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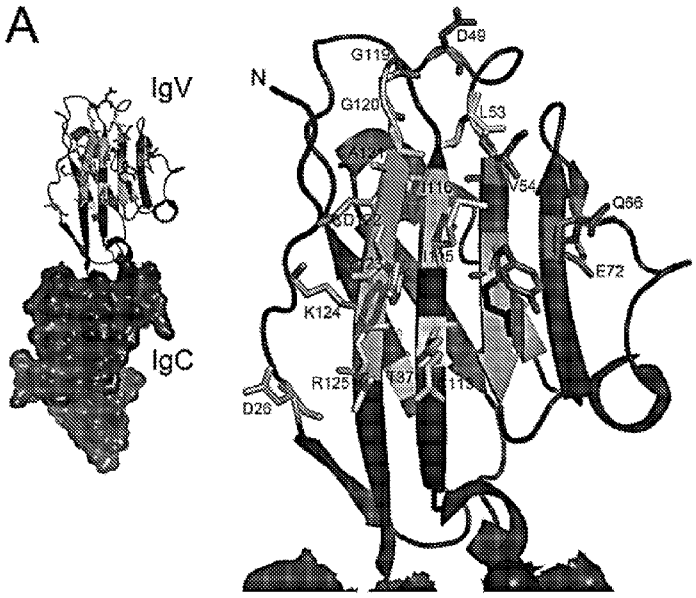
(54) Title: VARIANT PD-L1 POLYPEPTIDES, T-CELL MODULATORY MULTIMERIC POLYPEPTIDES, AND METHODS OF USE THEREOF

FIG. 8



(57) Abstract: The present disclosure provides variant PD-L1 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.

[Continued on next page]

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VARIANT PD-L1 POLYPEPTIDES, 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. 62/338,128, filed May 18, 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 is 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 PD-L1 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] 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.

[0005] FIG. 2A-2M provide an amino acid sequence of a wild-type mouse PD-L1 polypeptide (FIG. 2A); an amino acid sequence of a wild-type human PD-L1 polypeptide (FIG. 2B); a sequence alignment of a mouse and a human PD-L1 amino acid sequence (FIG. 2C); and examples of variant PD-L1 polypeptides (FIG. 2D-2M).

[0006] FIG. 3A-3D provides amino acid sequences of mouse PD-1 (FIG. 3A), human PD-1 (FIG. 3B), mouse B7-1 (FIG. 3C), and human B7-1 (FIG. 3D).

[0007] FIG. 4A-4C provide amino acid sequences of immunoglobulin Fc polypeptides.

[0008] FIG. 5A-5C provide amino acid sequences of human leukocyte antigen (HLA) Class I heavy chain polypeptides. Signal sequences are underlined.

[0009] 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:3), *Pan troglodytes* (NP_001009066.1; SEQ ID NO:4), *Macaca mulatta* (NP_001040602.1; SEQ ID NO:5), *Bos taurus* (NP_776318.1; SEQ ID NO:6) and *Mus musculus* (NP_033865.2; SEQ ID NO:7). Amino acids 1-20 are a signal peptide.

[0010] FIG. 7A-7C depict screening of PD-L1 mutants using a high-throughput microbead binding FACS assay (FIG. 7A and 7B); and FACS microbead binding data for PD-L1 mutants (FIG. 7C).

[0011] FIG. 8A-8D depict characterization of PD-L1 mutants with altered binding to PD-1 or B7-1.

[0012] FIG. 9A-9B depicts PD-1 competing with B7-1 for binding to PD-L1.

[0013] FIG. 10 provides Table 1.

[0014] FIG. 11 provides Table 2.

[0015] FIG. 12 depicts the effect of a PD-L1/synTac on pathogenic epitope-specific $CD8^+$ T cells *in vivo*.

DEFINITIONS

[0016] 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.

[0017] 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.

[0018] 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.

[0019] 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.

[0020] "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^{-3}. Preferred K_D values are 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.

[0021] 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.

[0022] "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.

[0023] "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.

[0024] A "modulatory domain" of a T-cell modulatory multimeric polypeptide of the present disclosure comprises a co-stimulatory polypeptide.

[0025] "Heterologous," as used herein, means a nucleotide or polypeptide that is not found in the native nucleic acid or protein, respectively.

[0026] "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.

[0027] 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.

[0028] 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.

[0029] 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.

[0030] 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.

[0031] 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.

[0032] 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.

[0033] 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.

[0034] 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.

[0035] 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 PD-L1 variant" includes a plurality of such variants 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.

[0036] 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.

[0037] 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

[0038] 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.

[0039] 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 (e.g., an immunomodulatory polypeptide present on a T-cell), compared to the affinity of a wild-type PD-L1 modulatory domain for the immunomodulatory polypeptide (e.g., PD-1 or B7-1). A synTac polypeptide of the present disclosure can modulate (e.g., inhibit) the activity of a target T-cell. A synTac polypeptide of the present disclosure provides for enhanced target cell specificity.

VARIANT IMMUNOMODULATORY POLYPEPTIDES

[0040] The present disclosure provides variant PD-L1 modulatory polypeptides. A wild-type amino acid sequence of human PD-L1 is provided in FIG. 2A.

[0041] Wild-type PD-L1 binds to PD1 and to B7-1. An amino acid sequence of a mouse PD-1 is provided in FIG. 3A; and an amino acid sequence of a human PD-1 is provided in FIG. 3B. An amino acid sequence of a mouse B7-1 is provided in FIG. 3C; and an amino acid sequence of a human B7-1 is provided in FIG. 3D. In some cases, variant PD-L1 polypeptide of the present disclosure binds to PD-1 with reduced affinity compared to binding of wild-type PD-L1 to PD1. In some cases, variant PD-L1 polypeptide of the present disclosure binds to B7-1 with reduced affinity compared to binding of wild-type PD-L1 to B7-1. In some cases, variant PD-L1 polypeptide of the present disclosure binds to PD-1 with substantially the same affinity as the binding affinity of wild-type PD-L1 to PD-1; and binds to B7-1 with reduced affinity

compared to binding of wild-type PD-L1 to B7-1. In some cases, variant PD-L1 polypeptide of the present disclosure binds to PD-1 with reduced affinity compared to binding of wild-type PD-L1 to PD1; and binds to B7-1 with reduced affinity compared to binding of wild-type PD-L1 to B7-1. In some cases, variant PD-L1 polypeptide of the present disclosure binds to PD-1 with reduced affinity compared to binding of wild-type PD-L1 to PD-1; and binds to B7-1 with substantially the same affinity as the binding affinity of wild-type PD-L1 to B7-1.

[0042] In some cases, a variant PD-L1 polypeptide of the present disclosure exhibits reduced binding affinity to PD-1, compared to the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A for PD-1 (e.g., a PD-1 polypeptide comprising the amino acid sequence depicted in FIG.3A). For example, in some cases, a variant PD-L1 polypeptide of the present disclosure binds PD-1 with a binding affinity that is less than the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A for a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3A. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure binds PD-1 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 PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A for PD-1 (e.g., a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3A).

[0043] In some cases, a variant PD-L1 polypeptide of the present disclosure exhibits reduced binding affinity to PD-1, compared to the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2B for PD-1 (e.g., a PD-1 polypeptide comprising the amino acid sequence depicted in FIG.3B). For example, in some cases, a variant PD-L1 polypeptide of the present disclosure binds PD-1 with a binding affinity that is less than the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2B for a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure binds PD-1 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 PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2B for PD-1 (e.g., a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B).

[0044] In some cases, a wild-type mouse PD-L1 ectodomain comprises the following amino acid sequence: FT ITAPKDLYVV EYGSNVTMEC RFPVERELDL LALVVYWEKE

DEQVIQFVAG EEDLKPQHSN FRGRASLPKD QLLKGNAALQ ITDVKLQDAG VYCCIISYGG ADYKRITLKV NAPYRKINQR ISVDPATSEH ELICQAEGYP EAEVIWTNSD HQPVSGKRSV TTSRTEGMLL NVTSSLRVNA TANDVFYCTF WRSQPGQNHT AELIIPELPA THPPQNR (SEQ ID NO:1).

[0045] In some cases, a wild-type human PD-L1 ectodomain comprises the following amino acid sequence: FT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV NAPYNKINQR ILVVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL FNVTSTLRIN TTTNEIFYCT FRRLDPEENH TAELVIPGNI LNVSIKI (SEQ ID NO:2).

[0046] In some cases, a variant PD-L1 polypeptide of the present disclosure exhibits reduced binding affinity to PD-1, compared to the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:1 for PD-1 (e.g., a PD-1 polypeptide comprising the amino acid sequence depicted in FIG.3A). For example, in some cases, a variant PD-L1 polypeptide of the present disclosure binds PD-1 with a binding affinity that is less than the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:1 for a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3A. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure binds PD-1 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 PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:1 for PD-1 (e.g., a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3A).

[0047] In some cases, a variant PD-L1 polypeptide of the present disclosure exhibits reduced binding affinity to PD-1, compared to the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2 for PD-1 (e.g., a PD-1 polypeptide comprising the amino acid sequence depicted in FIG.3B). For example, in some cases, a variant PD-L1 polypeptide of the present disclosure binds PD-1 with a binding affinity that is less than the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2 for a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure binds PD-1 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 PD-L1

polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2 for PD-1 (e.g., a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B).

[0048] In some cases, a variant PD-L1 polypeptide of the present disclosure exhibits reduced binding affinity to PD-1, as described above; and retains at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, of the binding affinity of a wild-type PD-L1 polypeptide for a wild-type B7-1 polypeptide. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure exhibits reduced binding affinity to PD-1, compared to the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2 for PD-1 (e.g., a PD-1 polypeptide comprising the amino acid sequence depicted in FIG.3B); and retains at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, of the binding affinity of a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[0049] In some cases, a variant PD-L1 polypeptide of the present disclosure exhibits from about 40% to about 60% reduced binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B), compared to the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2 for the PD-1 polypeptide; and retains at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, of the binding affinity of a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[0050] In some cases, a variant PD-L1 polypeptide of the present disclosure exhibits from about 40% to about 60% reduced binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3A), compared to the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:1 for the PD-1 polypeptide; and retains at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, of the binding affinity of a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in SEQ ID NO:1) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3C).

[0051] In some cases, a variant PD-L1 polypeptide of the present disclosure has a binding affinity to PD-1that is from 1nM to 1mM. In some cases, a variant PD-L1 polypeptide of the present disclosure has a binding affinity to PD-1that is from 100 nM to 100 μM. As another example, in some cases, a variant PD-L1 polypeptide of the present disclosure has a binding affinity for PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG.

3) 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.

[0052] A variant PD-L1 polypeptide of the present disclosure can have a single amino acid substitution relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide of the present disclosure has from 2 to 10 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide of the present disclosure has 2 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide of the present disclosure has 3 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide of the present disclosure has 4 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide of the present disclosure has 5 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide of the present disclosure has 6 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide of the present disclosure has 7 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide of the present disclosure has 8 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide of the present disclosure has 9 amino acid

substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide of the present disclosure has 10 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2).

[0053] A variant PD-L1 polypeptide of the present disclosure can have a length of from 200 amino acids to 240 amino acids. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure has a length of from 200 amino acids to 220 amino acids, or from 220 amino acids to 240 amino acids. In some cases, a variant PD-L1 polypeptide of the present disclosure has a length of from 200 amino acids to 219 amino acids. In some cases, a variant PD-L1 polypeptide of the present disclosure has a length of 219 amino acids.

D26 substitution

[0054] In some cases, a variant PD-L1 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 26 is an amino acid other than an aspartic acid, e.g., where amino acid 26 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 PD-L1 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 26 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant PD-L1 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 26 is Ala, Gly, Val, Leu, Ile, or Arg. In some cases, a variant PD-L1 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 26 is Ala. In some cases, a variant PD-L1 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 26 is Gly. In some cases, a variant PD-L1 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 26 is Val. In some cases, a variant PD-L1 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 26 is Leu. In some cases, a variant

PD-L1 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 26 is Ile. In some cases, a variant PD-L1 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 26 is Arg. In some cases, the variant PD-L1 polypeptide exhibits from about 40% to about 60% reduced binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B), compared to the binding affinity of a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B (or set forth in SEQ ID NO:2) for the PD-1 polypeptide; and retains at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, of the binding affinity of a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B or SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[0055] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, with an amino acid substitution at D26. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at D8. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is any amino acid other than aspartic acid; for example, amino acid 26 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 PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Ala, Gly, Val, Leu, or Ile. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Ala, Gly, Val, Leu, Ile, or Arg. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Ala instead of Asp. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Val instead of Asp. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Leu instead of Asp. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Gly instead of Asp. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Ile instead of Asp. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Arg instead of Asp.

[0056] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at D8. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is any amino acid other than aspartic acid; for example, amino acid 8 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 PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Ala, Gly, Val, Leu, or Ile instead of Asp. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Ala, Gly, Val, Leu, Ile, or Arg instead of Asp. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Ala instead of Asp. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Val instead of Asp. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Leu instead of Asp. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Gly instead of Asp. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Ile. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Arg instead of Asp.

[0057] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2D. In some cases, variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2E. In some cases, variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2F. In some cases, variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2G.

T37 substitution

[0058] In some cases, a variant PD-L1 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 37 is an amino acid other than threonine, e.g., where amino acid 37 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant PD-L1 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 37 is Gly, Ala, Val, Leu, Ile, Arg, Lys, or His.

In some cases, a variant PD-L1 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 37 is Arg, Lys, or His. In some cases, a variant PD-L1 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 37 is Gly, Ala, Val, Leu, or Ile. In some cases, a variant PD-L1 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 37 is Arg. In some cases, a variant PD-L1 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 37 is Lys. In some cases, a variant PD-L1 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 37 is His. In some cases, a variant PD-L1 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 37 is Gly. In some cases, a variant PD-L1 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 37 is Ala. In some cases, a variant PD-L1 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 37 is Val. In some cases, a variant PD-L1 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 37 is Leu. In some cases, a variant PD-L1 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 37 is Ile. In some cases, the variant PD-L1 polypeptide exhibits from about 15% to about 35% of the binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B) exhibited by a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B (or set forth in SEQ ID NO:2) for the PD-1 polypeptide; and exhibits reduced binding affinity to B7-1 (e.g., exhibits from about 70% to about 90% reduced binding affinity to B7-1) compared to the binding affinity of a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 3B or in SEQ

ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[0059] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, with an amino acid substitution at T37. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at T19. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is any amino acid other than threonine; for example, amino acid 37 can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Gly, Ala, Val, Leu, Ile, Arg, His, or Lys, instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Gly, Ala, Val, Leu, or Ile, instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Arg, His, or Lys, instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Arg instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Lys instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is His instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Gly instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Ala instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Val instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Leu instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Ile instead of Thr.

[0060] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at T19. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is any amino acid other than threonine; for example, amino acid 19 can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant PD-L1

polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Gly, Ala, Val, Leu, Ile, Arg, His, or Lys instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Gly, Ala, Val, Leu, or Ile, instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Arg, His, or Lys instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Arg instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Lys instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is His instead of 19. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Gly instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Ala instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Val instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Leu instead of Thr. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Ile instead of Thr.

