (GST), thioredoxin, cellulose binding domain, RYIRS (SEQ ID NO:98), Phe-His-His-Thr (SEQ ID NO:99), chitin binding domain, S-peptide, T7 peptide, SH2 domain, C-end RNA tag, WEAAAREACCRECCARA (SEQ ID NO:100), 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.

Exemplary multimeric polypeptides

[00196] Exemplary multimeric polypeptides of the present disclosure are described below.

N19

[00197] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is an amino acid other than an asparagine, e.g., where amino acid 19 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 19 is Ala, Val, Gly, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is an amino acid other than an asparagine, e.g., where amino acid 19 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 19 is Ala, Val, Gly, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the

present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is an amino acid other than an asparagine, e.g., where amino acid 19 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 19 is Ala, Val, Gly, Leu, or Ile. 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 19 is an amino acid other than an asparagine, e.g., where amino acid 19 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 19 is Ala, Val, Gly, Leu, or Ile.

N63

[00198] 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 β2M polypeptide; and iii) a variant CD80 polypeptide an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is an amino acid other than an asparagine, e.g., where amino acid 63 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 63 is Ala, Gly, Val, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is an amino acid other than an asparagine, e.g., where amino acid 63 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln,

Lys, Arg, His, Asp, or Glu, e.g., where amino acid 63 is Ala, Gly, Val, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00199] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, the first, second, and third variant CD80 polypeptides each comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is an amino acid other than an asparagine, e.g., where amino acid 63 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 63 is Ala, Gly, Val, Leu, or Ile.

[00200] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, the first, second, and third variant CD80 polypeptides each comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is an amino acid other than an asparagine, e.g., where amino acid 63 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 63 is Ala, Gly, Val, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00201] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, the first, second, and third variant CD80 polypeptides each comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence

depicted in FIG. 2D, where amino acid 63 is an amino acid other than an asparagine, e.g., where amino acid 63 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 63 is Ala, Gly, Val, Leu, or Ile.

[00202] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; iv) a third variant CD80 polypeptide of the present disclosure; v) a Class I MHC heavy chain; and vi) an Fc polypeptide. In some cases, the first, second, and third variant CD80 polypeptides each comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 63 is an amino acid other than an asparagine, e.g., where amino acid 63 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 63 is Ala, Gly, Val, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

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[00203] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is an amino acid other than an isoleucine, e.g., where amino acid 67 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 67 is Val, Gly, or Leu; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is an amino acid other than an isoleucine, e.g., where amino acid 67 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 67 is Val, Gly, or Leu; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00204] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is an amino acid other than an isoleucine, e.g., where amino acid 67 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 67 is Ala, Val, Gly, or Leu.

[00205] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vi) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is an amino acid other than an isoleucine, e.g., where amino acid 67 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 67 is Ala, Val, Gly, or Leu. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00206] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is an amino acid other than an isoleucine, e.g., where amino acid 67 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 67 is Ala, Val, Gly, or Leu.

[00207] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 67 is an amino acid other than an isoleucine, e.g., where amino acid 67 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 67 is Ala, Val, Gly, or Leu. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

K86

[00208] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is an amino acid other than a lysine, e.g., where amino acid 86 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu, e.g., where amino acid 86 is Ala, Val, Gly, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is an amino acid other than a lysine, e.g., where amino acid 86 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu, e.g., where amino acid 86 is Ala, Val, Gly, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00209] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the

present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is an amino acid other than a lysine, e.g., where amino acid 86 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu, e.g., where amino acid 86 is Ala, Val, Gly, Leu, or Ile.

[00210] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is an amino acid other than a lysine, e.g., where amino acid 86 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu, e.g., where amino acid 86 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00211] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is an amino acid other than a lysine, e.g., where amino acid 86 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu, e.g., where amino acid 86 is Ala, Val, Gly, Leu, or Ile.

[00212] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80

polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 86 is an amino acid other than a lysine, e.g., where amino acid 86 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu, e.g., where amino acid 86 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

Q157

[00213] an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is an amino acid other than a glutamine, e.g., where amino acid 157 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu.

[00214] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is an amino acid other than a glutamine, e.g., where amino acid 157 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 157 is Ala, Val, Gly, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is an amino acid other than a glutamine, e.g., where amino acid 157 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 157 is Ala, Val, Gly, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00215] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the

present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is an amino acid other than a glutamine, e.g., where amino acid 157 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 157 is Ala, Val, Gly, Leu, or Ile.

[00216] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is an amino acid other than a glutamine, e.g., where amino acid 157 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 157 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00217] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is an amino acid other than a glutamine, e.g., where amino acid 157 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 157 is Ala, Val, Gly, Leu, or Ile.

[00218] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i)

a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 157 is an amino acid other than a glutamine, e.g., where amino acid 157 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 157 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

D158

[00219] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is an amino acid other than an aspartic acid, e.g., where amino acid 158 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu, e.g., where amino acid 158 is Ala, Val, Gly, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is an amino acid other than an aspartic acid, e.g., where amino acid 158 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu, e.g., where amino acid 158 is Ala, Val, Gly, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00220] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at

least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is an amino acid other than an aspartic acid, e.g., where amino acid 158 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu, e.g., where amino acid 158 is Ala, Val, Gly, Leu, or Ile.

[00221] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is an amino acid other than an aspartic acid, e.g., where amino acid 158 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu, e.g., where amino acid 158 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00222] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is an amino acid other than an aspartic acid, e.g., where amino acid 158 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu, e.g., where amino acid 158 is Ala, Val, Gly, Leu, or Ile.

[00223] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vi) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence

having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 158 is an amino acid other than an aspartic acid, e.g., where amino acid 158 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu, e.g., where amino acid 158 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

L25

[00224] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is an amino acid other than a leucine, e.g., where amino acid 25 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 25 is Ala, Val, Gly, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is an amino acid other than a leucine, e.g., where amino acid 25 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 25 is Ala, Val, Gly, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00225] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is an amino acid other than a leucine, e.g., where amino acid 25 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 25 is Ala, Val, Gly, or Ile.

[00226] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is an amino acid other than a leucine, e.g., where amino acid 25 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 25 is Ala, Val, Gly, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00227] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is an amino acid other than a leucine, e.g., where amino acid 25 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 25 is Ala, Val, Gly, or Ile.

[00228] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 25 is an amino acid other than a leucine, e.g., where amino acid 25 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 25 is Ala, Val, Gly, or Ile. In some

cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

Y31

[00229] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is an amino acid other than a tyrosine, e.g., where amino acid 31 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 31 is Ala, Val, Gly, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is an amino acid other than a tyrosine, e.g., where amino acid 31 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 31 is Ala, Val, Gly, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00230] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is an amino acid other than a tyrosine, e.g., where amino acid 31 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 31 is Ala, Val, Gly, Leu, or Ile.

[00231] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant

CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is an amino acid other than a tyrosine, e.g., where amino acid 31 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 31 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00232] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is an amino acid other than a tyrosine, e.g., where amino acid 31 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 31 is Ala, Val, Gly, Leu, or Ile.

[00233] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 31 is an amino acid other than a tyrosine, e.g., where amino acid 31 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 31 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

Q33

[00234] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is an amino acid other than a glutamine, e.g., where amino acid 33 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 33 is Ala, Val, Gly, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is an amino acid other than a glutamine, e.g., where amino acid 33 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 33 is Ala, Val, Gly, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00235] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is an amino acid other than a glutamine, e.g., where amino acid 33 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 33 is Ala, Val, Gly, Leu, or Ile.

[00236] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In

some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is an amino acid other than a glutamine, e.g., where amino acid 33 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 33 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00237] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is an amino acid other than a glutamine, e.g., where amino acid 33 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 33 is Ala, Val, Gly, Leu, or Ile.

[00238] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 33 is an amino acid other than a glutamine, e.g., where amino acid 33 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 33 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

M38

[00239] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at

least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is an amino acid other than a methionine, e.g., where amino acid 38 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 38 is Ala, Val, Gly, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is an amino acid other than a methionine, e.g., where amino acid 38 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 38 is Ala, Val, Gly, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00240] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is an amino acid other than a methionine, e.g., where amino acid 38 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 38 is Ala, Val, Gly, Leu, or Ile.

[00241] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is an

amino acid other than a methionine, e.g., where amino acid 38 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 38 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00242] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is an amino acid other than a methionine, e.g., where amino acid 38 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 38 is Ala, Val, Gly, Leu, or Ile.

[00243] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 38 is an amino acid other than a methionine, e.g., where amino acid 38 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 38 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

V39

[00244] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is an amino acid other than a valine, e.g., where amino acid 39 is Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln,

Lys, Arg, His, Asp, or Glu, e.g., where amino acid 39 is Ala, Gly, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is an amino acid other than a valine, e.g., where amino acid 39 is Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 39 is Ala, Gly, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00245] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is an amino acid other than a valine, e.g., where amino acid 39 is Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 39 is Ala, Gly, Leu, or Ile.

[00246] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is an amino acid other than a valine, e.g., where amino acid 39 is Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 39 is Ala, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00247] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is an amino acid other than a valine, e.g., where amino acid 39 is Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 39 is Ala, Gly, Leu, or Ile.

[00248] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 39 is an amino acid other than a valine, e.g., where amino acid 39 is Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 39 is Ala, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

I49

[00249] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is an amino acid other than an isoleucine, e.g., where amino acid 49 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 49 is Ala, Val, Gly, or Leu; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is an amino acid other than an isoleucine, e.g., where amino acid 49 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 49 is Ala, Val, Gly, or Leu; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00250] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is an amino acid other than an isoleucine, e.g., where amino acid 49 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 49 is Ala, Val, Gly, or Leu.

[00251] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is an amino acid other than an isoleucine, e.g., where amino acid 49 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 49 is Ala, Val, Gly, or Leu. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00252] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide

of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is an amino acid other than an isoleucine, e.g., where amino acid 49 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 49 is Ala, Val, Gly, or Leu.