I54 substitution

[0061] In some cases, a variant PD-L1 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 54 is an amino acid other than isoleucine, e.g., where amino acid 54 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 PD-L1 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 54 is an amino acid other than isoleucine or valine, e.g., where amino acid 54 is Gly, Ala, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant PD-L1 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 54 is Ala, Gly, Leu, Glu, or Asp. In some cases, a variant PD-L1 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 54 is Glu or Asp. In some cases, a variant PD-L1 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 54 is Ala. In some cases, a variant PD-L1 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 54 is Gly. In some cases, a variant PD-L1 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 54 is Leu. In some cases, a variant PD-L1 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 54 is Asp. In some cases, a variant PD-L1 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 54 is Glu. In some cases, the variant PD-L1 polypeptide exhibits from about 70% to about 100% of the binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 2B) exhibited by a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B (or set forth in SEQ ID NO:2) for the PD-1 polypeptide; and exhibits reduced binding affinity to B7-1 (e.g., exhibits from about 40% to about 90% reduced binding affinity to B7-1) compared to the binding affinity of a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 3B or in SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[0062] In some cases, a variant PD-L1 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. 2A, where amino acid 54 is an amino acid other than valine, e.g., where amino acid 54 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 PD-L1 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. 2A, where amino acid 54 is an amino acid other than isoleucine or valine, e.g., where amino acid 54 is Gly, Ala, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant PD-L1 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. 2A, where amino acid 54 is Ala, Gly, Leu, Glu, or Asp. In some cases, a variant PD-L1 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. 2A, where amino acid 54 is Glu or Asp. In some cases, a variant PD-L1 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. 2A, where amino acid 54 is Ala. In some cases, a variant PD-L1 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. 2A, where amino acid 54 is Gly. In some cases, a variant PD-L1 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. 2A, where amino acid 54 is Leu. In some cases, a variant PD-L1 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. 2A, where amino acid 54 is Asp. In some cases, a variant PD-L1 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. 2A, where amino acid 54 is Glu. In some cases, the variant PD-L1 polypeptide exhibits from about 70% to about 100% of the binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3A) exhibited by a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2A (or set forth in SEQ ID NO:1) for the PD-1 polypeptide; and exhibits reduced binding affinity to B7-1 (e.g., exhibits from about 40% to about 90% reduced binding affinity to B7-1) compared to the binding affinity of a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2A or in SEQ ID NO:1) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3C).

[0063] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, with an amino acid substitution at I54. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at I36. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is any amino acid other than isoleucine; for example, amino acid 54 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 PD-L1 polypeptide of the present disclosure

comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is any amino acid other than isoleucine or valine; for example, amino acid 54 can be Gly, Ala, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is Ala, Gly, Leu, or Asp, instead of Ile. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is Ala instead of Ile. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is Leu instead of Ile. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is Gly instead of Ile. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is Asp instead of Ile.

[0064] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at V54. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V36. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is any amino acid other than valine; for example, amino acid 54 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 PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is any amino acid other than isoleucine or valine; for example, amino acid 54 can be Gly, Ala, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Ala, Gly, Leu, Glu, or Asp, instead of Val. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Glu or Asp, instead of Val. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Ala instead of Val. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Leu instead of Val. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Gly instead of Val. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Asp instead of Val. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Glu instead of Val.

[0065] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Ile-36. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is any amino acid other than isoleucine; for example, amino acid 36 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 PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is any amino acid other than isoleucine or valine; for example, amino acid 36 can be Gly, Ala, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is Ala, Gly, Leu, or Asp instead of Ile. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is Ala instead of Ile. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is Leu instead of Ile. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is Gly instead of Ile. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is Asp instead of Ile.

[0066] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V36. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is any amino acid other than valine; for example, amino acid 36 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 PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is any amino acid other than isoleucine or valine; for example, amino acid 36 can be Gly, Ala, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Ala, Gly, Leu, Glu, or Asp instead of Val. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Glu or Asp instead of Val. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Ala instead of Val. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Leu instead of Val. In some cases,

a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Gly instead of Val. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Asp instead of Val. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Glu instead of Val.

[0067] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2H. In some cases, variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2I.

Q66 substitution

[0068] In some cases, a variant PD-L1 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 66 is an amino acid other than glutamine, e.g., where amino acid 66 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 PD-L1 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 66 is Glu or Asp. In some cases, a variant PD-L1 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 66 is Glu. In some cases, a variant PD-L1 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 66 is Asp. In some cases, the variant PD-L1 polypeptide exhibits from about 80% to about 100% of the binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B) exhibited by a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B (or set forth in SEQ ID NO:2) for the PD-1 polypeptide; and exhibits reduced binding affinity to B7-1 (e.g., exhibits from about 40% to about 90% reduced binding affinity to B7-1) compared to the binding affinity of a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 3B or in SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[0069] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, with an amino acid substitution at Q66. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Q48. For example, in some cases,

a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is any amino acid other than glutamine; for example, amino acid 66 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 PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Ala, Gly, Leu, Glu, or Asp, instead of Gln. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Glu or Asp, instead of Gln. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Ala instead of Gln. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Leu instead of Gln. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Gly instead of Gln. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Asp instead of Gln. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Glu instead of Gln.

[0070] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Q48. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is any amino acid other than glutamine; for example, amino acid 48 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 PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Ala, Gly, Leu, Glu, or Asp instead of Gln. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Glu or Asp instead of Gln. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Ala instead of Gln. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Leu instead of Gln. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Gly instead of Gln. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Asp instead of Gln. In some cases, a variant PD-L1 polypeptide of the present

disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Glu instead of Gln.

[0071] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2J. In some cases, variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2K.

E72 substitution

[0072] In some cases, a variant PD-L1 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 72 is an amino acid other than glutamic acid, e.g., where amino acid 72 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. In some cases, a variant PD-L1 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 72 is Arg, Lys, or His. In some cases, a variant PD-L1 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 72 is Asp, Arg, Lys, or His. In some cases, a variant PD-L1 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 72 is Arg. In some cases, a variant PD-L1 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 72 is Lys. In some cases, a variant PD-L1 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 72 is His. In some cases, a variant PD-L1 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 72 is Asp. In some cases, the variant PD-L1 polypeptide exhibits from about 30% to about 60% of the binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B) exhibited by a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B (or set forth in SEQ ID NO:2) for the PD-1 polypeptide; and exhibits reduced binding affinity to B7-1 (e.g., exhibits from about 40% to about 90% reduced binding affinity to B7-1) compared to the binding affinity of a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino

acid sequence set forth in FIG. 3B or in SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[0073] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, with an amino acid substitution at E72. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at E54. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 72 is any amino acid other than glutamic acid; for example, amino acid 72 can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 72 is Asp, Arg, His, or Lys, instead of Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 72 is Arg, His, or Lys, instead of Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 72 is Arg instead of Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 72 is Lys instead of Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 72 is His instead of Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 72 is Asp instead of Glu.

[0074] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at E54. For example, in some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is any amino acid other than glutamic acid; for example, amino acid 54 can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is Asp, Arg, His, or Lys instead of Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is Arg, His, or Lys instead of Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is Arg instead of Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is Lys instead of Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where

amino acid 54 is His instead of Glu. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is Asp instead of Glu.

[0075] In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2L. In some cases, variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2M.

FUSION POLYPEPTIDES

[0076] The present disclosure provides PD-L1 fusion polypeptides. A fusion polypeptide of the present disclosure comprises: a) a variant PD-L1 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 PD-L1 polypeptide. In some cases, the heterologous fusion partner is fused to the C-terminus of the variant PD-L1 polypeptide. In some cases, a PD-L1 fusion polypeptide of the present disclosure comprises a first heterologous fusion partner fused to the N-terminus of the variant PD-L1 polypeptide, and a second heterologous fusion partner fused to the C-terminus of the variant PD-L1 polypeptide.

[0077] The total length of a PD-L1 fusion polypeptide of the present disclosure can range from 245 amino acids to 2000 amino acids. For example, a PD-L1 fusion polypeptide of the present disclosure can range from 245 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.

[0078] Suitable fusion partners include, but are not limited to, a transmembrane domain; an immunoglobulin Fc region (e.g., an IgG 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

[0079] 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 various 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." Where a T-cell modulatory multimeric polypeptide of the present disclosure comprises a PD-L1 immunomodulatory polypeptide (e.g., a variant PD-L1 immunomodulatory polypeptide of the present disclosure), such a T-cell modulatory multimeric polypeptide is also referred to herein as a "PD-L1/synTac."

[0080] In some cases, a synTac polypeptide of the present disclosure comprises a variant PD-L1 immunomodulatory polypeptide of the present disclosure. In some cases, a synTac polypeptide of the present disclosure comprises a variant PD-L1 immunomodulatory polypeptide comprising an amino acid substitution as depicted in FIG. 10 or FIG. 11. Thus, in some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of D26 of the amino acid sequence depicted in FIG. 2B; or D8 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of T37 of the amino acid sequence depicted in FIG. 2B; or T19 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of D49 of the amino acid sequence depicted in FIG. 2B; or D31 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of L53 of the amino acid sequence depicted in FIG. 2B; or L35 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of I54 (V54 in mouse PD-L1) of the amino acid sequence depicted in FIG. 2B; or I36 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of Y56 of the amino acid sequence depicted in FIG. 2B; or Y38 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of Y56 of the amino acid sequence depicted in FIG. 2B; or Y38 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of Q66 of the amino acid sequence depicted in FIG. 2B; or Q48 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of Q66 of the amino acid sequence depicted in FIG. 2B; or Q48 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of E72 of the amino acid sequence depicted in FIG. 2B; or E54 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a

substitution of M115 (I115 of mouse PD-L1) of the amino acid sequence depicted in FIG. 2B; or M97 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of I116 of the amino acid sequence depicted in FIG. 2B; or I98 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of G119 of the amino acid sequence depicted in FIG. 2B; or G101 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of G120 of the amino acid sequence depicted in FIG. 2B; or G102 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of G120 of the amino acid sequence depicted in FIG. 2B; or G102 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of A121 of the amino acid sequence depicted in FIG. 2B; or A103 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of D122 of the amino acid sequence depicted in FIG. 2B; or D104 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of Y123 of the amino acid sequence depicted in FIG. 2B; or Y105 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of K124 of the amino acid sequence depicted in FIG. 2B; or K106 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of R125 of the amino acid sequence depicted in FIG. 2B; or K107 of the amino acid sequence set forth in SEQ ID NO:2.

[0081] As noted above, in some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure exhibits reduced binding affinity for PD1, compared to the binding affinity of wild-type PD-L1 to PD1. In some cases, a multimeric polypeptide of the present disclosure that comprises a variant PD-L1 polypeptide of the present disclosure also exhibits reduced binding affinity to PD1, compared to a control multimeric polypeptide comprising a wild-type PD-L1 (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or comprising the amino acid sequence depicted in SEQ ID NO:1 or SEQ ID NO:2).

[0082] In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure binds to B7-1 with reduced affinity compared to binding affinity of wild-type PD-L1 for B7-1. In some cases, a multimeric polypeptide of the present disclosure that comprises a variant PD-L1 polypeptide of the present disclosure also exhibits reduced binding affinity to B7-1, compared to a control multimeric polypeptide comprising a wild-type PD-L1 (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or comprising the amino acid sequence depicted in SEQ ID NO:1 or SEQ ID NO:2).

[0083] In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure binds to PD-1 with substantially the same affinity as the binding affinity of wild-type PD-L1 to PD-1; and binds to B7-1 with reduced affinity compared to binding of wild-type PD-L1 to B7-1. In some cases, a multimeric polypeptide of the present disclosure that comprises a variant PD-L1 polypeptide of the present disclosure also exhibits substantially the same affinity for PD-1 as a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or comprising the amino acid sequence depicted in SEQ ID NO:1 or SEQ ID NO:2); and also binds B7-1 with reduced binding affinity for B7-1, compared to a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or comprising the amino acid sequence depicted in SEQ ID NO:1 or SEQ ID NO:2).

[0084] In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure binds to PD-1 with reduced affinity compared to binding of wild-type PD-L1 to PD1; and binds to B7-1 with reduced affinity compared to binding of wild-type PD-L1 to B7-1. In some cases, a multimeric polypeptide of the present disclosure that comprises a variant PD-L1 polypeptide of the present disclosure also exhibits reduced binding affinity to B7-1, compared to a control multimeric polypeptide comprising a wild-type PD-L1 (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or comprising the amino acid sequence depicted in SEQ ID NO:1 or SEQ ID NO:2); and also binds B7-1 with reduced binding affinity for B7-1, compared to a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or comprising the amino acid sequence depicted in SEQ ID NO:1 or SEQ ID NO:2).

[0085] In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure binds to PD-1 with reduced affinity compared to binding of wild-type PD-L1 to PD-1; and binds to B7-1 with substantially the same affinity as the binding affinity of wild-type PD-L1 to B7-1. In some cases, a multimeric polypeptide of the present disclosure that comprises a variant PD-L1 polypeptide of the present disclosure also exhibits reduced binding

affinity to B7-1, compared to a control multimeric polypeptide comprising a wild-type PD-L1 (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or comprising the amino acid sequence depicted in SEQ ID NO:1 or SEQ ID NO:2); and also exhibits substantially the same affinity for B7-1 as a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or comprising the amino acid sequence depicted in SEQ ID NO:1 or SEQ ID NO:2).

[0086] In some cases, a synTac polypeptide of the present disclosure exhibits reduced binding affinity to PD1, compared to the binding affinity of a control synTac polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or FIG. 2B, or SEQ ID NO:1 or SEQ ID NO:2, for PD1. For example, in some cases, a synTac polypeptide of the present disclosure binds PD1 with a binding affinity that is less than the binding affinity of a control synTac polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A for a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3A. For example, in some cases, a synTac polypeptide of the present disclosure binds PD1 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 PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A for PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3A). As another example, in some cases, a synTac polypeptide of the present disclosure binds PD1 with a binding affinity that is less than the binding affinity of a control synTac polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2B for a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3B. For example, in some cases, a synTac polypeptide of the present disclosure binds PD1 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 PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2B for PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3B).

[0087] In some cases, a synTac polypeptide of the present disclosure exhibits reduced binding affinity to PD1, compared to the binding affinity of a control synTac polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for PD1. For

example, in some cases, a synTac polypeptide of the present disclosure binds PD1 with a binding affinity that is less than the binding affinity of a control synTac polypeptide comprises a PD-L1 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3A. For example, in some cases, a synTac polypeptide of the present disclosure binds PD1 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 PD-L1 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3A).

[0088] In some cases, a synTac polypeptide of the present disclosure exhibits reduced binding affinity to PD1, compared to the binding affinity of a control synTac polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:2 for PD1. For example, in some cases, a synTac polypeptide of the present disclosure binds PD1 with a binding affinity that is less than the binding affinity of a control synTac polypeptide comprises a PD-L1 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:2 for a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3B. For example, in some cases, a synTac polypeptide of the present disclosure binds PD1 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 PD-L1 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:2 for PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3B).

[0089] In some cases, a synTac polypeptide of the present disclosure exhibits reduced binding affinity to B7-1, compared to the binding affinity of a control synTac polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or FIG. 2B, or SEQ ID NO:1 or SEQ ID NO:2, for B7-1. For example, in some cases, a synTac polypeptide of the present disclosure binds B7-1with a binding affinity that is less than the binding affinity of a control synTac polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A for a B7-1 polypeptide comprising the amino acid sequence depicted in FIG. 3C. For example, in some cases, a synTac polypeptide of the present disclosure binds B7-1 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 PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A for B7-1 (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG. 3C). As another example, in some cases, a synTac polypeptide of the present disclosure binds B7-1 with a binding affinity that is less than the binding affinity of a control synTac polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2B for a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3D. For example, in some cases, a synTac polypeptide of the present disclosure binds B7-1with 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 PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2B for B7-1 (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG. 3D).

[0090] In some cases, a synTac polypeptide of the present disclosure exhibits reduced binding affinity to B7-1, compared to the binding affinity of a control synTac polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for B7-1. For example, in some cases, a synTac polypeptide of the present disclosure binds B7-1 with a binding affinity that is less than the binding affinity of a control synTac polypeptide comprises a PD-L1 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for a B7-1 polypeptide comprising the amino acid sequence depicted in FIG. 3C. For example, in some cases, a synTac polypeptide of the present disclosure binds B7-1 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 PD-L1 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:1 for B7-1 (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG. 3C).

[0091] In some cases, a synTac polypeptide of the present disclosure exhibits reduced binding affinity to B7-1, compared to the binding affinity of a control synTac polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:2 for B7-1. For example, in some cases, a synTac polypeptide of the present disclosure binds B7-1 with a

binding affinity that is less than the binding affinity of a control synTac polypeptide comprises a PD-L1 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:2 for a B7-1 polypeptide comprising the amino acid sequence depicted in FIG. 3D. For example, in some cases, a synTac polypeptide of the present disclosure binds B7-1 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 PD-L1 polypeptide comprising the amino acid sequence depicted in SEQ ID NO:2 for B7-1 (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG. 3D).

[0092] As noted above, in some cases, a multimeric polypeptide of the present disclosure that comprises a variant PD-L1 polypeptide of the present disclosure exhibits substantially the same affinity for B7-1 (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG. 3C or FIG. 3D) as a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or comprising the amino acid sequence depicted in SEQ ID NO:1 or SEQ ID NO:2). For example, in some cases, a multimeric polypeptide of the present disclosure that comprises a variant PD-L1 polypeptide of the present disclosure exhibits at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, of the affinity for B7-1 (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG. 3C or FIG. 3D) as a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or comprising the amino acid sequence depicted in SEQ ID NO:1 or SEQ ID NO:2).

[0093] As noted above, in some cases, a multimeric polypeptide of the present disclosure that comprises a variant PD-L1 polypeptide of the present disclosure exhibits substantially the same affinity for PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3A or FIG. 3B) as a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or 2B, or comprising the amino acid sequence depicted in SEQ ID NO:1 or SEQ ID NO:2). For example, in some cases, a multimeric polypeptide of the present disclosure that comprises a variant PD-L1 polypeptide of the present disclosure exhibits at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, of the affinity for PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3A or FIG. 3B) as a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide

comprising the amino acid sequence depicted in FIG. 2A or 2B, or comprising the amino acid sequence depicted in SEQ ID NO:1 or SEQ ID NO:2).

[0094] In some cases, a synTac polypeptide of the present disclosure has a binding affinity for PD1 that is from 1 nM to about 1mM. In some cases, a synTac polypeptide of the present disclosure has a binding affinity for PD1 that is from 100 nM to about 100 μM. In some cases, a synTac polypeptide of the present disclosure has a binding affinity for PD1 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 PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3A or FIG. 3B) 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 PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3A or FIG. 3B) 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 PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3A or FIG. 3B) 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 PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3A or FIG. 3B) 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 PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3A or FIG. 3B) 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 PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3A or FIG. 3B) 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 PD1 (e.g., a PD1 polypeptide comprising the amino acid sequence depicted in FIG. 3A or FIG. 3B) 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.

[0095] A variant PD-L1 polypeptide present in a synTac polypeptide of the present disclosure can have a single amino acid substitution relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1

polypeptide comprising the amino acid sequence depicted in FIG. 2A or FIG. 2B or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide present in a synTac polypeptide of the present disclosure has from 2 to 10 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or FIG. 2B or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide present in a synTac polypeptide of the present disclosure has 2 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or FIG. 2B or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide present in a synTac polypeptide of the present disclosure has 3 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or FIG. 2B or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide present in a synTac polypeptide of the present disclosure has 4 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or FIG. 2B or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide present in a synTac polypeptide of the present disclosure has 5 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or FIG. 2B or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide present in a synTac polypeptide of the present disclosure has 6 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or FIG. 2B or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide present in a synTac polypeptide of the present disclosure has 7 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or FIG. 2B or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide present in a synTac polypeptide of the present disclosure has 8 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or FIG. 2B or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide present in a synTac polypeptide of the present disclosure has 9 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or FIG. 2B or as set forth in SEQ ID NO:1 or SEQ ID NO:2). In some cases, a variant PD-L1 polypeptide present in a synTac polypeptide of the present disclosure has 10 amino acid substitutions relative to a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence depicted in FIG. 2A or FIG. 2B or as set forth in SEQ ID NO:1 or SEQ ID NO:2).

[0096] 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 PD-L1 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 PD-L1 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, a multimeric polypeptide of the present disclosure includes two or more variant PD-L1 immunomodulatory polypeptides 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 variant PD-L1 immunomodulatory polypeptides comprise the same amino acid sequence as one another. Where a multimeric polypeptide of the present disclosure includes two or more variant PD-L1 immunomodulatory polypeptides, in some cases, the two or more variant PD-L1 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.

[0097] 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 PD-L1 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 PD-L1 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 PD-L1 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 PD-L1 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.

[0098] 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.

[0099] 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 PD-L1 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 PD-L1 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 PD-L1 polypeptide of the present disclosure; iv) a second variant PD-L1 polypeptide of the present disclosure; and v) a third variant PD-L1 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 PD-L1 polypeptides have the same amino acid sequence. In some cases, the first, second, and third variant PD-L1 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 PD-L1 polypeptide of the present disclosure; ii) a second variant PD-L1 polypeptide of the present disclosure; and iii) a third variant PD-L1 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 PD-L1 polypeptides have the same amino acid sequence. In some cases, the first, second, and third variant PD-L1 polypeptides differ from one another in amino acid sequence.

Linkers

[00100] 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 PD-L1 polypeptide and a second variant PD-L1 polypeptide; or a between a second variant PD-L1 polypeptide and a third variant PD-L1 polypeptide.

[00101] 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.

[00102] Exemplary linkers include glycine polymers $(G)_n$, glycine-serine polymers (including, for example, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO:8) and $(GGGS)_n$ (SEQ ID NO:9), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers can be used; both Gly and Ser

are relatively unstructured, and therefore can serve as a neutral tether between components. Glycine polymers can be used; glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, *Rev. Computational Chem.* 11173-142 (1992)). Exemplary linkers can comprise amino acid sequences including, but not limited to, GGSG (SEQ ID NO:10), GGSGG (SEQ ID NO:11), GSGSG (SEQ ID NO:12), GSGGG (SEQ ID NO:13), GGGSG (SEQ ID NO:14), GSSSG (SEQ ID NO:15), 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, where n is 4. In some cases, a linker comprises the amino acid sequence (GSSSS)n, where n is 5. Exemplary linkers can include, e.g., $((Gly_4)Ser)n$ (SEQ ID NO:45), where n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. For example, in some cases, a linker comprises the amino acid sequence (GGGGS)n, where n is 4. In some cases, a linker comprises the amino acid sequence (GGGGS)n, where n is 5.

[00103] 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 G**C**GASGGGGSGGGGS (SEQ ID NO:16).

Epitopes

[00104] 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.

[00105] 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.

[00106] Suitable epitopes include, but are not limited to, epitopes present in an autoimmune-associated antigen. Autoimmune antigens include, but are not limited to, myelin basic protein (MBP); proteolipid protein (PLP); myelin oligodendrocyte glycoprotein (MOG), myelin-associated oligodendrocytic basic protein cardiac myosin; outer surface protein (OSP); myelin associated glycoprotein (MAG); neurofilaments; interferon omega; transglutaminase; aromatic acid carboxylase; 17-hydroxylase; 21-hydroxylase, cardiolipin; pyruvate dehydrogenase; β2 glycoprotein I; phosphatidylserine; apoH; Annexin A5; LKM-1; soluble liver antigen; carbonic anhydrase; gpIIb-IIIa or 1b-IX; type XVII collagen; tissue transglutaminase; gliadin; GD1a; GQ1b; BP-1; BP-2; epidermal transglutaminase; histidine-tRNA; signal recognition peptide; Mi-2; Jo1; Glutamic acid decarboxylase, HSP60; HSP70; HSP90; IGRP; insulin; carboxypeptidase H; insulinoma antigen-2; IA-2beta; ICA69; ZnT8; chromogranin A; IAPP; sc170; topoisomerase; histones; Basement Membrane Collagen Type IV; enolase; thyroid peroxidase; thyroglobulin; complement component 3; voltage-gated calcium channels; Q-type calcium channel, synaptogagmin, muscarinic acetylcholine receptor M1; SMA; LKM-1; LKM-2; LKM-3; soluble liver antigen; SLA; LP; major peripheral myelin protein P0; myeloperoxidase; GQ1b; U1-RNP; Kir4.1; nicotinic acetylcholine receptor; MuSK protein; hypocretin; orexin; keratin; AQP4; Yo; Hu; glutamate receptor; Desmoglein 3; p62; sp100, Ro; LA; glycoproteins IIb-IIIa or Ib-IX; ADAMTS13; cardiolipin; β2 glycoprotein I; HPA-1a; HPA-5b; IFN-gamma, IL-1, TNF-alpha; and GMCSF. Autoimmune antigens also include autoantigens relevant in type 1 diabetes, multiple sclerosis, or systemic lupus erythematosus. Pancreatic beta cell antigen islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) peptide known as $IGRP_{206-214}$ can be used as an autoimmune epitope, e.g., in the context of type 1 diabetes; the amino acid sequence of $IGRP_{206-214}$ is VYLKTNVFL (SEQ ID NO:43#) (see, e.g., Krishnamurthy et al. (2008) *J. Immunol.* 180:4458; and Han et al. (2005) *J. Clin. Invest.* 115:1879). Other suitable IGRP peptides are disclosed in, e.g., Jarchum et al. (2008) *Clin. Immunol.* 127:359. Suitable autoantigen epitopes in the context of type 1 diabetes include peptide epitopes of preproinsulin; for example ALWGPDPAAA (SEQ ID NO:44#) (see, e.g., Skowera et al. (2008) *J. Clin. Invest.* 118:3390).

[00107] Autoimmune antigens and associated autoimmune disorders include, for example, myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG), in each case associated with multiple sclerosis (MS); CD44, preproinsulin, proinsulin, insulin, glutamic acid decaroxylase (GAD65), tyrosine phosphatase-like insulinoma antigen 2 (IA2), zinc transporter ((ZnT8), and heat shock protein 60 (HSP6O), in each case associated with diabetes Type I; interphotoreceptor retinoid-binding protein (IRBP) associated with autoimmune uveitis; acetylcholine receptor AchR, and insulin-like

growth factor-1 receptor (IGF-1R), in each case associated with Myasthenia gravis; M-protein from beta-hemolytic streptocci (pseudo-autoantigen) associated with Rheumatic Fever; Macrophage migration inhibitory factor associated with Arthritis; Ro/La RNP complex, alpha- and beta-fodrin, islet cell autoantigen, poly(ADP)ribose polymerase (PARP), NuMA, NOR-90, Ro60 autoantigen, and p27 antigen, in each case associated with Sjogren's syndrome; Ro60 autoantigen, low-density lipoproteins, Sm antigens of the U-1 small nuclear ribonucleoprotein complex (B/B', D1, D2, D3, E, F, G), and RNP ribonucleoproteins, in each case associated with lupus erythematosus; oxLDL, beta(2)GPI, HSP6O/65, and oxLDL/beta(2)GPI, in each case associated with Atherosclerosis; cardiac beta(1)-adrenergic receptor associated with idiopathic dilated cardiomyopathy (DCM); histidyl-tRNA synthetase (HisRS) associated with myositis; topoisomerase I associated with scleroderma; IL-17; or heat shock proteins.

MHC polypeptides

[00108] 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 (e.g., rabbits), non-human primates, canines (e.g., dogs), felines (e.g., cats), ungulates (e.g., equines, bovines, ovines, caprines, camels, 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).

[00109] 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.

[00110] 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.

[00111] 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 FIG. 5A.

[00112] 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: GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPE YWDGETRKVKAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGY HQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWL RRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQT QDTELVETRPAGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID NO:17).

[00113] 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 FIG. 5B.

[00114] 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 FIG. 5C.

[00115] 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:

[00116] GPHSLRYFVTAVSRPGLGEPRFIAVGYVDDTQFVRFDSDADNPRFEPRAPWME QEGPEYWEEQTQRAKSDEQWFRVSLRTAQRYYNQSKGGSHTFQRMFGCDVGSDWRL LRGYQQFAYDGRDYIALNEDLKTWTAADTAALITRRKWEQAGDAEYYRAYLEGECV EWLRRYLELGNETLLRTDSPKAHVTYHPRSQVDVTLRCWALGFYPADITLTWQLNGE DLTQDMELVETRPAGDGTFQKWAAVVVPLGKEQNYTCHVHHKGLPEPLTLRW (SEQ ID NO:18).

[00117] 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.

[00118] 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.

[00119] 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.

[00120] 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 FIG. 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.

[00121] In some cases, a β2M polypeptide comprises the amino acid sequence: IQRTPKIQVY S**R**HPAENGKS NFLNCYVSGF HPSDIEVDLLKNGERIEKVE HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSQP KIVKWDRDM. In some cases, a β2M polypeptide comprises the amino acid sequence: IQRTPKIQVY S**C**HPAENGKS

NFLNCYVSGF HPSDIEVDLLKNGERIEKVE HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSQP KIVKWDRDM.

[00122] In some cases, an HLA Class I heavy chain polypeptide comprises the amino acid sequence:

GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPE YWDGETRKVKAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGSDWRFLRGY HQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWL RRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQT QDTELVETRP**A**GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID NO:19).

[00123] In some cases, an HLA Class I heavy chain polypeptide comprises the amino acid sequence:

[00124] GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIE QEGPEYWDGETRKVKAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGSDWR FLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGT CVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRD GEDQTQDTELVETRP**C**GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID NO:20).

[00125] In some cases, the β2M polypeptide comprises the following amino acid sequence:

[00126] IQRTPKIQVY S**C**HPAENGKS NFLNCYVSGF HPSDIEVDLLKNGERIEKVE HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSQP KIVKWDRDM (SEQ ID NO:42); and the HLA ClassI heavy chain polypeptide of a multimeric polypeptide of the present disclosure comprises the following amino acid sequence:

[00127] GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIE QEGPEYWDGETRKVKAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGSDWR FLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGT CVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRD GEDQTQDTELVETRP**C**GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRWEP (SEQ ID NO:21), where the Cys residues that are underlined and in bold form a disulfide bond with one another in the multimeric polypeptide.

Immunomodulatory polypeptides

[00128] A multimeric polypeptide of the present disclosure comprises a variant PD-L1 polypeptide, as described above. Thus, a multimeric polypeptide of the present disclosure comprises the variant PD-L1 polypeptide present in the first polypeptide or the second polypeptide of a multimeric polypeptide of the present disclosure.

D26 substitution

[00129] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 26 is an amino acid other than an aspartic acid, e.g., where amino acid 26 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 PD-L1 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 26 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 26 is Ala, Gly, Val, Leu, Ile, or Arg. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 26 is Ala. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 26 is Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 26 is Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 26 is Leu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 26 is Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at

least 85%, at 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 26 is Arg. In some cases, a multimeric polypeptide of the present disclosure exhibits from about 40% to about 60% reduced binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B), compared to the binding affinity of control multimeric polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B (or set forth in SEQ ID NO:2) for the PD-1 polypeptide; and retains at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, of the binding affinity of a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B or SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[00130] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, with an amino acid substitution at D26. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at D8. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is any amino acid other than aspartic acid; for example, amino acid 26 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Ala, Gly, Val, Leu, or Ile instead of Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Ala, Gly, Val, Leu, Ile, or Arg, instead of Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Ala instead of Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Val instead of Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Leu instead of Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Gly instead of Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B,

where amino acid 26 is Ile instead of Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 26 is Arg instead of Asp.