[00253] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 49 is an amino acid other than an isoleucine, e.g., where amino acid 49 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 49 is Ala, Val, Gly, or Leu. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

Y53

[00254] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is an amino acid other than a tyrosine, e.g., where amino acid 53 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 53 is Ala, Val, Gly, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is an amino acid other than a

tyrosine, e.g., where amino acid 53 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 53 is Ala, Val, Gly, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00255] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is an amino acid other than a tyrosine, e.g., where amino acid 53 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 53 is Ala, Val, Gly, Leu, or Ile.

[00256] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is an amino acid other than a tyrosine, e.g., where amino acid 53 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 53 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00257] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence

depicted in FIG. 2Z, where amino acid 53 is an amino acid other than a tyrosine, e.g., where amino acid 53 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 53 is Ala, Val, Gly, Leu, or Ile.

[00258] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 53 is an amino acid other than a tyrosine, e.g., where amino acid 53 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 53 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

D60

[00259] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is an amino acid other than an aspartic acid, e.g., where amino acid 60 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu, e.g., where amino acid 60 is Ala, Val, Gly, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is an amino acid other than an aspartic acid, e.g., where amino acid 60 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu, e.g., where amino acid 60 is Ala, Val, Gly, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00260] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is an amino acid other than an aspartic acid, e.g., where amino acid 60 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu, e.g., where amino acid 60 is Ala, Val, Gly, Leu, or Ile.

[00261] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is an amino acid other than an aspartic acid, e.g., where amino acid 60 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu, e.g., where amino acid 60 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00262] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is an amino acid other than an aspartic acid, e.g., where amino acid 60 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu, e.g., where amino acid 60 is Ala, Val, Gly, Leu, or Ile.

[00263] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 60 is an amino acid other than an aspartic acid, e.g., where amino acid 60 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu, e.g., where amino acid 60 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

F108

[00264] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is an amino acid other than a phenylalanine, e.g., where amino acid 108 is Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 108 is Ala, Val, Gly, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is an amino acid other than a phenylalanine, e.g., where amino acid 108 is Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 108 is Ala, Val, Gly, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00265] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the

present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is an amino acid other than a phenylalanine, e.g., where amino acid 108 is Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 108 is Ala, Val, Gly, Leu, or Ile.

[00266] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is an amino acid other than a phenylalanine, e.g., where amino acid 108 is Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 108 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00267] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is an amino acid other than a phenylalanine, e.g., where amino acid 108 is Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 108 is Ala, Val, Gly, Leu, or Ile.

[00268] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i)

a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 108 is an amino acid other than a phenylalanine, e.g., where amino acid 108 is Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 108 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

D111

[00269] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is an amino acid other than a proline, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 111 is Ala, Val, Gly, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is an amino acid other than a proline, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 111 is Ala, Val, Gly, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00270] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at

least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is an amino acid other than a proline, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 111 is Ala, Val, Gly, Leu, or Ile.

[00271] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is an amino acid other than a proline, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 111 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00272] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is an amino acid other than a proline, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 111 is Ala, Val, Gly, Leu, or Ile.

[00273] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence

having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is an amino acid other than a proline, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 111 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

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[00274] 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 β2M polypeptide; and iii) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 156 is an amino acid other than a serine, e.g., where amino acid 156 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 156 is Ala, Val, Gly, Leu, or Ile; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc 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; and ii) a β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a variant CD80 polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is an amino acid other than a proline, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 111 is Ala, Val, Gly, Leu, or Ile; ii) a Class I MHC heavy chain; and iii) an Fc polypeptide.

[00275] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a second variant CD80 polypeptide of the present disclosure; and v) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is an amino acid other than a proline, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 111 is Ala, Val, Gly, Leu, or Ile.

[00276] 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 β2M polypeptide; iii) a first variant CD80 polypeptide of the present disclosure; iv) a linker; v) a second variant CD80 polypeptide of the present disclosure; vi) a linker; and vii) a third variant CD80 polypeptide of the present disclosure; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a Class I MHC heavy chain; and ii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is an amino acid other than a proline, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 111 is Ala, Val, Gly, Leu, or Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00277] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a second variant CD80 polypeptide of the present disclosure; and iii) a third variant CD80 polypeptide of the present disclosure; iv) a Class I MHC heavy chain; and v) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is an amino acid other than a proline, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 111 is Ala, Val, Gly, Leu, or Ile.

[00278] 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 β2M polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a first variant CD80 polypeptide of the present disclosure; ii) a linker; iii) a second variant CD80 polypeptide of the present disclosure; iv) a linker; v) a third variant CD80 polypeptide of the present disclosure; vi) a Class I MHC heavy chain; and vii) an Fc polypeptide. In some cases, each of the first, second, and third variant CD80 polypeptides comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 111 is an amino acid other than a proline, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, e.g., where amino acid 111 is Ala, Val, Gly, Leu, or

Ile. In some cases, the linker comprises a (GSSSS)n (SEQ ID NO:73) sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

NUCLEIC ACIDS

[00279] The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a variant CD80 polypeptide of the present disclosure. The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a CD80 fusion polypeptide of the present disclosure.

[00280] 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.

Separate nucleic acids encoding individual polypeptide chains of a multimeric polypeptide

[00281] 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.

[00282] 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 (e.g., a variant CD80 polypeptide of the present disclosure); 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.

[00283] 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 (e.g., a variant CD80 polypeptide of the present disclosure); 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

[00284] 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.

[00285] 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.

[00286] 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 (e.g., a variant CD80 polypeptide of the present disclosure); 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.g., a variant CD80 polypeptide of the present disclosure); 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.g., a variant CD80 polypeptide of the present disclosure); 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 β2-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.

[00287] Suitable MHC polypeptides are described above. In some cases, the first MHC polypeptide is a β2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide. In some cases, the β2-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:52, or at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:87). 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 amino acids 25-365 of the amino acid sequence set forth in SEQ ID NO:49. In some cases, the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to amino acids 25-362 of the amino acid sequence set forth in SEQ ID NO:50. In some cases, the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to amino acids 25-366 of the amino acid sequence set forth in SEQ ID NO:51. 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.

[00288] 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 4A-4C.

[00289] Suitable immunomodulatory polypeptides are described above.

[00290] 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:78); b) ENLYTQS (SEQ ID NO:79); c) DDDDK (SEQ ID NO:101); d) LVPR (SEQ ID NO:80); and e) GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:81). In some cases, the proteolytically cleavable linker comprises an amino acid sequence selected from: a) LEVLFQGP (SEQ ID NO:78); b) ENLYTQS (SEQ ID NO:79); c) a furin cleavage site; d) LVPR (SEQ ID NO:80); e) GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:81); f) GSGEGRGSLLTCGDVEENPGP (SEQ ID NO:82); g) GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO:83); and h) GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:84).

[00291] 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

[00292] 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.

[00293] 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 Opthalmol 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 Opthalmol 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.

[00294] 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.

[00295] 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).

[00296] 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.

[00297] 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

[00298] The present disclosure provides a genetically modified host cell, where the host cell is genetically modified with a nucleic acid of the present disclosure.

[00299] 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. CCLI.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, and the like.

[00300] In some cases, the host cell is a mammalian cell that has been genetically modified such that it does not synthesize endogenous MHC β2-M.

METHODS OF PRODUCING A MULTIMERIC POLYPEPTIDE

[00301] 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.

[00302] 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.

[00303] 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.

[00304] 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

[00305] The present disclosure provides compositions, including pharmaceutical compositions, comprising a variant CD80 polypeptide of the present disclosure. 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

[00306] 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, $MgCl_2$, KCl, $MgSO_4$, 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.

[00307] 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.

[00308] 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.

[00309] 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.

[00310] 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.

[00311] Where a multimeric polypeptide of the present disclosure is administered as an injectable (e.g. subcutaneously, intraperitoneally, intramuscularly, and/or intravenously) 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.

[00312] 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.

[00313] 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.

[00314] 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.

[00315] The present disclosure provides compositions, including pharmaceutical compositions, comprising a variant CD80 polypeptide of the present disclosure. A composition can comprise: a) a variant CD80 polypeptide of the present disclosure; and b) an excipient, as described above for the multimeric polypeptides. In some cases, the excipient is a pharmaceutically acceptable excipient.

Compositions comprising a nucleic acid or a recombinant expression vector

[00316] 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.

[00317] 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-hydroxyehtylpiperazine-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)methyl-glycine (Tricine), tris(hydroxymethyl)-aminomethane (Tris), etc.). Suitable salts include, e.g., NaCl, $MgCl_2$, KCl, $MgSO_4$, etc.

[00318] 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.

[00319] 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.

[00320] 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.

[00321] 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.

[00322] 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, which is incorporated herein by reference in its entirety.

[00323] 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.

[00324] 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, which is incorporated herein by reference in its entirety.

[00325] 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, triethanoiamine, N,N-dimethylacetamide, N,N-dimethylformamide, 2-pyrrolidone and derivatives thereof, tetrahydrofurfuryl alcohol, and AZONE™.

METHODS OF MODULATING T CELL ACTIVITY

[00326] 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*.

[00327] In some cases, e.g., where the target T cell is a $CD8^+$ T cell, the multimeric polypeptide comprises Class I MHC polypeptides (e.g., β2-microglobulin and Class I MHC heavy chain). In some cases, e.g., where the target T cell is a $CD4^+$ T cell, the multimeric polypeptide comprises Class II MHC polypeptides (e.g., Class II MHC α chain; Class II MHC β chain).

[00328] 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.

[00329] 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.

[00330] 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

[00331] The present invention 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.

[00332] 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.

[00333] 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.

[00334] 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 (or tumor volume) 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 (or tumor volume) 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.

[00335] 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.

[00336] 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.

[00337] 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.

[00338] 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.

[00339] 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.

[00340] 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

[00341] Suitable formulations are described above, where suitable formulations include a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: 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.

[00342] Suitable pharmaceutically acceptable excipients are described above.

Dosages

[00343] 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 μg to 10 mg per kilogram of body weight per minute.

[00344] In some cases, a suitable dose of a multimeric polypeptide of the present disclosure is from 0.01 μg to 100 g per kg of body weight, from 0.1 μg to 10 g per kg of body weight, from 1 μg to 1 g per kg of body weight, from 10 μg to 100 mg per kg of body weight, from 100 μg to 10 mg per kg of body weight, or from 100 μg 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 μg to 100 g per kg of body weight, from 0.1 μg to 10 g per kg of body weight, from 1 μg to 1 g per kg of body weight, from 10 μg to 100 mg per kg of body weight, from 100 μg to 10 mg per kg of body weight, or from 100 μg to 1 mg per kg of body weight.