[00131] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at D8; i.e., where amino acid 8 is other than an aspartic acid. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is any amino acid other than aspartic acid; for example, amino acid 8 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Ala, Gly, Val, Leu, or Ile instead of Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Ala, Gly, Val, Leu, Ile, or Arg instead of Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Ala instead of Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Val instead of Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Leu instead of Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Gly instead of Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Ile. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is Arg instead of Asp.

[00132] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2D. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2E. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino

acid sequence depicted in FIG. 2F. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2G.

T37 substitution

[00133] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is an amino acid other than threonine, e.g., where amino acid 37 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is Gly, Ala, Val, Leu, Ile, Arg, Lys, or His. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is Arg, Lys, or His. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is Gly, Ala, Val, Leu, or Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is Arg. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is Lys. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is His. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present dislosure comprises an amino acid sequence having at least 70%, at

least 75%, at least 80%, at least 85%, at 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 37 is Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is Ala. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is Leu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is Ile. In some cases, a multimeric polypeptide of the present disclosure exhibits from about 15% to about 35% of the binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B) exhibited by a control multimeric polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B (or set forth in SEQ ID NO:2) for the PD-1 polypeptide; and exhibits reduced binding affinity to B7-1 (e.g., exhibits from about 70% to about 90% reduced binding affinity to B7-1) compared to the binding affinity of a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 3B or in SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[00134] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, with an amino acid substitution at T37. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at T19. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is any amino acid other than threonine; for example, amino acid 37 can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met,

Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Gly, Ala, Val, Leu, Ile, Arg, His, or Lys, instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Gly, Ala, Val, Leu, or Ile, instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Arg, His, or Lys, instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Arg instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Lys instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is His instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Gly instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Ala instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Val instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Leu instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 37 is Ile instead of Thr.

[00135] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at T19; i.e., where amino acid 19 is other than threonine. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is any amino acid other than threonine; for example, amino acid 19 can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure

comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Gly, Ala, Val, Leu, Ile, Arg, His, or Lys instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Gly, Ala, Val, Leu, or Ile, instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Arg, His, or Lys instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Arg instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Lys instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is His instead of 19. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Gly instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Ala instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Val instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Leu instead of Thr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is Ile instead of Thr.

I54 substitution

[00136] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 54 is an amino acid other than isoleucine, e.g., where amino acid 54 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 54 is an amino acid other than isoleucine or

valine, e.g., where amino acid 54 is Gly, Ala, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 54 is Ala, Gly, Leu, Glu, Arg, or Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 54 is Glu or Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 54 is Ala. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 54 is Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 54 is Leu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 54 is Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 54 is Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 54 is Arg. In some cases, a multimeric polypeptide of the present disclosure exhibits from about 70% to about 100% of the binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 2B) exhibited by a control multimeric polypeptide comprising a PD-L1 polypeptide

comprising the amino acid sequence set forth in FIG. 2B (or set forth in SEQ ID NO:2) for the PD-1 polypeptide; and exhibits reduced binding affinity to B7-1 (e.g., exhibits from about 40% to about 90% reduced binding affinity to B7-1) compared to the binding affinity of a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 3B or in SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[00137] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2A, where amino acid 54 is an amino acid other than valine, e.g., where amino acid 54 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2A, where amino acid 54 is an amino acid other than isoleucine or valine, e.g., where amino acid 54 is Gly, Ala, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2A, where amino acid 54 is Ala, Gly, Leu, Glu, Arg, or Asp. I In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2A, where amino acid 54 is Glu or Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2A, where amino acid 54 is Ala. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2A, where amino acid 54 is Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino

acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2A, where amino acid 54 is Leu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2A, where amino acid 54 is Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2A, where amino acid 54 is Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2A, where amino acid 54 is Arg. In some cases, a multimeric polypeptide of the present disclosure exhibits from about 70% to about 100% of the binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3A) exhibited by a control multimeric polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2A (or set forth in SEQ ID NO:1) for the PD-1 polypeptide; and exhibits reduced binding affinity to B7-1 (e.g., exhibits from about 40% to about 90% reduced binding affinity to B7-1) compared to the binding affinity of a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2A or in SEQ ID NO:1) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3C).

[00138] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, with an amino acid substitution at I54. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at I36. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is any amino acid other than isoleucine; for example, amino acid 54 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is any amino acid other than isoleucine or

valine; for example, amino acid 54 can be Gly, Ala, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is Ala, Gly, Leu, Arg, or Asp, instead of Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is Ala instead of Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is Leu instead of Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is Gly instead of Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is Asp instead of Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 54 is Arg instead of Ile.

[00139] In some cases, the variant PD-L1 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 V54. In some cases, the variant PD-L1 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 V36. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is any amino acid other than valine; for example, amino acid 54 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is any amino acid other than isoleucine or valine; for example, amino acid 54 can be Gly, Ala, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Ala, Gly, Leu, Glu, Arg, or Asp, instead of Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Glu or Asp, instead of Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Ala instead of Val. In some cases, the variant PD-L1 polypeptide

present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Leu instead of Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Gly instead of Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Asp instead of Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Glu instead of Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, where amino acid 54 is Arg instead of Val.

[00140] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Ile-36; i.e., where amino acid 36 is other than isoleucine. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is any amino acid other than isoleucine; for example, amino acid 36 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is any amino acid other than isoleucine or valine; for example, amino acid 36 can be Gly, Ala, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is Ala, Gly, Leu, Arg, or Asp instead of Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is Ala instead of Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is Leu instead of Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is Gly instead of Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is Asp instead of Ile. In some cases, the variant PD-L1 polypeptide

present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 36 is Arg instead of Ile.

[00141] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V36. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is any amino acid other than valine; for example, amino acid 36 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is any amino acid other than isoleucine or valine; for example, amino acid 36 can be Gly, Ala, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Ala, Gly, Leu, Glu, or Asp instead of Val. In some cases, a variant PD-L1 polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Glu or Asp instead of Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Ala instead of Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Leu instead of Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Gly instead of Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Asp instead of Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Glu instead of Val. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, where amino acid 36 is Arg instead of Val.

[00142] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2H. In some cases,

the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2I.

Q66 substitution

[00143] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 66 is an amino acid other than glutamine, e.g., where amino acid 66 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 66 is Glu or Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 66 is Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 66 is Asp. In some cases, a multimeric polypeptide of the present disclosure exhibits from about 80% to about 100% of the binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B) exhibited by a control multimeric polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B (or set forth in SEQ ID NO:2) for the PD-1 polypeptide; and exhibits reduced binding affinity to B7-1 (e.g., exhibits from about 40% to about 90% reduced binding affinity to B7-1) compared to the binding affinity of a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 3B or in SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[00144] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, with an amino acid substitution at Q66. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Q48. For example, in some cases, the variant PD-L1

polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is any amino acid other than glutamine; for example, amino acid 66 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Ala, Gly, Leu, Glu, or Asp, instead of Gln. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Glu or Asp, instead of Gln. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Ala instead of Gln. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Leu instead of Gln. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Gly instead of Gln. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Asp instead of Gln. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 66 is Glu instead of Gln.

[00145] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Q48; i.e., where amino acid 48 is other than glutamine. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is any amino acid other than glutamine; for example, amino acid 48 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Ala, Gly, Leu, Glu, or Asp instead of Gln. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Glu or Asp instead of Gln. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Ala instead of Gln. In

some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Leu instead of Gln. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Gly instead of Gln. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Asp instead of Gln. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 48 is Glu instead of Gln.

[00146] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2J. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2K.

E72 substitution

[00147] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 72 is an amino acid other than glutamic acid, e.g., where amino acid 72 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 72 is Arg, Lys, or His. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 72 is Asp, Arg, Lys, or His. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 72 is Arg. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 72 is Lys. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 72 is His. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 72 is Asp. In some cases, a multimeric polypeptide of the present disclosure exhibits from about 30% to about 60% of the binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B) exhibited by a control multimeric polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B (or set forth in SEQ ID NO:2) for the PD-1 polypeptide; and exhibits reduced binding affinity to B7-1 (e.g., exhibits from about 40% to about 90% reduced binding affinity to B7-1) compared to the binding affinity of a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 3B or in SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[00148] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, with an amino acid substitution at E72. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at E54. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 72 is any amino acid other than glutamic acid; for example, amino acid 72 can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 72 is Asp, Arg, His, or Lys, instead of Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 72 is Arg, His, or Lys, instead of Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 72 is Arg instead of Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino

acid sequence set forth in FIG. 2B, where amino acid 72 is Lys instead of Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 72 is His instead of Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 72 is Asp instead of Glu.

[00149] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at E54; i.e., where amino acid 54 is other than a glutamic acid. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is any amino acid other than glutamic acid; for example, amino acid 54 can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is Asp, Arg, His, or Lys instead of Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is Arg, His, or Lys instead of Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is Arg instead of Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is Lys instead of Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is His instead of Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 54 is Asp instead of Glu.

[00150] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2L. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence depicted in FIG. 2M.

Y56

[00151] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 56 is an amino acid other than tyrosine, e.g., where amino acid 56 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 56 is Ala, Gly, Val, Leu, or Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 56 is Asp or Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 56 is Arg, His, or Lys. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 56 is Ala, Asp, or Arg. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 56 is Arg. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 56 is Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 56 is Ala. In some cases, a multimeric polypeptide of the present disclosure exhibits from about 50% to about

100% of the binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B) exhibited by a control multimeric polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B (or set forth in SEQ ID NO:2) for the PD-1 polypeptide; and exhibits reduced binding affinity to B7-1 (e.g., exhibits from about 60% to about 95% reduced binding affinity to B7-1) compared to the binding affinity of a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 3B or in SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[00152] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, with an amino acid substitution at Y56. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Y38. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 56 is any amino acid other than tyrosine; for example, amino acid 56 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 56 is Ala, Val, Gly, Leu, or Ile, instead of Tyr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 56 is Arg, His, or Lys, instead of Tyr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 56 is Asp or Glu, instead of Tyr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 56 is Arg instead of Tyr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 56 is Asp instead of Tyr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 56 is Ala instead of Tyr.

[00153] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid

substitution at Y38; i.e., where amino acid 38 is other than tyrosine. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 38 is any amino acid other than tyrosine; for example, amino acid 38 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 38 is Arg, His, or Lys instead of Tyr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 38 is Asp or Glu instead of Tyr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 38 is Ala, Gly, Val, Leu, or Ile instead of Tyr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 38 is Arg instead of Tyr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 38 is Ala instead of Tyr. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 38 is Asp instead of Tyr.

G119

[00154] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 119 is an amino acid other than glycine, e.g., where amino acid 119 is 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 119 is Ala, Val, Leu, or Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 119 is Asp or Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure

comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 119 is Arg, His, or Lys. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 119 is Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 119 is Arg. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 119 is Ala. In some cases, a multimeric polypeptide of the present disclosure exhibits from about 20% to about 50%, or from about 50% to 100%, of the binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B) exhibited by a control multimeric polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B (or set forth in SEQ ID NO:2) for the PD-1 polypeptide; and exhibits reduced binding affinity to B7-1 (e.g., exhibits from about 60% to about 95% reduced binding affinity to B7-1) compared to the binding affinity of a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 3B or in SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[00155] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, with an amino acid substitution at G119. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G101. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 119 is any amino acid other than glycine; for example, amino acid 119 can be 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 119 is Ala, Val, Leu, or Ile, instead of Gly. In some cases, the

variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 119 is Arg, His, or Lys, instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 119 is Asp or Glu, instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 119 is Arg instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 119 is Asp instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 119 is Ala instead of Gly.

[00156] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G101; i.e., where amino acid 101 is other than glycine. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 101 is any amino acid other than glycine; for example, amino acid 101 can be 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 101 is Arg, His, or Lys instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 101 is Asp or Glu instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 101 is Ala, Val, Leu, or Ile instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 101 is Arg instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 101 is Ala instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 101 is Asp instead of Gly.

G120

[00157] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 120 is an amino acid other than glycine, e.g., where amino acid 120 is 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 120 is Ala, Val, Leu, or Ile. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 120 is Asp or Glu. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 120 is Arg, His, or Lys. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 120 is Asp. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 120 is Arg. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 120 is Ala. In some cases, a multimeric polypeptide of the present disclosure exhibits from about 20% to about 50%, or from about 50% to 100%, of the binding affinity to PD-1 (e.g., to a PD-1 polypeptide comprising the amino acid sequence depicted in FIG. 3B) exhibited by a control multimeric polypeptide comprising a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 2B (or set forth in SEQ ID NO:2) for the PD-1 polypeptide; and exhibits reduced binding

affinity to B7-1 (e.g., exhibits from about 60% to about 95% reduced binding affinity to B7-1) compared to the binding affinity of a control multimeric polypeptide comprising a wild-type PD-L1 polypeptide (e.g., a PD-L1 polypeptide comprising the amino acid sequence set forth in FIG. 3B or in SEQ ID NO:2) for a wild-type B7-1 polypeptide (e.g., a B7-1 polypeptide comprising the amino acid sequence depicted in FIG.3D).

[00158] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, with an amino acid substitution at G120. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G102. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 120 is any amino acid other than glycine; for example, amino acid 120 can be 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 120 is Ala, Val, Leu, or Ile, instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 120 is Arg, His, or Lys, instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 120 is Asp or Glu, instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 120 is Arg instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 120 is Asp instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where amino acid 120 is Ala instead of Gly.

[00159] In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G102; i.e., where amino acid 102 is other than glycine. For example, in some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 102 is any amino acid other than glycine; for example, amino acid 101 can be 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 PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 102 is Arg, His, or Lys instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 102 is Asp or Glu instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 102 is Ala, Val, Leu, or Ile instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 102 is Arg instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 102 is Ala instead of Gly. In some cases, the variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, where amino acid 102 is Asp instead of Gly.

Multiple variant PD-L1 immunomodulatory domains

[00160] In some cases, a multimeric polypeptide of the present disclosure includes a single variant PD-L1 immunomodulatory polypeptide.

[00161] In some cases, a multimeric polypeptide of the present disclosure includes two variant PD-L1 immunomodulatory polypeptides. In some cases, the two variant PD-L1immunomodulatory polypeptides are in tandem in a polypeptide chain. In some cases, the two variant PD-L1 immunomodulatory polypeptides are in separate polypeptide chains. In some cases, the two variant PD-L1 immunomodulatory polypeptides are in separate polypeptide chains of the multimeric polypeptide. In some cases, the two variant PD-L1 polypeptides have the same amino acid sequence as one another. In some cases, the two variant PD-L1 polypeptides have different amino acid sequences (e.g., the two differ from one another by at least one amino acid).

[00162] In some cases, a multimeric polypeptide of the present disclosure includes three variant PD-L1 immunomodulatory polypeptides. In some cases, the three variant PD-L1 immunomodulatory polypeptides are in tandem in a polypeptide chain. In some cases, one of the three variant PD-L1 immunomodulatory polypeptides is on a separate polypeptide chain of the multimeric polypeptide from the other two variant PD-L1 immunomodulatory polypeptides. In some cases, the three variant PD-L1 polypeptides have the same amino acid sequence as one another. In some cases, each of the three variant PD-L1 polypeptides has a different amino acid sequence (e.g., each differs from the other two by at least one amino acid).