[00345] 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.

[00346] 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).

[00347] 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

[00348] 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.

[00349] 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.

[00350] 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.

[00351] 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.

[00352] 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 invention include, but are not necessarily limited to, enteral, parenteral, or inhalational routes.

[00353] 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

[00354] 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.

EXAMPLES

[00355] 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 and characterization of synTac polypeptides with variant CD80

[00356] synTac multimeric polypeptides were generated, in which a first polypeptide ("Syn289") was heterodimerized with a second polypeptide ("Syn290," "Syn291," "Syn292," "Syn293," "Syn294," "Syn295," or "Syn296").

[00357] Syn289 comprises, in order from N-terminus to C-terminus: a) an ovalbumin (OVA) T-cell epitope; and b) a β2M polypeptide.

[00358] The second polypeptide of the synTac comprised an MHC Class I heavy chain; thus, the second polypeptide was referred to as the "heavy chain" (H chain) of the synTac. The second polypeptide comprised, in order from N-terminus to C-terminus: a) a CD80 ectodomain; b) an MHC Class I heavy chain; and c) an IgG2a Fc polypeptide. The CD80 ectodomain polypeptide present in Syn290 was wild-type CD80 ectodomain (SEQ ID NO:1). The CD80 ectodomain polypeptide present in Syn 291, Syn292, Syn293, Syn294, Syn295, and Syn296 comprised a single amino acid substitution compared to wild-type CD80 ectodomain. The single amino acid substitutions are set out in Table 1.

Table 1

synTac H chain	CD80 ectodomain
290	Wild-type
291	N19A
292	N63A
293	I67A
294	K86A
295	Q157A
296	D158A

[00359] The first and the second polypeptide chains were disulfide linked to one another via Cys-12 in the β2M polypeptide of the first polypeptide and Cys-236 of the MHC Class I heavy chain of the second polypeptide.

[00360] The resulting synTac heterodimers were cultured *in vitro* with ovalbumin-specific T cells for 3 days, at concentrations of 0, 1, 3.17, 10.01, 31.65, and 100 nM synTac. Controls included: a) medium alone; b) phorbol 12-myristate 13-acetate (PMA) and the ionophore A23187; and c) an anti-CD3 antibody and an anti-CD28 antibody.

[00361] After 3 days, the concentration of IFN-γ, IL-2, IL-6, and TNF in the culture medium was determined. In addition, the viability of the ovalbumin-specific T cells, and the proliferation of the ovalbumin-specific T cells, was determined.

[00362] The data are depicted in FIG. 14 through FIG. 19.

[00363] As shown in FIG. 14 through FIG. 19, synTac polypeptides that include variant CD80 polypeptide induce production of IL-2 (a cellular fitness cytokine); induce production of cytotoxic cytokines TNFα and IFN-γ; and also induce proliferation and enhance viability of epitope-specific T cells.

Example 2: *In vivo* effect of a CD80/synTac

[00364] A synTac comprising a human papilloma virus (HPV) E7 antigenic peptide and a CD80 K86A variant of the present disclosure (referred to as "CUE:CD80 (K86A)" in FIG. 20) was administered at 2.5 mg/kg by intraperitoneal (IP) injection into mice bearing flank engrafted HPV^+ TC-1 lung carcinoma. As a control, phosphate buffered saline (PBS) was administered to mice bearing the same tumor. As shown in FIG. 20, tumor volume was decreased in mice treated with CUE:CD80 (K86A), compared to mice treated with PBS.

[00365] 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 true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. A variant CD80 immunomodulatory polypeptide comprising an amino acid sequence having at least 85% amino acid sequence identity to the CD80 amino acid sequence depicted in FIG. 2A or to the CD80 amino acid sequence set forth in SEQ ID NO:1,

wherein the variant CD80 immunomodulatory polypeptide has one or more amino acid substitutions relative to the CD80 amino acid sequence depicted in FIG. 2A or to the CD80 amino acid sequence set forth in SEQ ID NO:1; and

wherein the variant CD80 immunomodulatory polypeptide exhibits reduced binding affinity to a CD86 polypeptide having an amino acid sequence depicted in one of FIG. 3A-C, compared to the binding affinity of the CD80 amino acid sequence depicted in FIG. 2A, or compared to the binding affinity of the CD80 amino acid sequence as set forth in SEQ ID NO:1, for the CD86 polypeptide.

2. The variant immunomodulatory polypeptide of claim 1, wherein the polypeptide comprises a substitution of amino acid N19, N63, I67, K86, Q157, D158, L25, Y31, Q33, M38, V39, I49, Y53, D60, F108, or S156.

3. The variant immunomodulatory polypeptide of claim 1, wherein the variant immunomodulatory polypeptide exhibits from less than 10% to less than 50% of binding affinity exhibited by to the CD80 amino acid sequence depicted in FIG. 2A, or as set forth in SEQ ID NO:1, for the CD86 polypeptide.

4. A multimeric polypeptide comprising:

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, 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,

wherein the immunomodulatory domain is a variant immunomodulatory polypeptide of any one of claims 1-3, and

wherein the multimeric polypeptide exhibits reduced binding affinity to a CD28 polypeptide having an amino acid sequence depicted in one of FIG. 3A-3C, compared to the binding affinity of a control multimeric polypeptide comprising an immunomodulatory domain comprising the CD80 amino acid sequence depicted in FIG. 2A, or compared to the binding affinity of a control multimeric polypeptide comprising an immunomodulatory domain comprising the CD80 amino acid sequence as set forth in SEQ ID NO:1, for the CD28 polypeptide.

5. The multimeric polypeptide of claim 4, wherein 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.

6. The multimeric polypeptide of claim 4, wherein 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.

7. The multimeric polypeptide of claim 4, wherein 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.

8. The multimeric polypeptide of claim 4, wherein 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.

9. The multimeric polypeptide of claim 4, wherein 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.

10. The multimeric polypeptide of claim 4, wherein 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.

11. The multimeric polypeptide of claim 4, wherein the non-Ig scaffold is an XTEN polypeptide, a transferrin polypeptide, an elastin-like polypeptide, a silk-like polypeptide, or a silk-elastin-like polypeptide.

12. The multimeric polypeptide of any one of claims 4-11, wherein the first MHC polypeptide is a β2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide.

13. The multimeric polypeptide of claim 12, wherein the β2-microglobulin polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to one of the amino acid sequences set forth in FIG. 6.

14. The multimeric polypeptide of claim 11, wherein the MHC class I heavy chain polypeptide is an HLA-A, an HLA-B, or an HLA-C heavy chain.

15. The multimeric polypeptide of claim 12, wherein 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 one of FIG. 5A-5C.

16. The multimeric polypeptide of any one of claims 4-11, wherein 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.

17. The multimeric polypeptide of any one of claims 4-16, wherein the epitope is a T-cell epitope.

18. The multimeric polypeptide of any one of claims 4-10 and 12-17, wherein 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.

19. The multimeric polypeptide of claim 18, wherein the Ig Fc polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to an amino acid sequence depicted in FIG. 4A-4C.

20. The multimeric polypeptide of any one of claims 4-19, wherein the first polypeptide and the second polypeptide are non-covalently associated.

21. The multimeric polypeptide of any one of claims 4-19, wherein the first polypeptide and the second polypeptide are covalently linked.

22. The multimeric polypeptide of claim 21, wherein the covalent linkage is via a disulfide bond.

23. The multimeric polypeptide of claim 22, wherein 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.

24. The multimeric polypeptide of any one of claims 4-11, comprising a first linker interposed between the epitope and the first MHC polypeptide.

25. The multimeric polypeptide of any one of claims 4-11, wherein the variant CD80 immunomodulatory polypeptide comprises a substitution of amino acid N19, N63, I67, K86, Q157, D158, L25, Y31, Q33, M38, V39, I49, Y53, D60, F108, or S156.

26. The multimeric polypeptide of any one of claims 4-25, comprising 2 or more immunomodulatory polypeptides.

27. The multimeric polypeptide of claim 26, wherein the 2 or more immunomodulatory polypeptides are in tandem.

28. The multimeric polypeptide of any one of claims 26 and 27, wherein 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.

29. The multimeric polypeptide of claim 28, wherein the third polypeptide is covalently linked to the first polypeptide.

30. The multimeric polypeptide of any one of claims 4-10 and 12-29, wherein 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.

31. 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 major histocompatibility complex (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;

wherein the immunomodulatory polypeptide is a variant immunomodulatory polypeptide of any one of claims 1-3; 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,

wherein the immunomodulatory polypeptide is a variant immunomodulatory polypeptide of any one of claims 1-3.

32. The nucleic acid of claim 31, wherein the first MHC polypeptide is a β2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide.

33. The nucleic acid of claim 32, wherein the β2-microglobulin polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to one of the amino acid sequences set forth in FIG. 6.

34. The nucleic acid of claim 31, wherein the MHC class I heavy chain polypeptide is an HLA-A, HLA-B, or HLA-C heavy chain.

35. The nucleic acid of claim 34, wherein 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 any one of FIG. 5A-5C.

36. The nucleic acid of claim 31, wherein 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.

37. The nucleic acid of claim 31, wherein the epitope is a T-cell epitope.

38. The nucleic acid of claim 31, 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.

39. The nucleic acid of claim 38, wherein 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 4A-4C.

40. The nucleic acid of claim 31, wherein the variant CD80 immunomodulatory polypeptide comprises a substitution of amino acid N19, N63, I67, K86, Q157, D158, L25, Y31, Q33, M38, V39, I49, Y53, D60, F108, or S156.

41. The nucleic acid of claim 31, wherein the multimeric polypeptide comprises a second immunomodulatory polypeptide selected from a CD7, CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, and HVEM.

42. The nucleic acid of claim 31, wherein the proteolytically cleavable linker or ribosome skipping signal comprises an amino acid sequence selected from:

a) LEVLFQGP (SEQ ID NO:78);

b) ENLYTQS (SEQ ID NO:79);

c) a furin cleavage site;

d) LVPR (SEQ ID NO:80);

e) GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:81);

f) GSGEGRGSLLTCGDVEENPGP (SEQ ID NO:82);

g) GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO:83); and

h) GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:84).

43. The nucleic acid of claim 31, wherein 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.

44. The nucleic acid of claim 43, wherein the first leader peptide and the second leader peptide is a β2-M leader peptide.

45. The nucleic acid of claim 31, wherein the nucleotide sequence is operably linked to a transcriptional control element.

46. The nucleic acid of claim 45, wherein the transcriptional control element is a promoter that is functional in a eukaryotic cell.

47. The nucleic acid of claim 31, wherein 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.

48. A recombinant expression vector comprising the nucleic acid of any one of claims 31-47.

49. The recombinant expression vector of claim 48, wherein the vector is a viral vector or a non-viral vector.

50. A host cell genetically modified with the recombinant expression vector of claim 48.

51. The host cell of claim 50, wherein the host cell is *in vitro*.

52. The host cell of claim 50, wherein the host cell is genetically modified such that the cell does not produce an endogenous MHC β2-microglobulin polypeptide.

53. The host cell of claim 50, wherein the host cell is a T lymphocyte.

54. 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,

wherein the immunomodulatory domain is a variant immunomodulatory polypeptide of any one of claims 1-3; 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.

55. 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, wherein the immunomodulatory domain is a variant immunomodulatory polypeptide of any one of claims 1-3;

ii) a second MHC polypeptide; and

iii) an Ig Fc polypeptide.

56. The composition of claim 54 or 55, wherein the first and/or the second nucleic acid is present in a recombinant expression vector.

57. A host cell genetically modified with the composition of any one of claims 54-56.

58. A method of producing the multimeric polypeptide of any one of claims 4-30, the method comprising:

a) culturing the host cell of any one of claims 50-53 and 57 *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.

59. The method of claim 58, wherein 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.

60. The method of claim 58, comprising eluting the immobilized multimeric polypeptide.

61. A method of selectively modulating the activity of an epitope-specific T cell, the method comprising contacting the T cell with the multimeric polypeptide of any one of claims 4-30, wherein said contacting selectively modulates the activity of the epitope-specific T cell.

62. The method of claim 61, wherein the immunomodulatory polypeptide is an activating polypeptide, and wherein the multimeric polypeptide activates the epitope-specific T cell.

63. The method of claim 61, wherein the immunomodulatory polypeptide is an inhibiting polypeptide, and wherein the multimeric polypeptide inhibits the epitope-specific T cell.

64. The method of claim 61, wherein said contacting is *in vitro*.

65. The method of claim 61, wherein said contacting is *in vivo*.

66. 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 the multimeric polypeptide of any one of claims 4-30 effective to selectively modulate the activity of an epitope-specific T cell in an individual.

67. The method of claim 66, wherein the immunomodulatory polypeptide is an activating polypeptide, and wherein the multimeric polypeptide activates the epitope-specific T cell.

68. The method of claim 67, wherein 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.

69. The method of claim 66, wherein the immunomodulatory polypeptide is an inhibitory polypeptide, and wherein the multimeric polypeptide inhibits activity of the epitope-specific T cell.

70. The method of claim 69, wherein the epitope is a self-epitope, and wherein said administering selectively inhibits the activity of a T cell specific for the self-epitope.

71. A method of treating an infection in an individual, the method comprising administering to the individual an effective amount of

a) the multimeric polypeptide of any one of claims 4-30; or

b) one or more recombinant expression vectors comprising nucleotide sequences encoding the multimeric polypeptide of any one of claims 4-30; or

c) one or more mRNAs comprising nucleotide sequences encoding the multimeric polypeptide of any one of claims 4-30.

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.

72. The method of claim 71, wherein the pathogen is a virus, a bacterium, or a protozoan.

73. The method of any one of claims 66-71, wherein said administering is subcutaneous.

74. The method of any one of claims 66-71, wherein said administering is intravenous.

75. The method of any one of claims 66-71, wherein said administering is intramuscular.

76. The method of any one of claims 66-71, wherein said administering is systemic.

77. The method of any one of claims 66-71, wherein said administering is distal to a treatment site.

78. The method of any one of claims 66-71, wherein said administering is local.

79. The method of any one of claims 66-71, wherein said administering is at or near a treatment site.

80. A composition comprising:

a) the multimeric polypeptide of any one of claims 4-30; and

b) a pharmaceutically acceptable excipient.

81. A composition comprising:

a) the nucleic acid of any one of claims 31-47 or the recombinant expression vector of claim 48 or 49; and

b) a pharmaceutically acceptable excipient.

FIG. 1A

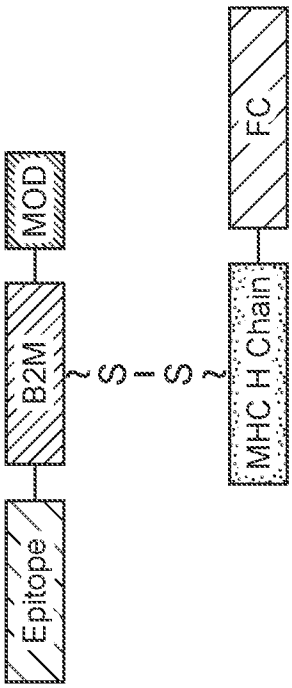

FIG. 1B

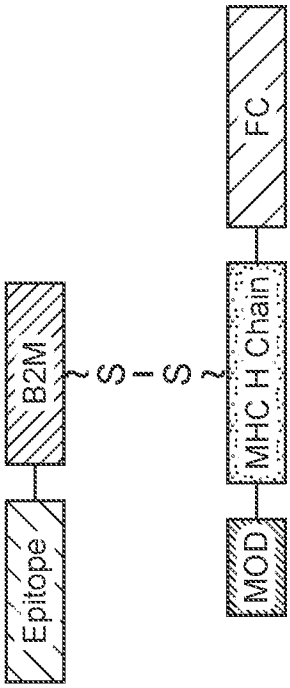

FIG. 1C

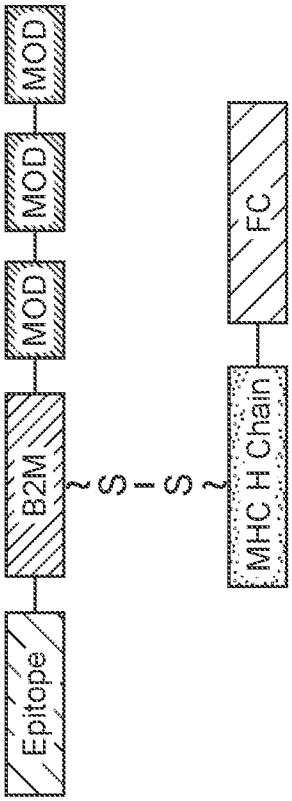

FIG. 1D

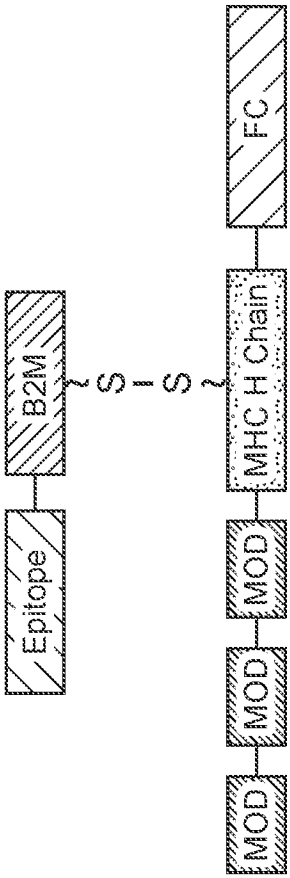

FIG. 2A

Homo sapiens
CD80 (B7-1)

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dnllpswait lisvngifvi ccltycfapr crerrrnerl rresvrpv

(SEQ ID NO:2)

FIG. 2B
N19

vihvtk evkevatlsc gh**x**vsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:3)

FIG. 2C
N19

vihvtk evkevatlsc gh**a**vsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:4)

FIG. 2D
N63

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifdit**x**nls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:5)

FIG. 2E
N63

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifdit**a**nls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:6)

FIG. 2F
I67

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls **x**vilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:7)

FIG. 2G
I67

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls **a**vilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:8)

FIG. 2H
K86

```
                    vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlx

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:9)
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FIG. 2I
K86

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                    vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvla

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:10)
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FIG. 2J
Q157

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs **x**dpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:11)

FIG. 2K
Q157

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs **a**dpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:12)

FIG. 2L
D158

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs q**x**petelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:13)

FIG. 2M
D158

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs q**a**petelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:14)

FIG. 2N
L25

vihvtk evkevatlsc ghnvsvee**x**a

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:15)

FIG. 2O
L25

vihvtk evkevatlsc ghnvsvee**a**a

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:16)

FIG. 2P
Y31

vihvtk evkevatlsc ghnvsveela

qtri**x**wqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:17)

FIG. 2Q
Y31

vihvtk evkevatlsc ghnvsveela

qtri**a**wqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:18)

FIG. 2R
Q33

vihvtk evkevatlsc ghnvsveela

qtriyw**x**kek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:19)

FIG. 2S
Q33

vihvtk evkevatlsc ghnvsveela

qtriyw**a**kek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:20)

FIG. 2T
M38

vihvtk evkevatlsc ghnvsveela

qtriywqkek k**x**vltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:21)

FIG. 2U
M38

vihvtk evkevatlsc ghnvsveela

qtriywqkek k**a**vltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:22)

FIG. 2V
V39

vihvtk evkevatlsc ghnvsveela

qtriywqkek km**x**ltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:23)

FIG. 2W
V39

vihvtk evkevatlsc ghnvsveela

qtriywqkek km**a**ltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:24)

FIG. 2X
I49

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mn**x**wpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:25)

FIG. 2Y
I49

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mn**a**wpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:26)

FIG. 2Z
Y53

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpe**x**knr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:27)

FIG. 2AA
Y53

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpe**a**knr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:28)

FIG. 2BB
D60

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tif**x**itnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:29)

FIG. 2CC
D60

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tif**a**itnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:30)

FIG. 2DD
F108

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka d**x**ptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:31)

FIG. 2EE
F108

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka d**a**ptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:32)

FIG. 2FF
S156

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttv**x** qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:33)

FIG. 2GG
S156

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttv**a** qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:34)

FIG. 2HH
P111

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfpt**x**sisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:35)

FIG. 2II
P111

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfpt**a**sisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:36)

FIG. 3A
CD28 isoform 1
Homo sapiens
Mature protein amino acids 19-220