Scaffold polypeptides

[00163] A T-cell modulatory multimeric polypeptide of the present disclosure comprises an Fc polypeptide, or another suitable scaffold polypeptide.

[00164] 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.

[00165] 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

[00166] 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 FIG. 4A-

4C. 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 FIG. 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 FIG. 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 FIG. 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 FIG. 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 FIG. 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 FIG. 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 FIG. 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

[00167] 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

[00168] Suitable epitope tags include, but are not limited to, hemagglutinin (HA; e.g., YPYDVPDYA (SEQ ID NO:22); FLAG (e.g., DYKDDDDK (SEQ ID NO:23); c-myc (e.g., EQKLISEEDL; SEQ ID NO:24), and the like.

Affinity domain

[00169] 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:25), HisX6 (HHHHHH) (SEQ ID NO:26), C-myc (EQKLISEEDL) (SEQ ID NO:27), Flag (DYKDDDDK) (SEQ ID NO:28), StrepTag (WSHPQFEK) (SEQ ID NO:29), hemagglutinin, e.g., HA Tag (YPYDVPDYA) (SEQ ID NO:30), glutathione-S-transferase (GST), thioredoxin, cellulose binding domain, RYIRS (SEQ ID NO:31), Phe-His-His-Thr (SEQ ID NO:32), chitin binding domain, S-peptide, T7 peptide, SH2 domain, C-end RNA tag, WEAAAREACCRECCARA (SEQ ID NO:33), 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

[00170] Exemplary multimeric polypeptides of the present disclosure are described below.

D26 substitution

[00171] 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 PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 26 is an amino acid other than an aspartic acid, e.g., where amino acid 26 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 26 is Ala or Arg; or a variant PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is an amino acid other than an aspartic acid, e.g., where amino acid 8 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 8 is Ala or Arg; 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 PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 26 is an amino acid other than an aspartic acid, e.g., where amino acid 26 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 26 is Ala or Arg; or a variant PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is an amino acid other than an aspartic acid, e.g., where amino acid 8 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 8 is Ala or Arg; 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 PD-L1 polypeptide of the present disclosure; iv) a second variant PD-L1 polypeptide of the present disclosure; and v) a third variant PD-L1 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 PD-L1 polypeptides comprises: i) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 26 is an amino acid other than an aspartic acid, e.g., where amino acid 26 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 26 is Ala or Arg; or ii) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least

90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is an amino acid other than an aspartic acid, e.g., where amino acid 8 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 8 is Ala or Arg. 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 PD-L1 polypeptide of the present disclosure; ii) a second variant PD-L1 polypeptide of the present disclosure; and iii) a third variant PD-L1 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 PD-L1 polypeptides comprises: i) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 26 is an amino acid other than an aspartic acid, e.g., where amino acid 26 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 26 is Ala or Arg; or ii) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is an amino acid other than an aspartic acid, e.g., where amino acid 8 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 8 is Ala or Arg. 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 PD-L1 polypeptide of the present disclosure; ii) a linker; iii) a second variant PD-L1 polypeptide of the present disclosure; iv) a linker; v) a third variant PD-L1 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 PD-L1 polypeptides comprises: i) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 26 is an amino acid other than an aspartic acid, e.g., where amino acid 26 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 26 is Ala or Arg; or ii) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 8 is an amino acid other than an aspartic acid, e.g., where amino acid 8 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 8 is Ala or Arg. In some cases, the

linker comprises a (GSSSS)n sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

T37 substitution

[00172] 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 PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is an amino acid other than threonine, e.g., where amino acid 37 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu; e.g., where amino acid 37 is Gly, Ala, Val, Leu, Ile, Arg, Lys, or His; or a variant PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is any amino acid other than threonine; for example, amino acid 19 can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu; e.g., where amino acid 19 is Gly, Ala, Val, Leu, Ile, Arg, Lys, or His; 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 PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is an amino acid other than threonine, e.g., where amino acid 37 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu; e.g., where amino acid 37 is Gly, Ala, Val, Leu, Ile, Arg, Lys, or His; or a variant PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is any amino acid other than threonine; for example, amino acid 19 can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu; e.g., where amino acid 19 is Gly, Ala, Val, Leu, Ile, Arg, Lys, or His; 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 PD-L1 polypeptide of the present disclosure; iv) a second variant PD-L1 polypeptide of the present disclosure; and v) a

third variant PD-L1 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 PD-L1 polypeptides comprises: i) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is an amino acid other than threonine, e.g., where amino acid 37 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu; e.g., where amino acid 37 is Gly, Ala, Val, Leu, Ile, Arg, Lys, or His; or ii) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is any amino acid other than threonine; for example, amino acid 19 can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu; e.g., where amino acid 19 is Gly, Ala, Val, Leu, Ile, Arg, Lys, or His. 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 PD-L1 polypeptide of the present disclosure; ii) a second variant PD-L1 polypeptide of the present disclosure; and iii) a third variant PD-L1 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 PD-L1 polypeptides comprises: i) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is an amino acid other than threonine, e.g., where amino acid 37 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu; e.g., where amino acid 37 is Gly, Ala, Val, Leu, Ile, Arg, Lys, or His; or ii) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is any amino acid other than threonine; for example, amino acid 19 can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu; e.g., where amino acid 19 is Gly, Ala, Val, Leu, Ile, Arg, Lys, or His. 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 PD-L1 polypeptide of the present disclosure; ii) a linker; iii) a second variant PD-L1 polypeptide of the present disclosure; iv) a linker; v) a third variant PD-L1 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 PD-

L1 polypeptides comprises: i) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 37 is an amino acid other than threonine, e.g., where amino acid 37 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu; e.g., where amino acid 37 is Gly, Ala, Val, Leu, Ile, Arg, Lys, or His; or ii) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 19 is any amino acid other than threonine; for example, amino acid 19 can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu; e.g., where amino acid 19 is Gly, Ala, Val, Leu, Ile, Arg, Lys, or His. In some cases, the linker comprises a (GSSSS)n sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

Y56 substitution

[00173] 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 PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 56 is an amino acid other than tyrosine, e.g., where amino acid 56 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 56 is Ala, Gly, Val, Leu, or Ile, where amino acid 56 is Asp or Glu, or where amino acid 56 is Arg, His, or Lys; or a variant PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 38 is an amino acid other than tyrosine, e.g., where amino acid 56 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 38 is Ala, Gly, Val, Leu, or Ile, where amino acid 38 is Asp or Glu, or where amino acid 38 is Arg, His, or Lys; 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 PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 56 is an amino acid other than tyrosine, e.g., where amino acid 56 is Gly, Ala, Val, Leu, Ile, Pro,

Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 56 is Ala, Gly, Val, Leu, or Ile, where amino acid 56 is Asp or Glu, or where amino acid 56 is Arg, His, or Lys; or a variant PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 38 is an amino acid other than tyrosine, e.g., where amino acid 38 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 38 is Ala, Gly, Val, Leu, or Ile, where amino acid 38 is Asp or Glu, or where amino acid 38 is Arg, His, or Lys; 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 PD-L1 polypeptide of the present disclosure; iv) a second variant PD-L1 polypeptide of the present disclosure; and v) a third variant PD-L1 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 PD-L1 polypeptides comprises: i) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 56 is an amino acid other than tyrosine, e.g., where amino acid 56 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 56 is Ala, Gly, Val, Leu, or Ile, where amino acid 56 is Asp or Glu, or where amino acid 56 is Arg, His, or Lys; or ii) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 38 is an amino acid other than tyrosine, e.g., where amino acid 38 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 38 is Ala, Gly, Val, Leu, or Ile, where amino acid 38 is Asp or Glu, or where amino acid 38 is Arg, His, or Lys. 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 PD-L1 polypeptide of the present disclosure; ii) a second variant PD-L1 polypeptide of the present disclosure; and iii) a third variant PD-L1 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 PD-L1 polypeptides comprises: i) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 56 is an amino acid other than tyrosine, e.g., where amino acid 56 is Gly, Ala, Val, Leu, Ile, Pro,

Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 56 is Ala, Gly, Val, Leu, or Ile, where amino acid 56 is Asp or Glu, or where amino acid 56 is Arg, His, or Lys; or ii) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 38 is an amino acid other than tyrosine, e.g., where amino acid 38 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 38 is Ala, Gly, Val, Leu, or Ile, where amino acid 38 is Asp or Glu, or where amino acid 38 is Arg, His, or Lys. 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 PD-L1 polypeptide of the present disclosure; ii) a linker; iii) a second variant PD-L1 polypeptide of the present disclosure; iv) a linker; v) a third variant PD-L1 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 PD-L1 polypeptides comprises: i) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 56 is an amino acid other than tyrosine, e.g., where amino acid 56 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 56 is Ala, Gly, Val, Leu, or Ile, where amino acid 56 is Asp or Glu, or where amino acid 56 is Arg, His, or Lys; or ii) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 38 is an amino acid other than tyrosine, e.g., where amino acid 38 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 38 is Ala, Gly, Val, Leu, or Ile, where amino acid 38 is Asp or Glu, or where amino acid 38 is Arg, His, or Lys. In some cases, the linker comprises a (GSSSS)n sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

G119 substitution

[00174] 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 PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 119 is an amino acid other than glycine, e.g., where amino acid 119 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu,

where amino acid 119 is Ala, Val, Leu, or Ile, where amino acid 119 is Arg, His, or Lys, or where amino acid 119 is Glu or Asp; or a variant PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 101 is an amino acid other than glycine, e.g., where amino acid 101 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 101 is Ala, Val, Leu, or Ile, where amino acid 101 is Arg, His, or Lys, or where amino acid 101 is Glu or Asp; 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 PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 119 is an amino acid other than glycine, e.g., where amino acid 119 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 119 is Ala, Val, Leu, or Ile, where amino acid 119 is Arg, His, or Lys, or where amino acid 119 is Glu or Asp; or a variant PD-L1 polypeptide comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 101 is an amino acid other than glycine, e.g., where amino acid 101 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 101 is Ala, Val, Leu, or Ile, where amino acid 101 is Arg, His, or Lys, or where amino acid 101 is Glu or Asp; 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 PD-L1 polypeptide of the present disclosure; iv) a second variant PD-L1 polypeptide of the present disclosure; and v) a third variant PD-L1 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 PD-L1 polypeptides comprises: i) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 119 is an amino acid other than glycine, e.g., where amino acid 119 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 119 is Ala, Val, Leu, or Ile, where amino acid 119 is Arg, His, or Lys, or where

amino acid 119 is Glu or Asp; or ii) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 101 is an amino acid other than glycine, e.g., where amino acid 101 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 101 is Ala, Val, Leu, or Ile, where amino acid 101 is Arg, His, or Lys, or where amino acid 101 is Glu or Asp. 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 PD-L1 polypeptide of the present disclosure; ii) a second variant PD-L1 polypeptide of the present disclosure; and iii) a third variant PD-L1 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 PD-L1 polypeptides comprises: i) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 119 is an amino acid other than glycine, e.g., where amino acid 119 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 119 is Ala, Val, Leu, or Ile, where amino acid 119 is Arg, His, or Lys, or where amino acid 119 is Glu or Asp; or ii) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 101 is an amino acid other than glycine, e.g., where amino acid 101 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 101 is Ala, Val, Leu, or Ile, where amino acid 101 is Arg, His, or Lys, or where amino acid 101 is Glu or Asp. 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 PD-L1 polypeptide of the present disclosure; ii) a linker; iii) a second variant PD-L1 polypeptide of the present disclosure; iv) a linker; v) a third variant PD-L1 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 PD-L1 polypeptides comprises: i) an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at 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 119 is an amino acid other than glycine, e.g., where amino acid 119 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 119 is Ala, Val, Leu, or Ile, where amino acid 119 is Arg, His, or Lys, or where amino acid 119 is Glu or Asp; or ii) an amino acid

sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, where amino acid 101 is an amino acid other than glycine, e.g., where amino acid 101 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu, where amino acid 101 is Ala, Val, Leu, or Ile, where amino acid 101 is Arg, His, or Lys, or where amino acid 101 is Glu or Asp. In some cases, the linker comprises a (GSSSS)n sequence, where n is 1, 2, 3, 4, or 5. In some cases, n is 4. In some cases, n is 5.

[00175] In any of the above-described embodiments, the variant PD-L1 polypeptide present in the multimeric polypeptide can comprise a substitution of an amino acid as set out in FIG. 10 or FIG. 11. The following are examples. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of D26 of the amino acid sequence depicted in FIG. 2B; or D8 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of T37 of the amino acid sequence depicted in FIG. 2B; or T19 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of D49 of the amino acid sequence depicted in FIG. 2B; or D31 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of L53 of the amino acid sequence depicted in FIG. 2B; or L35 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of I54 (V54 in mouse PD-L1) of the amino acid sequence depicted in FIG. 2B; or I36 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of Y56 of the amino acid sequence depicted in FIG. 2B; or Y38 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of Y56 of the amino acid sequence depicted in FIG. 2B; or Y38 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of Q66 of the amino acid sequence depicted in FIG. 2B; or Q48 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of Q66 of the amino acid sequence depicted in FIG. 2B; or Q48 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of E72 of the amino acid sequence depicted in FIG. 2B; or E54 of the amino acid

sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of M115 (I115 of mouse PD-L1) of the amino acid sequence depicted in FIG. 2B; or M97 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of I116 of the amino acid sequence depicted in FIG. 2B; or I98 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of G119 of the amino acid sequence depicted in FIG. 2B; or G101 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of G120 of the amino acid sequence depicted in FIG. 2B; or G102 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of G120 of the amino acid sequence depicted in FIG. 2B; or G102 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of A121 of the amino acid sequence depicted in FIG. 2B; or A103 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of D122 of the amino acid sequence depicted in FIG. 2B; or D104 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of Y123 of the amino acid sequence depicted in FIG. 2B; or Y105 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of K124 of the amino acid sequence depicted in FIG. 2B; or K106 of the amino acid sequence set forth in SEQ ID NO:2. In some cases, a variant PD-L1 polypeptide present in a multimeric polypeptide of the present disclosure comprises a substitution of R125 of the amino acid sequence depicted in FIG. 2B; or K107 of the amino acid sequence set forth in SEQ ID NO:2.

NUCLEIC ACIDS

[00176] The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a variant PD-L1 polypeptide of the present disclosure. The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a PD-L1 fusion polypeptide of the present disclosure.

[00177] 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

[00178] 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. Thus, the present disclosure provides a composition comprising a first nucleic acid and a second nucleic acid, where the first nucleic acid comprises a nucleotide sequence encoding a first polypeptide chain of a multimeric polypeptide of the present disclosure, and wehre teh second nucleic acid comprises a nucleotide sequence encoding a second polypeptide chain of a multimeric polypeptide of the present disclosure.

[00179] 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 PD-L1 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.

[00180] 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 PD-L1 polypeptide of the present disclosure); b) a second MHC polypeptide; and c) an Ig Fc polypeptide. Suitable T-cell epitopes, MHC polypeptides, variant PD-L1 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

[00181] 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 include 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.

[00182] 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.

[00183] 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 PD-L1 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 PD-L1 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 PD-L1 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.

[00184] 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%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to any one of the amino acid sequences depicted in FIG. 6. In some cases, the MHC class I heavy chain polypeptide is an HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-K, or HLA-L heavy chain. In some cases, the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 85%

amino acid sequence identity to the amino acid sequence set forth in one of FIG. 5A-C. 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.

[00185] 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%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to an amino acid sequence depicted in FIG. 4A-4C.

[00186] Suitable variant PD-L1 immunomodulatory polypeptides are described above.

[00187] 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:34); b) ENLYTQS (SEQ ID NO:35); c) DDDDK (SEQ ID NO:36); d) LVPR (SEQ ID NO:37); and e) GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:38).