```
  1 mlrlllalnl fpsiqvtgnk ilvkqspmlv aydnavnlsc kysynlfsre fraslhkgld
 61 savevcvvyg nysqqlqvys ktgfncdgkl gnesvtfylq nlyvnqtdiy fckievmypp
121 pyldneksng tiihvkgkhl cpsplfpgps kpfwvlvvvg gvlacysllv tvafiifwvr
181 skrsrllhsd ymnmtprrpg ptrkhyqpya pprdfaayrs
```
(SEQ ID NO: 37)

FIG. 3B
CD28 isoform 2
Homo sapiens
Mature protein amino acids 19-123

```
  1 mlrlllalnl fpsiqvtgnk ilvkqspmlv aydnavnlsw khlcpsplfp gpskpfwvlv
 61 vvggvlacys llvtvafiif wvrskrsrll hsdymnmtpr rpgptrkhyq pyapprdfaa
121 yrs (SEQ ID NO: 38)
```

FIG. 3C
CD28 isoform 2
Homo sapiens
Mature protein amino acids 19-101

```
  1 mlrlllalnl fpsiqvtgkh lcpsplfpgp skpfwvlvvv ggvlacysll vtvafiifwv
 61 rskrsrllhs dymnmtprrp gptrkhyqpy apprdfaayr s (SEQ ID NO:39)
```

FIG. 3D
CTLA4
Homo sapiens

```
  1 maclgfqrhk aqlnlatrtw pctllfflif ipvfckamhv aqpavvlass rgiasfvcey
 61 aspgkatevr vtvlrqadsq vtevcaatym mgneltfldd sictgtssgn qvnltiqglr
121 amdtglyick velmypppyy lgignqtqiy vidpepcpds dfllwilaav ssglffysfl
181 ltavslskml kkrspltqgv yvkmpptepe cekqfqpyfi pin (SEQ ID NO:40)
```

Figure 4A
GenBank 3S7G_A
Homo sapiens **IgG1** Fc (SEQ ID NO:41)
227 aa

```
  1 dkthtcppcp apellggpsv flfppkpkdt lmisrtpevt cvvvdvshed pevkfnwyvd
 61 gvevhnaktk preeqynsty rvvsvltvlh qdwlngkeyk ckvsnkalpa piektiskak
121 gqprepqvyt lppsrdeltk nqvsltclvk gfypsdiave wesngqpenn ykttppvlds
181 dgsfflyskl tvdksrwqqg nvfscsvmhe alhnhytqks lslspgk
```

GenBank AAN76044
Homo sapiens **IgG2** Fc (amino acids 99-325) (SEQ ID NO:42)
227 aa

```
  1 stkgpsvfpl apcsrstses taalgclvkd yfpepvtvsw nsgaltsgvh tfpavlqssg
 61 lyslssvvtv pssnfgtqty tcnvdhkpsn tkvdktverk ccvecppcpa ppvagpsvfl
121 fppkpkdtlm isrtpevtcv vvdvshedpe vqfnwyvdgv evhnaktkpr eeqfnstfrv
181 vsvltvvhqd wlngkeykck vsnkglpapi ektisktkgq prepqvytlp psreemtknq
241 vsltclvkgf ypsdiavewe sngqpennyk ttppmldsdg sfflyskltv dksrwqqgnv
301 fscsvmheal hnhytqksls lspgk
```

GenBank AAW65947
Homo sapiens **IgG3** Fc (amino acids 19-246) (SEQ ID NO:43)
238 aa

```
  1 hkpsntkvdk rvelktplgd tthtcppcpa pellggpsvf lfppkpkdtl misrtpevtc
 61 vvvdvshedp evkfnwyvdg vevhnaktkp reeqynstyr vvsvltvlhq dwlngkeykc
121 kvsnkalpap iektiskakg qprepqvytl ppsrdeltkn qvsltclvkg fypsdiavew
181 esngqpenny kttppvldsd gsfflysklt vdksrwqqgn vfscsvmhea lhnhytqksl
241 slspgk
```

Figure 4B

GenBank AAA52770
Homo sapiens **IgD** Fc (amino acids 162-383) (SEQ ID NO:44)
222 aa

```
  1 ptkapdvfpi isgcrhpkdn spvvlaclit gyhptsvtvt wymgtqsqpq rtfpeiqrrd
 61 syymtssqls tplqqwrqge ykcvvqhtas kskkeifrwp espkaqassv ptaqpqaegs
121 lakattapat trntgrggee kkkekekeeq eeretktpec pshtqplgvy lltpavqdlw
181 lrdkatftcf vvgsdlkdah ltwevagkvp tggveeglle rhsngsqsqh srltlprslw
241 nagtsvtctl nhpslppqrl malrepaaqa pvklslnlla ssdppeaasw llcevsgfsp
301 pnillmwled qrevntsgfa parpppqprs ttfwawsvlr vpappspqpa tytcvvshed
361 srtllnasrs levsyvtdhg pmk
```

GenBank 0308221A
Homo sapiens **IgM** Fc (SEQ ID NO:45)
276 aa

```
  1 vtstltikzs dwlgesmftc rvdhrgltfq qnassmcvpd qdtairvfai ppsfasiflt
 61 kstkltclvt dlttybsvti swtreengav kthtnisesh pnatfsavge asicedbdws
121 gerftctvth tdlpsplkqt isrpkgvalh rpbvyllppa rzzlnlresa titclvtgfs
181 padvfvewmq rgeplspqky vtsapmpepq apgryfahsi ltvseeewnt ggtytcvvah
241 ealpnrvter tvdkstgkpt lynvslvmsd tagtcy
```

Figure 4C

GenBank P01876
Homo sapiens **IgA** Fc (amino acids 120-353) (SEQ ID NO:46)
234 aa

```
  1 asptspkvfp lslcstqpdg nvviaclvqg ffpqeplsvt wsesgqgvta rnfppsqdas
 61 gdlyttssql tlpatqclag ksvtchvkhy tnpsqdvtvp cpvpstpptp spstpptpsp
121 scchprlslh rpaledlllg seanltctlt glrdasgvtf twtpssgksa vqgpperdlc
181 gcysvssvlp gcaepwnhgk tftctaaype sktpltatls ksgntfrpev hllpppseel
241 alnelvtltc largfspkdv lvrwlqgsqe lprekyltwa srqepsqgtt tfavtsilrv
301 aaedwkkgdt fscmvgheal plaftqktid rlagkpthvn vsvvmaevdg tcy
```

GenBank 1F6A_B
Homo sapiens **IgE** Fc (amino acids 6-222) (SEQ ID NO:47)
212 aa

```
  1 adpcdsnprg vsaylsrpsp fdlfirkspt itclvvdlap skgtvnltws rasgkpvnhs
 61 trkeekqrng tltvtstlpv gtrdwieget yqcrvthphl pralmrsttk tsgpraapev
121 yafatpewpg srdkrtlacl iqnfmpedis vqwlhnevql pdarhsttqp rktkgsgffv
181 fsrlevtrae weqkdeficr avheaaspsq tvqravsvnp gk
```

GenBank P01861
Homo sapiens **IgG4** Fc (amino acids 100-327) (SEQ ID NO:48)
228 aa

```
  1 astkgpsvfp lapcsrstse staalgclvk dyfpepvtvs wnsgaltsgv htfpavlqss
 61 glyslssvvt vpssslgtkt ytcnvdhkps ntkvdkrves kygppcpscp apeflggpsv
121 flfppkpkdt lmisrtpevt cvvvdvsqed pevqfnwyvd gvevhnaktk preeqfnsty
181 rvvsvltvlh qdwlngkeyk ckvsnkglps siektiskak gqprepqvyt lppsqeemtk
241 nqvsltclvk gfypsdiave wesngqpenn ykttppvlds dgsfflysrl tvdksrwqeg
301 nvfscsvmhe alhnhytqks lslslgk
```

Figure 5A
Homo sapiens
GenBank NP_001229687
HLA-A
Amino acids 25-365 (SEQ ID NO:49)

```
  1 mavmaprtll lllsgalalt qtwagshsmr yfftsvsrpg rgeprfiavg yvddtqfvrf
 61 dsdaasqkme prapwieqeg peywdqetrn mkahsqtdra nlgtlrgyyn qsedgshtiq
121 imygcdvgpd grflrgyrqd aydgkdyial nedlrswtaa dmaaqitkrk weavhaaeqr
181 rvylegrcvd glrrylengk etlqrtdppk thmthhpisd heatlrcwal gfypaeitlt
241 wqrdgedqtq dtelvetrpa gdgtfqkwaa vvvpsgeeqr ytchvqhegl pkpltlrwel
301 ssqptipivg iiaglvllga vitgavvaav mwrrkssdrk ggsytqaass dsaqgsdvsl
361 tackv
```

Figure 5B
Homo sapiens
GenBank NP_005505
HLA-B
Amino acids 25-362 (SEQ ID NO:50)

```
  1 mlvmaprtvl lllsaalalt etwagshsmr yfytsvsrpg rgeprfisvg yvddtqfvrf
 61 dsdaaspree prapwieqeg peywdrntqi ykaqaqtdre slrnlrgyyn qseagshtlq
121 smygcdvgpd grllrghdqy aydgkdyial nedlrswtaa dtaaqitqrk weaareaeqr
181 raylegecve wlrrylengk dkleradppk thvthhpisd heatlrcwal gfypaeitlt
241 wqrdgedqtq dtelvetrpa gdrtfqkwaa vvvpsgeeqr ytchvqhegl pkpltlrwep
301 ssqstvpivg ivaglavlav vvigavvaav mcrrkssggk ggsysqaacs dsaqgsdvsl
361 ta
```

Figure 5C

Homo sapiens

GenBank NP_001229971

HLA-C

Amino acids 25-366 (SEQ ID NO:51)

```
  1 mrvmaprall lllsggla1t etwacshsmr yfdtavsrpg rgeprfisvg yvddtqfvrf
 61 dsdaasprge prapwveqeg peywdretqn ykrqaqadrv slrnlrgyyn qsedgshtlq
121 rmygcdlgpd grllrgydqs aydgkdyial nedlrswtaa dtaaqitqrk leaaraaeql
181 raylegtcve wlrrylengk etlqraeppk thvthhplsd heatlrcwal gfypaeitlt
241 wqrdgedqtq dtelvetrpa gdgtfqkwaa vvvpsgqeqr ytchmqhegl qeplt1swep
301 ssqptipimg ivaglavlvv lavlgavvta mmcrrkssgg kggscsqaac snsaqgsdes
361 litcka
```

FIG. 6

```
NP_004039.1          MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLL 60
NP_001009066.1       MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLL 60
NP_001040602.1       MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPPENGKPNFLNCYVSGFHPSDIEVDLL 60
NP_776318.1          MARFVALVLLGLLSLSGLDAIQRPPKIQVYSRHPPEDGKPNYLNCYVYGFHPPQIEIDLL 60
NP_033865.2          MARSVTLVFLVLVSLTGLYAIQKTPQIQVYSRHPPENGKPNILNCYVTQFHPPHIEIQML 60
                     *:* *;*..* *:**:** ***:.*:********.*:**.* *****  ***..**:::*

NP_004039.1          KNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM 119
NP_001009066.1       KNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM 119
NP_001040602.1       KNGEKMGKVEHSDLSFSKDWSFYLLYYTEFTPNEKDEYACRVNHVTLSGPRTVKWDRDM 119
NP_776318.1          KNGEKI-KSEQSDLSFSKDWSFYLLSHAEFTPNSKDQYSCRVKHVTLEQPRIVKWDRDL 118
NP_033865.2          KNGKKIPKVEMSDMSFSKDWSFYILAHTEFTPTETDTYACRVKHASMAEPKTVYWDRDM 119
                     ***:;: * * **:**********:* ;:****...* *:***:*.::  *: * ****:
```

Figure 7A
PD-L1
Mus musculus
NP_068693
Amino acids 19-290

```
  1 mrifagiift acchllraft itapkdlyvv eygsnvtmec rfpvereldl lalvvyweke
 61 deqviqfvag eedlkpqhsn frgraslpkd qllkgnaalq itdvklqdag vycciisygg
121 adykritlkv napyrkinqr isvdpatseh elicqaegyp eaeviwtnsd hqpvsgkrsv
181 ttsrtegmll nvtsslrvna tandvfyctf wrsqpgqnht aeliipelpa thppqnrthw
241 vllgsillfl ivvstvllfl rkqvrmldve kcgvedtssk nrndtqfeet
```

(SEQ ID NO:57)

Figure 7B
PD-L1
Homo sapiens
NP_054852
Amino acids 19-290

```
  1 mrifavfifm tywhllnaft vtvpkdlyvv eygsnmtiec kfpvekqldl aalivyweme
 61 dkniiqfvhg eedlkvqhss yrqrarllkd qlslgnaalq itdvklqdag vyrcmisygg
121 adykritvkv napynkinqr ilvvdpvtse heltcqaegy pkaeviwtss dhqvlsgktt
181 ttnskreekl fnvtstlrin tttneifyct frrldpeenh taelvipelp lahppnerth
241 lvilgaillc lgvaltfifr lrkgrmmdvk kcgiqdtnsk kqsdthleet
```

(SEQ ID NO:58)

Figure 8

4-1-BBL
Homo sapiens
GenBank NP_003802
Amino acids 80-254 = ectodomain

```
  1 meyasdasld peapwppapr aracrvlpwa lvagllllll laaacavfla cpwavsgara
 61 spgsaasprl regpelspdd paglldlrqg mfaqlvaqnv llidgplswy sdpglagvsl
121 tgglsykedt kelvvakagv yyvffqlelr rvvagegsgs vslalhlqpl rsaagaaala
181 ltvdlppass earnsafgfq grllhlsagq rlgvhlhtea rarhawqltq gatvlglfrv
241 tpeipaglps prse
```

(SEQ ID NO:59)

Figure 9
Homo sapiens
ICOS-L
GenBank NP_056074
Amino acids 19-302

```
  1 mrlgspgllf llfsslradt qekevramvg sdvelscacp egsrfdlndv yvywqtsesk
 61 tvvtyhipqn sslenvdsry rnralmspag mlrgdfslrl fnvtpqdeqk fhclvlsqsl
121 gfqevlsvev tlhvaanfsv pvvsaphsps qdeltftcts ingyprpnvy winktdnsll
181 dqalqndtvf lnmrglydvv svlriartps vnigccienv llqqnltvgs qtgndigerd
241 kitenpvstg eknaatwsil avlcllvvva vaigwvcrdr clqhsyagaw avspeteltg
301 hv
```

(SEQ ID NO:60)

Figure 10
Homo sapiens
GenBank NP_003317
OX4L

```
  1 mervqpleen vgnaarprfe rnklllvasv iqglglllcf tyiclhfsal qvshrypriq
 61 sikvqfteyk kekgfiltsq kedeimkvqn nsviincdgf ylislkgyfs qevnislhyq
121 kdeeplfqlk kvrsvnslmv asltykdkvy lnvttdntsl ddfhvnggel ilihqnpgef
181 cvl
```

(SEQ ID NO:61)

Figure 11
Homo sapiens
GenBank NP_079515
PD-L2
Amino acids 20-273

```
  1 miflllmlsl elqlhqiaal ftvtvpkely iiehgsnvtl ecnfdtgshv nlgaitaslq
 61 kvendtsphr eratlleeql plgkasfhip qvqvrdegqy qciiiygvaw dykyltlkvk
121 asyrkinthi lkvpetdeve ltcqatgypl aevswpnvsv pantshsrtp eglyqvtsvl
181 rlkpppgrnf scvfwnthvr eltlasidlq sqmeprthpt wllhifipfc iiafifiatv
241 ialrkqlcqk lysskdttkr pvtttkrevn sai
```

(SEQ ID NO:62)

Figure 12
Homo sapiens
GenBank NP_787058
CD86 (B7-2)
Amino acids 31-329

```
  1 mdpqctmgls nilfvmafll sgaaplkiqa yfnetadlpc qfansqnqsl selvvfwqdq
 61 enlvlnevyl gkekfdsvhs kymgrtsfds dswtlrlhnl qikdkglyqc iihhkkptgm
121 irihqmnsel svlanfsqpe ivpisniten vyinltcssi hgypepkkms vllrtknsti
181 eydgimqksq dnvtelydvs islsvsfpdv tsnmtifcil etdktrllss pfsieledpq
241 pppdhipwit avlptviicv mvfclilwkw kkkkrprnsy kcgtntmere eseqtkkrek
301 ihipersdea qrvfksskts scdksdtcf
```

(SEQ ID NO:63)

FIG. 13
Fas ligand (FasL)
Homo sapiens
GenBank NP_000630
Amino acids 1-281