[00188] 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

[00189] 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.

[00190] 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.

[00191] 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.

[00192] 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).

[00193] 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.

[00194] 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

[00195] The present disclosure provides a genetically modified host cell, where the host cell is genetically modified with a nucleic acid of the present disclosure.

[00196] 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), Chinese hamster ovary (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.

[00197] 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

[00198] 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.

[00199] 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.

[00200] 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.

[00201] 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

[00202] The present disclosure provides compositions, including pharmaceutical compositions, comprising a variant PD-L1 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

[00203] 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.

[00204] 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.

[00205] 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.

[00206] 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.

[00207] 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.

[00208] 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.

[00209] 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.

[00210] 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.

[00211] 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.

[00212] The present disclosure provides compositions, including pharmaceutical compositions, comprising a variant PD-L1 polypeptide of the present disclosure. A composition can comprise: a) a variant PD-L1 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

[00213] 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.

[00214] 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.

[00215] 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.

[00216] 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.

[00217] 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.

[00218] 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.

[00219] 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.

[00220] 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.

[00221] 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.

[00222] 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™.

TREATMENT METHODS

[00223] 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.

[00224] 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.

[00225] In some cases, an immunomodulatory polypeptide (e.g., a variant PD-L1 polypeptide of the present disclosure) present in a multimeric polypeptide of the present disclosure is an inhibitory polypeptide, and the multimeric polypeptide comprising the variant PD-L1 polypeptide inhibits activity of an 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.

[00226] 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 a variant PD-L1 polypeptide of the present disclosure. 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 and/or activity of 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 and/or activity 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.

[00227] Autoimmune disorders that are amenable to treatment with a method of the present disclosure include, but are not limited to, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune oophoritis

and orchitis, autoimmune thrombocytopenia, Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac sprue-dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatrical pemphigoid, CREST syndrome (also known as limited cutaneous form of systemic sclerosis), cold agglutinin disease, Crohn's disease, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, glomerulonephritis, Graves' disease, Guillain-Barré, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), irritable bowel disease (IBD), IgA neuropathy, juvenile arthritis, lichen planus, lupus erthematosus, Meniere's disease, mixed connective tissue disease, multiple sclerosis, type 1 diabetes mellitus, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychrondritis, polyglandular syndromes, polymyalgia rheumatics, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynauld's phenomenon, Reiter's syndrome, rheumatoid arthritis, sarcoidosis, scleroderma, Sjögren's syndrome, stiff-man syndrome, systemic lupus erythematosus, lupus erythematosus, takayasu arteritis, temporal arteristis/giant cell arteritis, ulcerative colitis, uveitis, vasculitides such as dermatitis herpetiformis vasculitis, vitiligo, and Wegener's granulomatosis.

[00228] In some cases, an immunomodulatory polypeptide (e.g., a variant PD-L1 polypeptide of the present disclosure) present in a multimeric polypeptide of the present disclosure is an inhibitory polypeptide, and the multimeric polypeptide comprising the variant PD-L1 polypeptide inhibits activity of an epitope-specific T cell. In some cases, the epitope is an epitope on an allograft (e.g., a skin allograft, a liver allograft, a kidney allograft, a heart allograft, a bone allograft, a cartilage allograft, a lung allograft, a cell allograft (e.g., a bone marrow allograft), etc.); and the multimeric polypeptide selectively inhibits the activity of a T cell specific for an antigen present on the allograft.

[00229] The present disclosure provides a method of inhibiting allograft rejection in an individual, the method comprising administering to an individual (e.g., an individual who is a recipient of an allograft; or an individual who is about to become an allograft recipient) 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 an epitope present on an allograft, and where the multimeric polypeptide comprises a variant PD-L1 polypeptide of the present disclosure. 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 and/or activity of alloreactive (allograft 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 and/or activity of alloreactive (allograft 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, increases the survival time of an allograft in the individual; e.g., the survival time of the allograft in the individual is increased by at least 25%, at least 50%, at least 2-fold, at least 5-fold, at least 10-fold, at least 50-fold, or at least 100-fold, compared to the allograft survival time in the individual 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, ameliorates one or more symptoms associated with allograft rejection in the individual.

[00230] 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

[00231] Suitable formulations are described above, where suitable formulations include a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: a) a multimeric polypeptide of the present disclosure; and b) a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: a) a nucleic acid comprising a nucleotide sequence encoding a multimeric polypeptide of the present disclosure; and b) a pharmaceutically acceptable excipient; in some instances, the nucleic acid is an mRNA. In some cases, a suitable formulation comprises: a) a first nucleic acid comprising a nucleotide sequence encoding the first polypeptide of a multimeric polypeptide of the present disclosure; b) a second nucleic acid comprising a nucleotide sequence encoding the second polypeptide of a multimeric polypeptide of the present disclosure; and c) a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: a) a recombinant expression vector comprising a nucleotide sequence encoding a multimeric polypeptide of the present disclosure; and b) a pharmaceutically acceptable excipient. In some cases, a suitable formulation comprises: a) a first recombinant expression vector comprising a nucleotide sequence encoding the first polypeptide of a multimeric polypeptide of the present disclosure; b) a second

recombinant expression vector comprising a nucleotide sequence encoding the second polypeptide of a multimeric polypeptide of the present disclosure; and c) a pharmaceutically acceptable excipient.

[00232] Suitable pharmaceutically acceptable excipients are described above.

Dosages

[00233] 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.

[00234] 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.

[00235] 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.

[00236] 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).

[00237] 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

[00238] 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.

[00239] 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.

[00240] 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.

[00241] 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.

[00242] 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.

[00243] 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

[00244] 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

[00245] 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 PD-L1 variants

MATERIALS AND METHODS

PD-L1 Mutagenesis

[00246] Full-length mouse PD-L1 was cloned into the SacI and BamHI sites of the Clontech N1 mCherry vector. The native leader peptide sequence was replaced by the EPO leader peptide sequence to improve localization and expression level. Site-specific mutagenesis was performed using high fidelity KOD polymerase, 2mM dNTPs and 4mM $MgCl_2$. Positions for mutagenesis were selected based on the crystal structure of complex formed by human PD-L1 and PD-1 (PDB: 3BIK). Equivalent surface accessible positions in mouse PD-L1 were identified by sequence alignment to human PD-L1 (36 positions total). Mutagenesis was attempted such that each chosen position was mutated to an Ala, Glu or Arg residue. The overall mutagenesis success rate was ~70%, and for some positions not all substitutions (A, E and R) were obtained. The sequence validated mutants were expression tested by transient transfection of 1 mL of suspension HEK 293 cells. Only those mutants that showed comparable expression to wild-type PD-L1 and correct membrane localization were subsequently utilized in the microarray and FACS binding studies yielding a final set of 60 PD-L1 mutants to assay.

Microbead FACS Binding Assay

[00247] PD-L1 mCherry mutant constructs were transiently transfected into HEK 293S cells and subsequently challenged with protein A microbeads (Milltenyi) pre-saturated with a 4:1 mixture of PD-1 Fc-fusion and FITC-Fc protein. A total Fc protein to bead ratio of 5ug/10uL microbeads was utilized on the basis of a previous report from Genentech (16). The FITC-Fc served to make the otherwise non-fluorescent microbeads show green fluorescence. For each titration experiment, 500μL of protein A microbeads were loaded with a mixture of 10 μg fluorescein isothiocyanate (FITC)-Fc and 40 μg of either PD-1-Fc or B7-1-Fc protein in a total volume of 5mL 1x phosphate buffered saline (PBS). The beads were incubated overnight (~16 hours) at 4°C. Loaded beads were stored for up to two weeks prior to use. Initial experiments determined that 75 μL of the loaded beads were sufficient to saturate 150,000 cells transfected with wild type PD-L1, (transfection efficiency being consistently 60-70%). For titration experiments, sets of wild type and mutant PD-L1 constructs were transfected in 24-well tissue culture plates containing 1mL of suspension HEK-293 cells. Three days post transfection cells were counted, diluted to $1x10^6$ cell/mL with 1x PBS with 2% BSA. 150K cells (150μL) were transferred to Eppendorf tubes, and 75μL of loaded microbeads added along with an additional 100μL 1x PBS with 2% bovine serum albumin (BSA). Reactions were mixed end over end for 1 hour at 4°C, 4',6-diamidino-2-phenylindole (DAPI) was added and samples were immediately analyzed by flow cytometry on a BD Aria III cytometer. Data were analyzed by gating first for live cells (DAPI negative) then for mCherry positive cells (PD-L1 expression). The percentage

of mCherry positive cells that were FITC positive (microbeads bound) was used as "percent bound". For each experiment, the percent bound was normalized to wild type binding.

Purification of Recombinant Fc-fusion protein

[00248] To clone mPD-L1 Fc-fusion protein, full-length wild type or mutant PD-L1 ectodomains (residues F19 - R237) were sub-cloned into a LIC vector containing a C-terminal his-tagged Fc domain (mIgG2a-10xHis). These constructs and an isotope only control were transiently expressed in 1L of HEK 293 suspension cells. Four days post transfection, the media was harvested, 50 mM MES was added to adjust the pH and 100 mM Arg-Cl (pH 7.6) was added to improve solubility. Fc-fusions were subsequently purified over Ni^{2+}-NTA resin (GE) using a batch binding method followed by gravity flow over a 600 mL capacity glass column with a 10 mL resin bed volume. The Ni^{2+}-nitroloacetic acid (NTA) resin was washed with 100 column volumes of wash buffer (50 mM MES pH 6.5, 100 mM Arg-Cl, 5 mM imidazole, 150 mM NaCl, 10% Glycerol) and the bound protein eluted with the same buffer containing 500 mM immidizole. Nickel elutes were concentrated and further purified by gel filtration on an S200 sephadex column (GE) in 50 mM MES pH 6.5, 100 mM Arg-Cl, 150 mM NaCl, 10% Glycerol. Wild-type mPD-1 Fc (residues L25 - Q167) and mB7-1 Fc (residues D37 - K245) constructs were cloned into a lentiviral expression LIC vector that also contains the mIgG2a-10xHis tag. The constructs were co-transfected with lentiviral packaging plasmids and viral supernatants collected after 2-days. Large-scale transductions were started in 125 mL baffled flasks with $20x10^6$ cells and 5-10 mL of viral supernatant. A complete media change was performed on day 3 post transduction and starting on day 5 the cultures were scaled up ending with to a final volume of 1.5L. Supernatants were collected for purification on day 12. Purification of supernatant obtained from the lentiviral produced PD-1 and B7-1 were purified as described for mPD-L1.

FACS Titration Assay

[00249] Fluorescence activated cell sorting (FACS) titration assays were performed with PD-1 Fc and B7-1 Fc fusion proteins purified in house as described above. HEK 293 suspension cells were transfected with the wild type or mutant PD-L1 constructs. Three days post transfection cells were counted and diluted to $1x10^6$ cells/mL in 1x PBS. Premixed reactions containing a final concentration of 1μM Fc-fusion protein and 1.5μM Alexa 488 goat anti-mouse secondary antibody were incubated on ice for 30 min. Subsequently, increasing amounts of the premixed reaction was added to wells of a 96-well plate and the volume adjusted to 50μL. 150 μL of diluted cells (150,000 cells total) were then added to the wells. Binding was performed at 4°C for 1 hour and the cells washed 3x with PBS by centrifugation and subsequently analyzed by FACS. Gated live cells were sub-gated for mCherry and mCherry positive cells sub-gated for Alexa-488. The percent bound represents the percentage of mCherry cells that were Alexa-488

positive. Data represents the average of three independent experiments fit to the single site binding equation $Y = B_{max}*X/(EC_{50} + X)$.

T-Cell Activation Assay

[00250] Spleens were harvested from C57BL/6 mice and $CD4^+$ T-cells isolated using mouse anti-CD4 microbeads (Milltenyi). The $CD4^+$ T-cells were collected in complete RPMI media supplemented with 10% fetal bovine serum (FBS), pen/strep antibiotics, 2mM L-glutamine and 0.1% BME. The cells were counted, stained with carboxyfluorescein *N*-succinimidyl ester (CFSE) (Invitrogen) using the manufacture's protocol and recounted. On the same day, 75,000 cells were plated per well in a 96-well TC plates in complete RPMI media and either left inactivated, activated with 33.3 nM (~ 5 ug/mL) anti-CD3, or activated with 33.3 nM anti-CD3 in the presence of a ~5-fold molar excess (174.3 nM) of either control Fc, WT PD-L1-Fc or mutant PD-L1 Fc proteins. Four days post activation, proliferation was determined by FACS by analyzing CSFE dilution by gating on the non-activated T-cells. The data from each experiment were normalized to the control Fc population and a total of three independent experiments were averaged.

PD-1/B7-1 Competition Binding Experiment

[00251] A mB7-1 hIgG1 Fc fusion construct was cloned which used the same erythropoietin (EPO) leader, mB7-1 ecto domain boundaries and linker sequence as the original mIgG2a construct described above. This construct was transiently expressed in HEK 293 cells and purified as described above for the other Fc fusion proteins used. For the completion experiment, HEK 293 suspension cells were transiently transfected with wild-type mPD-L1 mCherry. Three days post transfection transfected cells were counted and diluted to 1×10^6 cells/mL. B7-1 hIgG1 fusion protein was added to 100,000 transfected cells at a final concentration of 5 nM dimer, in either the absence or presence of increasing concentrations of PD-1 mIgG2a protein (0.01 – 250 nM dimer). Parallel experiments were carried out in which purified mIgG2a isotype control was titrated at equivalent molar concentrations. Protein binding was carried out at 22°C shaking at 900 rpm on a 96-well plate shaker for 1 hour. After binding, plates were washed two times with 1X PBS with 0.2% BSA and anti-human (H + L) Alexa 488 labeled secondary antibody (Invitrogen) was added at 0.01 µg/µL (1 µg total) and incubated for 30 min. Cells were subsequently washed two more times with 1X PBS with 0.2% BSA. Samples were immediately analyzed by FACS and the data gated for the percent of mCherry positive cells (FL4 - PD-L1 expression) that were also Alexa 488 positive (FL1 – B7-1 Binding). Competition data was normalized to 5 nM B7-1 binding in the absence of mPD-1 and plotted as a function of log [mPD-1]. Average data from three independent experiments was fit using a one-site competition model equation $Y = min + (max – min) / (1 + 10^{x\text{-}logEC50})$.

RESULTS

Mechanistic dissection by microarray analysis

[00252] To generate selective PD-L1 reagents, the X-ray structure of the PD-1:PD-L1 complex was used as a framework to identify residues for mutagenesis – identifying 36 solvent exposed residues within the PD-L1 Ig variable domain (24). Each residue was changed to an alanine, arginine and glutamic acid to sample a range of side chain physico-chemical characteristic properties. The cell microarray platform was used initially to challenge a set of wild-type and mutant PD-L1 constructs with PD-1 or B7-1 Fc-fusion protein. These experiments identified mutants that affected only PD-1 binding (D122A, Y123A, Y123R, K124A, K124D, R125A, R125D), only B7-1 binding (Y56A, Y56D, E72R, G119D, G120D) or both (L53R, G119R, A121R) (Table 1; provided in FIG. 10). However, consistent quantification of the PD-1/B7-1 binding proved difficult using the cell microarrays for the following reasons: (1) the lower affinity of B7-1 for PD-L1 reduced the signal to noise for these arrays compared to those challenged with PD-1; (2) the complete loss of binding was easily identified but modest reductions in binding were often more variable; (3) the inherent slide to slide variably associated with independently printed, transfected and treated slides added to signal to noise variations and made direct comparisons more difficult.