```
  1 mqqpfnypyp qiywvdssas spwappgtvl pcptsvprrp gqrrpppppp ppplpppppp
 61 pplpplplpp lkkrgnhstg lcllvmffmv lvalvglglg mfqlfhlqke laelrestsq
121 mhtasslekq ighpspppek kelrkvahlt gksnsrsmpl ewedtygivl lsgvkykkgg
181 lvinetglyf vyskvyfrgq scnnlplshk vymrnskypq dlvmmegkmm sycttgqmwa
241 rssylgavfn ltsadhlyvn vselslvnfe esqtffglyk l
```

(SEQ ID NO:64)

FIG. 14

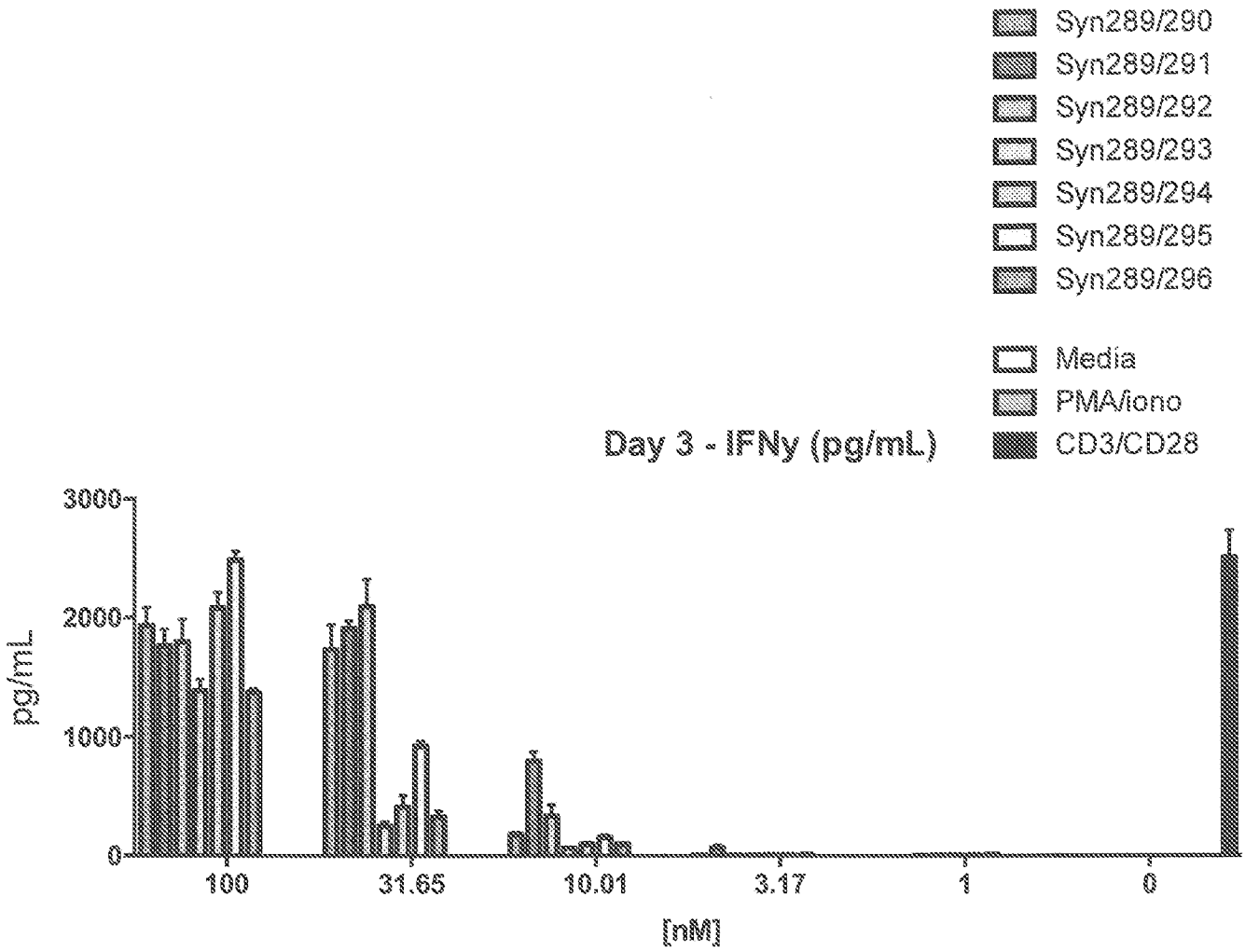
Syn289/290
Syn289/291
Syn289/292
Syn289/293
Syn289/294
Syn289/295
Syn289/296
Media
PMA/iono
CD3/CD28
Day 3 - IFNy (pg/mL)
3000
2000
1000
0
pg/mL
100
31.65
10.01
3.17
1
0
[nM]

FIG. 15

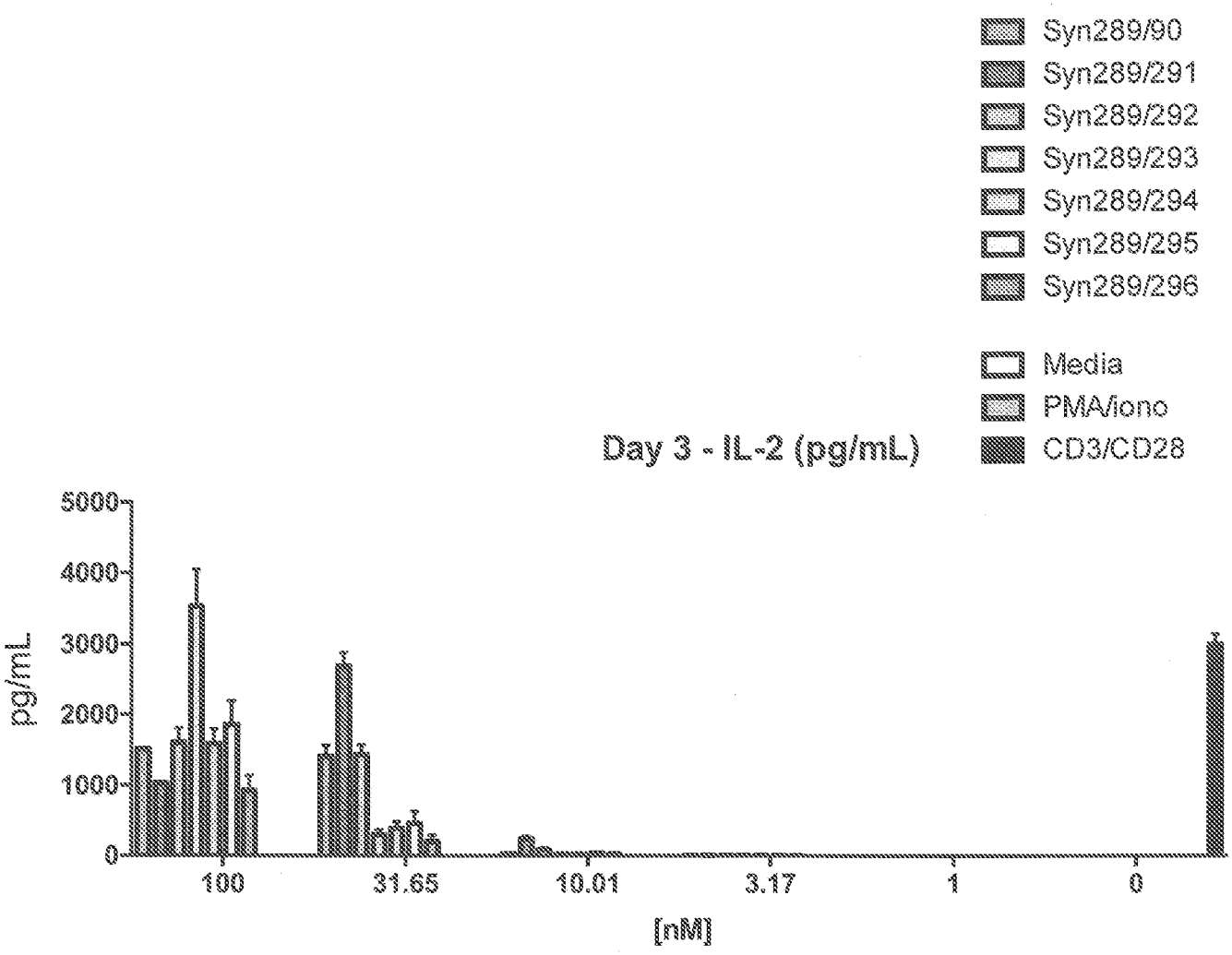
Syn289/90
Syn289/291
Syn289/292
Syn289/293
Syn289/294
Syn289/295
Syn289/296
Media
PMA/iono
CD3/CD28
Day 3 - IL-2 (pg/mL)
pg/mL
5000
4000
3000
2000
1000
0
100
31.65
10.01
3.17
1
0
[nM]

FIG. 16

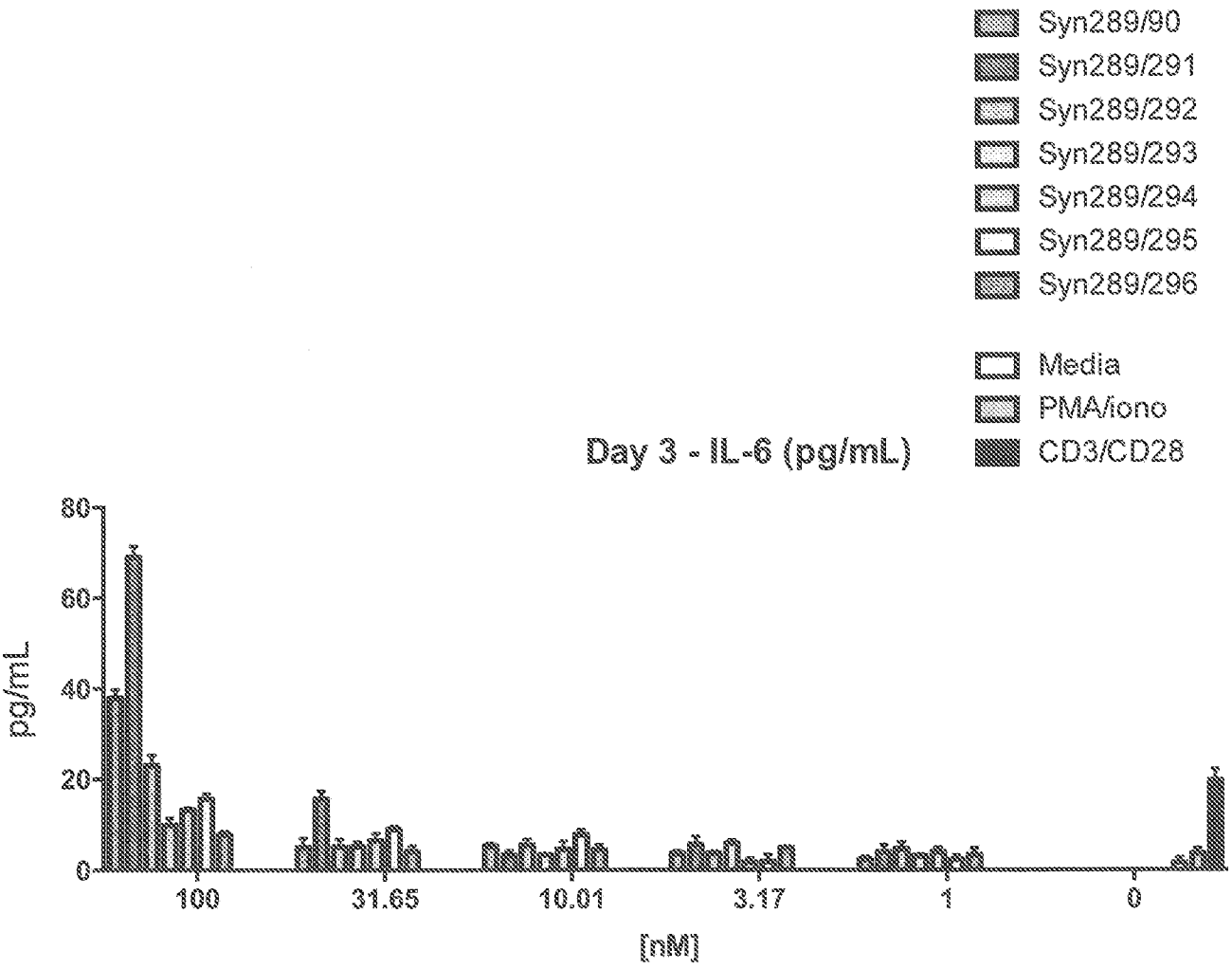