Validation by FACS analysis

[00253] To validate and more quantitatively evaluate the binding characteristics of PD-L1 mutants we implemented a high-throughput fluorescence activated cell sorting (FACS) assay, which enables the interrogation of 96 samples every ~15 minutes. This FACs platform affords an enhanced dynamic range compared to cell microarrays. Notably, the mode of query protein presentation is modified. While bivalent Ig-fusions, as used in the microarray platform, are effective for the identification of interactions with moderate affinities, weaker interactions might be missed. To support detection of the wide range of apparent affinities anticipated in analysis of the library of PD-L1 mutants the higher valency afforded by magnetic microbead capture and presentation was exploited (Fig. 7A). For example probing the microarray presenting PD-L1 required higher concentrations of B7-1 Fc than PD-1 Fc, resulting in greater background signal. The increase in dynamic range observed using the FACS microbeads assay is at least in part due to the reduction in background due to non-specific binding. This is likely for two reasons: (1) no secondary antibody is used in the microbead assay; (2) higher avidity means lower amounts of protein can be used to challenge the cells. The microbead assay has the added benefit of not requiring any wash steps, which minimizes loss of bound sample and makes the assay a much more direct measure of protein binding. Additionally, for some lower affinity interactions, such as that between B7-1 and PD-L1, achieving saturation with soluble

B7-1 Fc is difficult in FACS assay, whereas B7-1 Fc conjugated microbeads resulted in significant improvement.

[00254] Briefly, HEK293 cell lines were individually transiently transfected with 55 different surface displayed mutant PD-L1-mCherry fusions. These cells were probed by flow cytometry for their ability to bind either FITC-loaded microbeads decorated with wild type PD-1 Ig-fusion or wild type B7-1 Ig-fusion proteins (Fig. 7B). Importantly, it is unlikely that these mutations caused global changes to the structure or stability of PD-L1, as the transient protein expression levels were similar to wild-type for all the mutants used for analysis. Also, fluorescence microscopy of the wild-type and mutant PD-L1 variants showed correct membrane localization of the C-terminal mCherry fusion protein suggesting the mutant proteins were being correctly folded, processed and inserted into the membrane. These studies resulted in the identification of PD-L1 mutants that either bound specifically to PD-1 (D49R, V54D, V54R, Y56A, Y56D, Y56R, Q66D, E72R, G119D, G120D) or B7-1 (D122A, Y123R, Y123A, K124A, K124D, K124R, R125A, R125D) or neither PD-1 or B7-1 (L53D, L53R, I115D, I116R, G119R, G120A, G120R, A121D, A121R, D122R). The affected residues were mapped onto the crystal structure of the PD-1:PD-L1 complex and shows the overlapping but distinct PD-L1 surfaces responsible for PD-1 and B7-1 binding. These results validated those obtained by the initial cell microarray experiments and provided a more quantitative assessment of PD-1 and B7-1 binding especially for those mutants that showed significantly reduced but not obliterated binding to PD-1 or B7-1 (Table 1 (FIG. 10), Table 2 (FIG. 11)). For example, in the cell microarray experiments levels of PD-1 and B7-1 binding to the V54D and Q66D PD-L1 mutants were similar whereas in the context of the microbead FACS assay these same two mutants showed wild-type levels of PD-1 binding and significantly reduced B7-1 binding.

[00255] Sequence alignment analysis of PD-L1 and PD-L2 also hints at the relative importance of these residues to PD-1 and B7-1 binding. In general the PD-1 binding specific residues are highly conserved in both PD-L1 and PD-L2, which is expected as both ligands bind to PD-1. However many of the identified B7-1-specific binding residues are only highly conserved in PD-L1 not PD-L2, which is logical as PD-L2 does not bind B7-1. This supports the data highlighting V54 and Y56 as especially critical for B7-1 binding.

Biological activity of PD-L1 mutants in a T-cell proliferation assay

[00256] High-throughput transient transfection of HEK 293 cells in 24-well suspension tissue culture plates was optimized for the production of recombinant secreted Fc-fusion proteins in amounts consistent with screening. Utilizing this method, Fc-fusion proteins for a subset of the PD-L1 mutants with altered binding characteristics were purified. Following small-scale nickel purification of the PD-L1 proteins analytical gel filtration demonstrated that the selected mutants behaved similar to wild type protein. Prior to activity testing in T cell proliferation

studies, the quality of each mutant protein was evaluated by FACS analysis for binding to HEK cells expressing surface-resident PD-1 or B7-1 (GFP fusions) to confirm that the soluble reagents behaved as expected (i.e. PD-L1_Y56A_Fc binds to cells expressing PD-1, but not to those expressing B7-1).

[00257] To characterize the biological activity of the functionally dissected PD-L1 mutants, an *in vitro* T-cell activation assay was used. This widely employed assay uses plate-bound anti-CD3 antibody to simulate activation of T-cells via the T-cell receptor. Anti-CD3 was co-plated in the presence of either IgG control, wild type PD-L1 or PD-L1 mutants and measured activation of CSFE labeled primary $CD4^+$ mouse T-cells. In the context of anti-CD3-mediated $CD4^+$ T-cell activation, wild type PD-L1 inhibits activation compared to isotype control (Fig. 8D) PD-L1 mutants with reduced levels of PD-1 binding showed a significantly reduced ability to inhibit T-cell activation. In contrast, PD-L1 mutants with reduced B7-1 activity elicited effects comparable to wild type PD-L1. These data suggest that under the *in vitro* experimental system employed, PD-L1-induced inhibition of $CD4^+$ T-cell activation occurs primarily via its interaction with PD-1. These data demonstrate the feasibility of generating mutants with specific biological activities that can aid in defining the distinct contributions of the PD-L1:PD-1 and PD-L1:B7-1 interactions to mammalian immunity.

PD-1 can compete with B7-1 for binding to PD-L1.

[00258] The mutagenesis data showed that the binding surfaces on PD-L1 that bind PD-1 and B7-1 overlap but are distinct suggesting that PD-1 and B7-1 should compete for binding to PD-L1. This hypothesis was tested using a cell based FACS competition assay utilizing B7-1 and PD-1 Fc-fusion protein that we purified using two different Fc fusion isotypes. With these reagents, increasing concentrations of PD-1 (mIgG2a) were titrated, while the loss of B7-1 (hIgG1) that was bound to HEK cells expressing wild-type PD-L1 was selectively monitored using an anti-human Alexa 488 secondary antibody (Fig 9A). The result shows that PD-1 binding efficiently displaces B7-1 from cells expressing PD-L1, which supports the finding, based on the PD-L1 mutagenesis,that the binding sites overlap.

[00259] **FIG. 7A-7C: Screening PD-L1 mutants using a high-throughput microbead binding FACS assay. A)** Schematic of the microbead FACS binding assay. **B)** Representative control microbead experiment. Cells expressing either mCherry alone (-control) or PD-L1 mCherry were challenged with microbeads conjugated with control Fc, PD-1 Fc or B7-1 Fc fusion protein. The FACS data was gated for all live cells and shows binding of both PD-1 and B7-1 coated microbeads (upper right quadrant) to cells expressing wild type PD-L1. **C)** FACS microbead binding data for a panel of 54 PD-L1 mutants. Data shows the fraction of mCherry positive cells (PD-L1 expressing) bound to microbeads coated in either PD-1 (Blue) or B7-1

(Red) with binding normalized to wild type. PD-1 and B7-1 binding was done in parallel triplicate experiments with error bars representing the standard deviation.

[00260] FIG. 8A-8D: Characterization of PD-L1 mutants with altered binding to PD-1 or B7-1. A) The crystal structure of the PD-1: PD-L1 complex (PDB: 3SBW) showing just the PD-L1 IgC and IgV domains. The IgV domain was enlarged and residue that when mutated resulted in altered binding are labeled and colored accordingly, green = PD-1 binding affected, red = B7-1 binding affected, gray = both PD-1 and B7-1 binding affected. B) Data obtained from FACS titration experiments in which cells expressing either wild type PD-L1 or a mutant were titrated with increasing concentrations of recombinant PD-1 or B7-1 Fc-fusion protein. Binding was detected using an anti-mouse Alexa 488 secondary antibody. Data points show the average of three independent experiments with error bars showing the standard deviation. Curves show the fit of the data to a single-site binding model. **C)** Table of EC_{50} and B_{max} values obtained from the FACS titration experiments in B. Stars denote those titrations for which binding was so low (baseline) that the data could not be fit. D) Data shows the fraction of CSFE labeled $CD4^+$ T-cells isolated from C57BL/6 mice activated after 4 days of stimulation with anti-CD3 in the presence of isotype control, wild type or mutant PD-L1 Fc-fusion protein. Activation was normalized to isotype control and represents three independent experiments.

[00261] FIG. 9A-9B: PD-1 competes with B7-1 for binding to PD-L1. A) Cartoon depiction of the competition assay. Briefly, HEK 293 cells transiently transfected with PD-L1 mCherry were incubated with mB7-1 hIgG1 protein in the absence or presence of increasing concentrations of mPD-1 mIgG2a. The amount of mB7-1 hIgG1 bound to cells was then monitored by FACS analysis using an anti-human Alexa 488 antibody. **B)** Heat map showing results from one representative experiment. In the presence of control mIgG2a no loss of mB7-1 hIgG1 binding was observed. The graph shows the average and standard deviation for data from three independent experiments. This data was fit using a one-site competition model equation in the software Prism and the calculated EC_{50} was 8.3 ± 1.5 nM.

Example 2: *In vivo* activity of a PD-L1/synTac

[00262] The NY8.3 TCR transgenic NOD model of type 1 diabetes (NOD 8.3) develops aggressive T cell autoimmunity directed against the pancreatic beta cell antigen $Igrp_{206-214}$ in the context of the MHC class I allele $H\text{-}2K^d$. NOD 8.3 mice have circulating transgenic (Igrp-specific) T cells at high frequency. A PD-L1/synTac bearing $Igrp_{206-214}/H\text{-}2K^d$ and PD-L1 (G119R variant) was administered to NOD 8.3 mice to determine the effect on the frequency of pathogenic transgenic T cells in the spleen. As shown in FIG. 12, a PD-L1(G119R)/synTac effects a dose dependent depletion of $Igrp_{206—214}/H\text{-}2K^d$ specific T cells, but not non-specific T cells.

[00263] 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 PD-L1 immunomodulatory polypeptide comprising an amino acid sequence having at least 85% amino acid sequence identity to the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, or to the PD-L1 amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2,

wherein the variant PD-L1 immunomodulatory polypeptide has one or more amino acid substitutions relative to the PD-L1 amino acid sequence depicted in FIG. 2A or to the PD-L1 amino acid sequence set forth in SEQ ID NO:1; and

wherein the variant PD-L1 immunomodulatory polypeptide exhibits:

a) reduced binding affinity to a PD1 polypeptide having an amino acid sequence depicted in FIG. 3A or 3B, compared to the binding affinity of the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, or compared to the binding affinity of the PD-L1 amino acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:2, for the PD1 polypeptide; and/or

b) reduced binding affinity to a B7-1 polypeptide having an amino acid sequence depicted in FIG. 3C or 3D, compared to the binding affinity of the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, or compared to the binding affinity of the PD-L1 amino acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:2, for the B7-1 polypeptide.

2. The variant immunomodulatory polypeptide of claim 1, wherein the polypeptide comprises a substitution of amino acid D26, T37, V54, Q66, or E72, based on the amino acid numbering set forth in FIG. 2A, or wherein the polypeptide comprises a substitution of amino acid D26, T37, I54, Q66, or E72, based on the amino acid numbering set forth in FIG. 2B.

3. The variant immunomodulatory polypeptide of claim 1 or claim 2, wherein the variant immunomodulatory polypeptide exhibits from less than 10% to less than 75% of binding affinity exhibited by a PD-L1 polypeptide comprising the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2, for the PD1 polypeptide.

4. The variant immunomodulatory polypeptide of any one of claims 1-3, wherein the variant immunomodulatory polypeptide exhibits from less than 10% to less than 75% of binding affinity exhibited by a PD-L1 polypeptide comprising the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2, for the B7-1 polypeptide.

5. The variant immunomodulatory polypeptide of any one of claims 1, 2, or 4, wherein the variant immunomodulatory polypeptide exhibits from 75% to greater than 95% of binding affinity exhibited by a PD-L1 polypeptide comprising the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2, for the PD1 polypeptide.

6. The variant immunomodulatory polypeptide of any one of claims 1-3 or 5, wherein the variant immunomodulatory polypeptide exhibits from 75% to greater than 95% of binding affinity exhibited by a PD-L1 polypeptide comprising the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, or as set forth in SEQ ID NO:1 or SEQ ID NO:2, for the B7-1 polypeptide.

7. 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 at least one of 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 comprises an amino acid sequence having at least 85% amino acid sequence identity to the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, to the PD-L1 amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2,

wherein the variant PD-L1 immunomodulatory polypeptide has one or more amino acid substitutions relative to the PD-L1 amino acid sequence depicted in FIG. 2A or to the PD-L1 amino acid sequence set forth in SEQ ID NO:1; and

wherein the variant PD-L1 immunomodulatory polypeptide exhibits:

a) reduced binding affinity to a PD1 polypeptide having an amino acid sequence depicted in FIG. 3A or 3B, compared to the binding affinity of the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, or compared to the binding affinity of the PD-L1 amino acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:2, for the PD1 polypeptide; and/or

b) reduced binding affinity to a B7-1 polypeptide having an amino acid sequence depicted in FIG. 3C or 3D, compared to the binding affinity of the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, or compared to the binding affinity of the PD-L1 amino acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:2, for the B7-1 polypeptide.

8. The multimeric polypeptide of claim 7, wherein the multimeric polypeptide exhibits reduced binding affinity to a PD1 polypeptide having an amino acid sequence depicted in FIG. 3A or 3B, compared to the binding affinity of a control multimeric polypeptide comprising an immunomodulatory domain comprising the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, or compared to the binding affinity of a control multimeric polypeptide comprising an immunomodulatory domain comprising the PD-L1 amino acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:2, for the PD1 polypeptide.

9. The multimeric polypeptide of claim 7, wherein the multimeric polypeptide comprises:

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

i) the epitope;

ii) the first MHC polypeptide; and

iii) the immunomodulatory domain; and

b) a second polypeptide comprising, in order from N-terminus to C-terminus:

i) the second MHC polypeptide; and

ii) the Ig Fc polypeptide.

10. The multimeric polypeptide of claim 7, wherein the multimeric polypeptide comprises:

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

i) the epitope; and

ii) the first MHC polypeptide; and

b) a second polypeptide comprising, in order from N-terminus to C-terminus:

i) the immunomodulatory domain;

iii) the second MHC polypeptide; and

ii) the Ig Fc polypeptide.

11. The multimeric polypeptide of claim 7, wherein the multimeric polypeptide comprises:

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

i) the epitope; and

ii) the first MHC polypeptide; and

b) a second polypeptide comprising, in order from N-terminus to C-terminus:

i) the second MHC polypeptide; and

ii) the Ig Fc polypeptide; and

iii) the immunomodulatory domain.

12. The multimeric polypeptide of claim 7, wherein the multimeric polypeptide comprises:

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

i) the epitope; and

ii) the first MHC polypeptide; and

b) a second polypeptide comprising, in order from N-terminus to C-terminus:

i) the second MHC polypeptide; and

ii) the immunomodulatory domain.

13. The multimeric polypeptide of claim 7, wherein the multimeric polypeptide comprises:

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

i) the epitope; and

ii) the first MHC polypeptide; and

b) a second polypeptide comprising, in order from N-terminus to C-terminus:

i) the immunomodulatory domain; and

ii) the second MHC polypeptide.