Syn289/90
Syn289/291
Syn289/292
Syn289/293
Syn289/294
Syn289/295
Syn289/296
Media
PMA/iono
CD3/CD28
Day 3 - IL-6 (pg/mL)
pg/mL
80
60
40
20
0
100
31.65
10.01
3.17
1
0
[nM]

FIG. 17

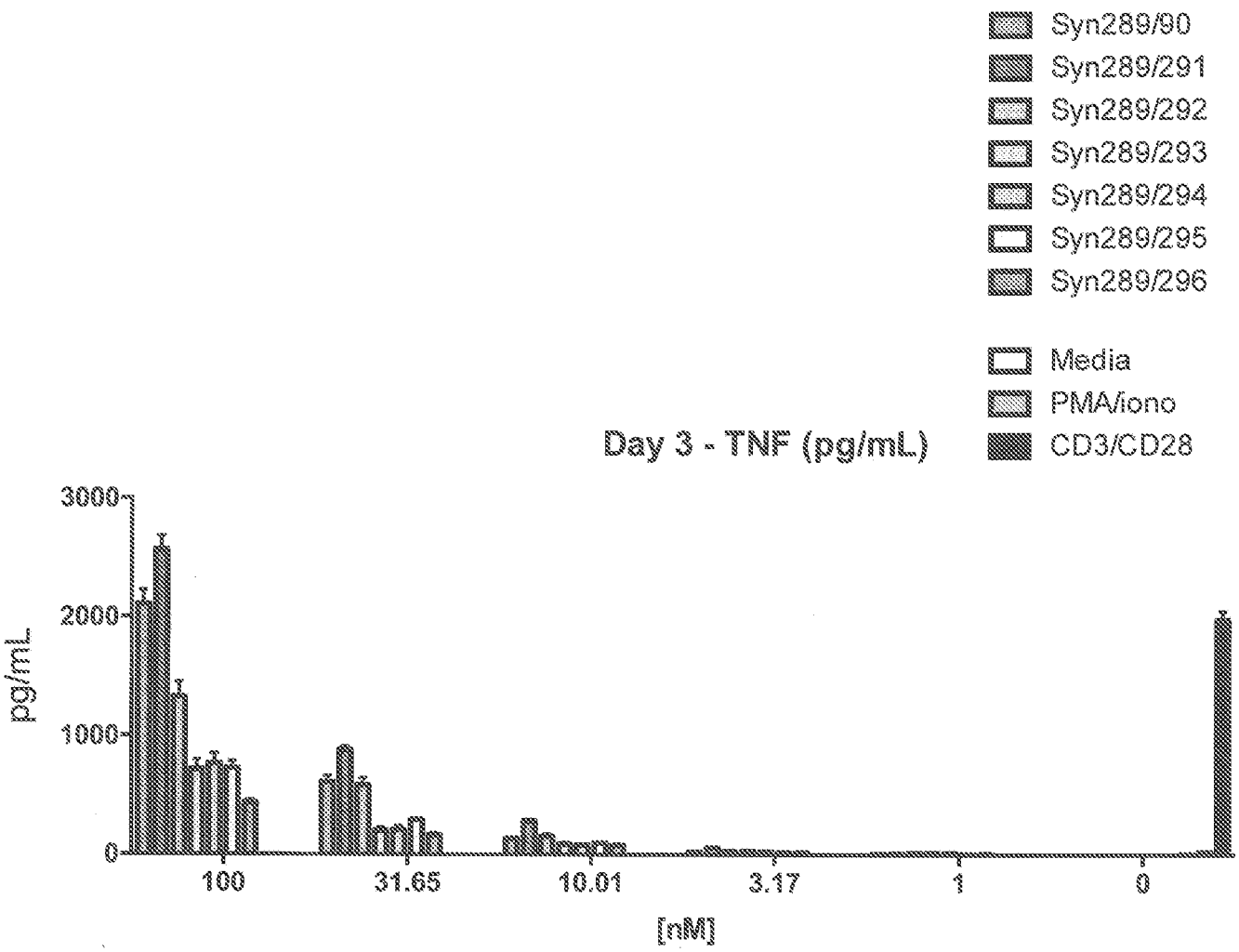
Syn289/90
Syn289/291
Syn289/292
Syn289/293
Syn289/294
Syn289/295
Syn289/296
Media
PMA/iono
CD3/CD28
Day 3 - TNF (pg/mL)
3000
2000
1000
0
pg/mL
100
31.65
10.01
3.17
1
0
[nM]

FIG. 18

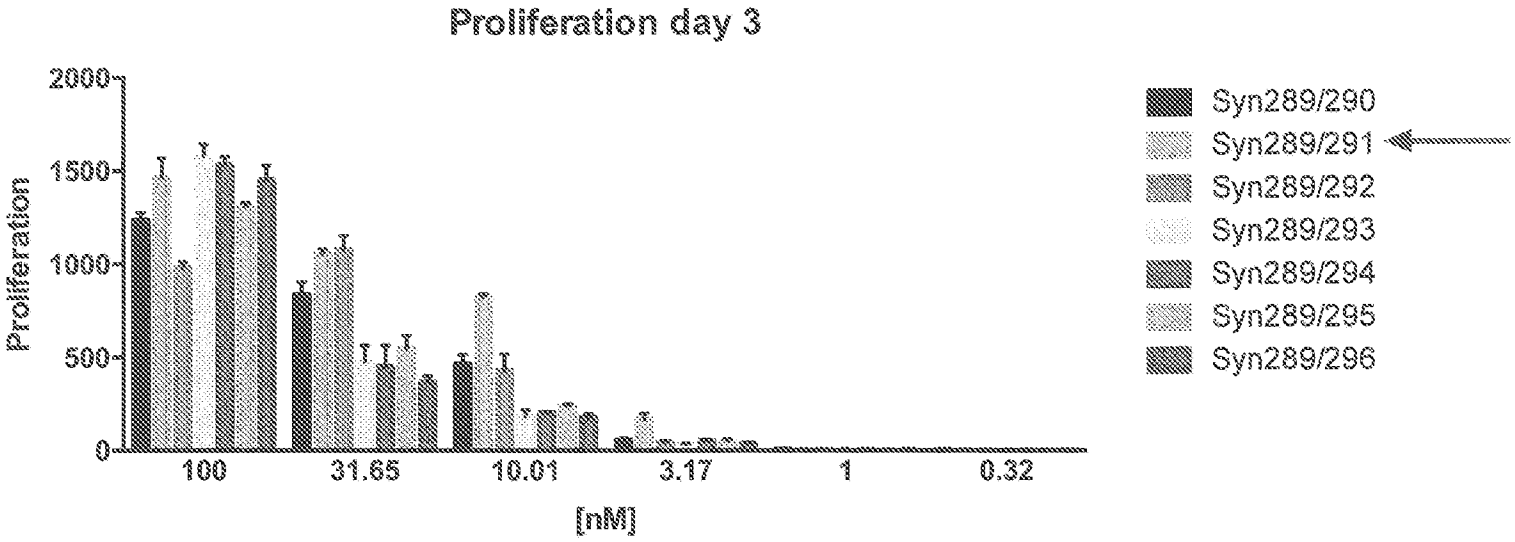
Proliferation day 3
Proliferation
2000
1500
1000
500
0
100
31.65
10.01
3.17
1
0.32
[nM]
Syn289/290
Syn289/291
Syn289/292
Syn289/293
Syn289/294
Syn289/295
Syn289/296

FIG. 19

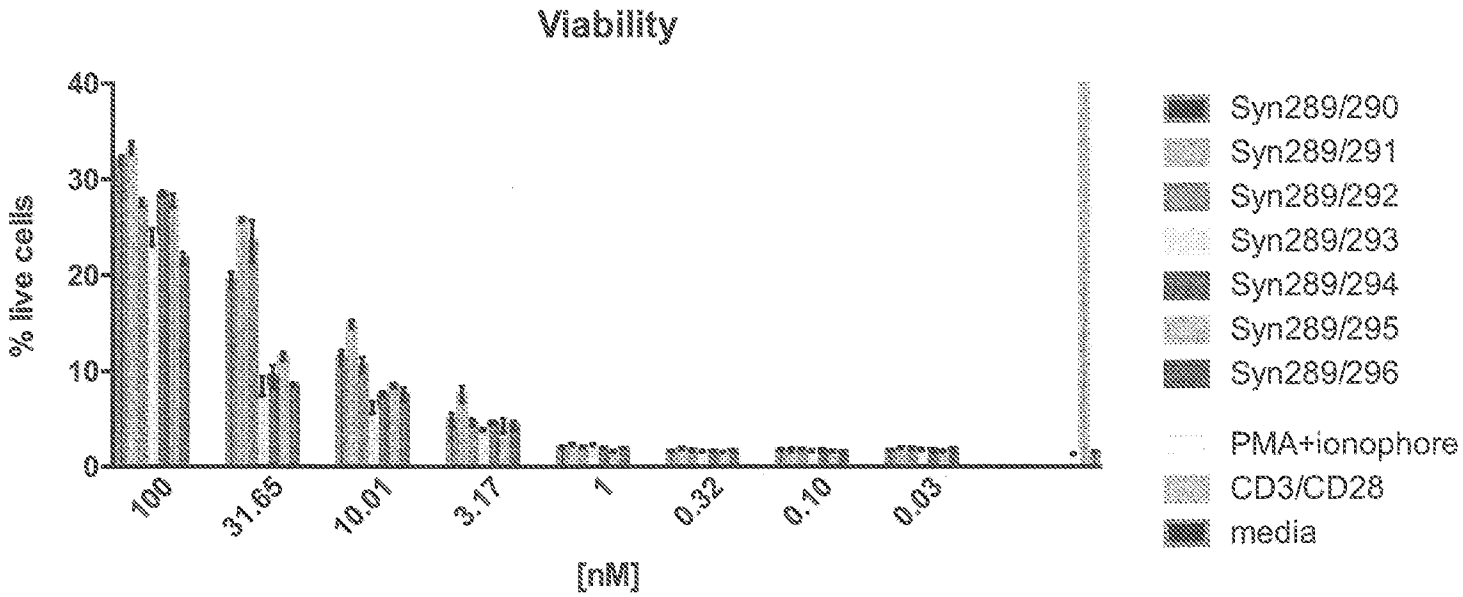
Viability
% live cells
40
30
20
10
0
100
31.65
10.01
3.17
1
0.32
0.10
0.03
[nM]
Syn289/290
Syn289/291
Syn289/292
Syn289/293
Syn289/294
Syn289/295
Syn289/296
PMA+ionophore
CD3/CD28
media

FIG. 20