14. The multimeric polypeptide of claim 7, wherein the multimeric polypeptide comprises:

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

i) the epitope;

ii) the first MHC polypeptide; and

iii) the immunomodulatory domain; and

b) a second polypeptide comprising, in order from N-terminus to C-terminus:

i) the second MHC polypeptide.

15. The multimeric polypeptide of claim 7, 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.

16. The multimeric polypeptide of any one of claims 7-15, wherein the first MHC polypeptide is a β2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide.

17. The multimeric polypeptide of claim 16, 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.

18. The multimeric polypeptide of claim 16, wherein the MHC class I heavy chain polypeptide is an HLA-A, an HLA-B, or an HLA-C heavy chain.

19. The multimeric polypeptide of claim 18, 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.

20. The multimeric polypeptide of any one of claims 7-16, 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.

21. The multimeric polypeptide of any one of claims 7-20, wherein the epitope is a T-cell epitope.

21. The multimeric polypeptide of any one of claims 7-11 and 15-18, 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.

22. The multimeric polypeptide of claim 21, 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.

23. The multimeric polypeptide of any one of claims 7-22, wherein the first polypeptide and the second polypeptide are non-covalently associated.

24. The multimeric polypeptide of any one of claims 7-22, wherein the first polypeptide and the second polypeptide are covalently linked.

25. The multimeric polypeptide of claim 24, wherein the covalent linkage is via a disulfide bond.

26. The multimeric polypeptide of claim 25, 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.

27. The multimeric polypeptide of any one of claims 7-14, comprising a first linker interposed between the epitope and the first MHC polypeptide.

28. The multimeric polypeptide of any one of claims 7-14, wherein the variant PD-L1 immunomodulatory polypeptide comprises a substitution of an amino acid selected from D26, T37, I54, Y56, Q66, E72, I115, G119, or G120, based on the amino acid numbering of the PD-L1 amino acid sequence depicted in FIG. 2B.

29. The multimeric polypeptide of any one of claims 7-28, comprising 2 or more variant PD-L1 immunomodulatory polypeptides.

30. The multimeric polypeptide of claim 29, wherein the 2 or more immunomodulatory polypeptides are in tandem.

31. The multimeric polypeptide of any one of claims 7-28, wherein the immunomodulatory polypeptide is an immunomodulatory polypeptide according to any one of claims 1-6.

32. The multimeric polypeptide of any one of claims 29-31, 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.

33. The multimeric polypeptide of claim 32, wherein the third polypeptide is covalently linked to the first polypeptide.

34. The multimeric polypeptide of any one of claims 7-13 and 15-33, 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.

35. 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 1-6; 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 1-6.

36. The nucleic acid of claim 35, wherein the first MHC polypeptide is a β2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide.

37. The nucleic acid of claim 36, 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.

38. The nucleic acid of claim 35, wherein the MHC class I heavy chain polypeptide is an HLA-A, HLA-B, or HLA-C heavy chain.

39. The nucleic acid of claim 38, 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.

40. The nucleic acid of claim 35, 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.

41. The nucleic acid of claim 35, wherein the epitope is a T-cell epitope.

42. The nucleic acid of claim 35, 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.

43. The nucleic acid of claim 42, 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.

44. The nucleic acid of claim 35, wherein the variant PD-L1 polypeptide comprises a substitution of amino acid D26, T37, V54, Q66, or E72, based on the amino acid numbering set forth in FIG. 2A, or wherein the wherein the polypeptide comprises a substitution of amino acid D26, T37, I54, Q66, or E72, based on the amino acid numbering set forth in FIG. 2B.

45. The nucleic acid of claim 35, wherein the multimeric polypeptide comprises two or more variant PD-L1 immunomodulatory polypeptides.

46. The nucleic acid of claim 35, wherein the proteolytically cleavable linker or ribosome skipping signal comprises an amino acid sequence selected from:

a) LEVLFQGP (SEQ ID NO:34);

b) ENLYTQS (SEQ ID NO:35);

c) a furin cleavage site;

d) LVPR (SEQ ID NO:37);

e) GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:38);

f) GSGEGRGSLLTCGDVEENPGP (SEQ ID NO:39);

g) GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO:40); and

h) GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:41).

47. The nucleic acid of claim 35, 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.

48. The nucleic acid of claim 47, wherein the first leader peptide and the second leader peptide is a β2-M leader peptide.

49. The nucleic acid of claim 35, wherein the nucleotide sequence is operably linked to a transcriptional control element.

50. The nucleic acid of claim 49, wherein the transcriptional control element is a promoter that is functional in a eukaryotic cell.

51. The nucleic acid of claim 35, 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.

52. A recombinant expression vector comprising the nucleic acid of any one of claims 35-51.

53. The recombinant expression vector of claim 52, wherein the vector is a viral vector or a non-viral vector.

54. A host cell genetically modified with the recombinant expression vector of claim 52.

55. The host cell of claim 54, wherein the host cell is *in vitro*.

56. The host cell of claim 54, wherein the host cell is genetically modified such that the cell does not produce an endogenous MHC β2-microglobulin polypeptide.

57. The host cell of claim 54, wherein the host cell is a T lymphocyte.

58. 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 1-6; 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.

59. 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 1-6;

ii) a second MHC polypeptide; and

iii) an Ig Fc polypeptide.

60. The composition of claim 58 or 59, wherein the first and/or the second nucleic acid is present in a recombinant expression vector.

61. A host cell genetically modified with the composition of any one of claims 58-60.

62. A method of producing the multimeric polypeptide of any one of claims 7-34, the method comprising:

a) culturing the host cell of any one of claims 54-57 and 61 *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.

63. The method of claim 62, 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.

64. The method of claim 62, comprising eluting the immobilized multimeric polypeptide.

65. 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 7-34, wherein said contacting selectively modulates the activity of the epitope-specific T cell.

66. The method of claim 65, wherein the immunomodulatory polypeptide is an inhibiting polypeptide, and wherein the multimeric polypeptide inhibits the epitope-specific T cell.

67. The method of claim 65, wherein said contacting is *in vitro*.

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

69. The method of claim 65, wherein said contacting is *ex vivo*.

70. 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 7-34 effective to selectively modulate the activity of an epitope-specific T cell in an individual.

71. The method of claim 70, wherein the immunomodulatory polypeptide is an inhibitory polypeptide, and wherein the multimeric polypeptide inhibits activity of the epitope-specific T cell.

72. The method of claim 71, wherein the epitope is a self-epitope, and wherein said administering selectively inhibits the activity of a T cell specific for the self-epitope.

73. The method of claim 71, wherein the epitope is an epitope present on an allograft, wherein said administering selectively inhibits the activity of a T cell specific for the allograft.

74. A method of treating an autoimmune disorder in an individual, the method comprising administering to the individual an effective amount of the multimeric polypeptide of any one of claims 7-34 effective to selectively inhibits the activity of a T-cell specific for a self epitope in the individual, thereby treating the autoimmune disorder.

75. The method of claim 74, wherein the autoimmune disease is multiple sclerosis, systemic lupus erythematosus, or autoimmune arthritis.

76. A method of inhibiting allograft rejection in an individual, the method comprising administering to the individual an effective amount of the multimeric polypeptide of any one of claims 7-34 effective to selectively inhibit the activity of a T-cell specific for an epitope on an allograft present in the individual, thereby inhibiting allograft rejection.

77. The method of claim 76, wherein the allograft is a kidney, a lung, skin, a liver, bone, cartilage, or a heart.

78. The method of any one of claims 70-77, wherein said administering is subcutaneous.

79. The method of any one of claims 70-77, wherein said administering is intravenous.

80. The method of any one of claims 70-77, wherein said administering is intramuscular.

81. The method of any one of claims 70-77, wherein said administering is systemic.

82. The method of any one of claims 70-77, wherein said administering is distal to a treatment site.

83. The method of any one of claims 70-77, wherein said administering is local.

84. The method of any one of claims 70-77, wherein said administering is at or near a treatment site.

85. A composition comprising:

a) the multimeric polypeptide of any one of claims 7-34; and

b) a pharmaceutically acceptable excipient.

86. A composition comprising:

a) the nucleic acid of any one of claims 35-51 or the recombinant expression vector of claim 52 or 53; and

b) a pharmaceutically acceptable excipient.

FIG. 1C

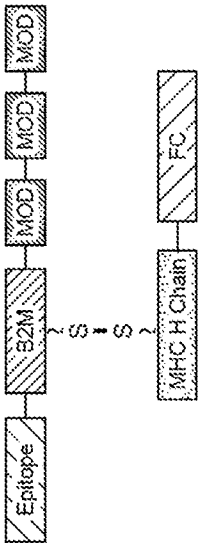


FIG. 1A

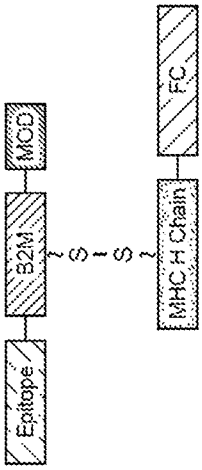


FIG. 1D

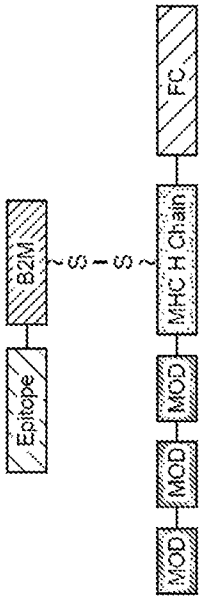


FIG. 1B

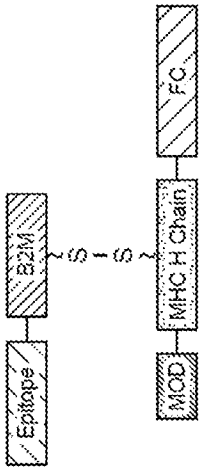


FIG. 2A
Mus musculus PD-L1
Amino acids 1-18 = signal
Amino acids 240-260 = transmembrane

```
        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:1)
```

FIG. 2B
Homo sapiens PD-L1

```
        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 taelvipgni
lnvsikiclt
      241 lspst (SEQ ID NO:2)
```

FIG. 2C

```
                    10        20        30        40        50
60
Mouse
MRIFAGIIFTACCHLLRA**FTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYWEKE**  60
            MRIFA  IF    HLL AFT+T PKDLYVVEYGSN+T+EC+FPVE++LDL
AL+VYWE E
Human
MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEME  60

Mouse
**DEQVIQFVAGEEDLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCCIISYGG**  120
            D+ +IQFV GEEDLK QHS++R RA L KDQL  GNAALQITDVKLQDAGVY
C+ISYGG
Human
DKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGG  120

Mouse       **ADYKRITLKVNAPYRKINQRI-**
**SVDPATSEHELICQAEGYPEAEVIWTNSDHQPVSGKRS**  179
            ADYKRIT+KVNAPY KINQRI  VDP TSEHEL CQAEGYP+AEVIWT+SDHQ
+SGK +
Human
ADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTT  180

Mouse
VTTSRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAELIIPELPATHPPQNR  237
             T S+ E  L NVTS+LR+N T N++FYCTF R  P +NHTAEL+IP
Human
TTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPGNILNVSIKI- 237
```

FIG. 2D

1 mrifavfifm tywhllnaft vtvpk**a**lyvv eygsnmtiec kfpvekqldl
aalivyweme
61 dkniiqfvhg eedlkvqhss yrqrarllkd qlslgnaalq itdvklqdag
vyrcmisygg
121 adykritvkv napynkinqr ilvvdpvtse heltcqaegy pkaeviwtss
dhqvlsgktt
181 ttnskreekl fnvtstlrin tttneifyct frrldpeenh taelvipgni
lnvsikiclt
241 lspst (SEQ ID NO:46)

FIG. 2E

FT VTVPK**A**LYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG
EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV
NAPYNKINQR ILVVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL
FNVTSTLRIN TTTNEIFYCT FRRLDPEENH TAELVIPGNI LNVSIKI (SEQ ID NO:47)

FIG. 2F

1 mrifavfifm tywhllnaft vtvpk**r**lyvv eygsnmtiec kfpvekqldl
aalivyweme
61 dkniiqfvhg eedlkvqhss yrqrarllkd qlslgnaalq itdvklqdag
vyrcmisygg
121 adykritvkv napynkinqr ilvvdpvtse heltcqaegy pkaeviwtss
dhqvlsgktt
181 ttnskreekl fnvtstlrin tttneifyct frrldpeenh taelvipgni
lnvsikiclt
241 lspst (SEQ ID NO:48)

FIG. 2G

FT VTVPK**R**LYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG
EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV
NAPYNKINQR ILVVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL
FNVTSTLRIN TTTNEIFYCT FRRLDPEENH TAELVIPGNI LNVSIKI (SEQ ID NO:49)

FIG. 2H

1 mrifavfifm tywhllnaft vtvpkdlyvv eygsnmtiec kfpvekqldl
aal**d**vyweme
61 dkniiqfvhg eedlkvqhss yrqrarllkd qlslgnaalq itdvklqdag
vyrcmisygg
121 adykritvkv napynkinqr ilvvdpvtse heltcqaegy pkaeviwtss
dhqvlsgktt
181 ttnskreekl fnvtstlrin tttneifyct frrldpeenh taelvipgni
lnvsikiclt
241 lspst (SEQ ID NO:50)

FIG. 2I

FT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL AAL**D**VYWEME DKNIIQFVHG
EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV
NAPYNKINQR ILVVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL
FNVTSTLRIN TTTNEIFYCT FRRLDPEENH TAELVIPGNI LNVSIKI (SEQ ID NO:51)

FIG. 2J

1 mrifavfifm tywhllnaft vtvpkdlyvv eygsnmtiec kfpvekqldl
aalivyweme
61 dkniid**f**vhg eedlkvqhss yrqrarllkd qlslgnaalq itdvklqdag
vyrcmisygg
121 adykritvkv napynkinqr ilvvdpvtse heltcqaegy pkaeviwtss
dhqvlsgktt
181 ttnskreekl fnvtstlrin tttneifyct frrldpeenh taelvipgni
lnvsikiclt
241 lspst (SEQ ID NO:52)

FIG. 2K

FT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNII**D**FVHG
EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV
NAPYNKINQR ILVVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL
FNVTSTLRIN TTTNEIFYCT FRRLDPEENH TAELVIPGNI LNVSIKI (SEQ ID NO:53)

FIG. 2L

```
        1 mrifavfifm tywhllnaft vtvpkdlyvv eygsnmtiec kfpvekqldl
aalivyweme
       61 dkniiqfvhg erdlkvqhss yrqrarllkd qlslgnaalq itdvklqdag
vyrcmisygg
      121 adykritvkv napynkinqr ilvvdpvtse heltcqaegy pkaeviwtss
dhqvlsgktt
      181 ttnskreekl fnvtstlrin tttneifyct frrldpeenh taelvipgni
lnvsikiclt
      241 lspst (SEQ ID NO:54)
```

FIG. 2M

FT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG
ERDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV
NAPYNKINQR ILVVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL
FNVTSTLRIN TTTNEIFYCT FRRLDPEENH TAELVIPGNI LNVSIKI (SEQ ID NO:55)

FIG. 3A
Mus musculus PD-1 (SEQ ID NO:56)

```
        1 mwvrqvpwsf twavlqlswq sgwllevpng pwrsltfypa wltvsegana
tftcslsnws
       61 edlmlnwnrl spsnqtekqa afcnglsqpv qdarfqiiql pnrhdfhmni
ldtrrndsgi
      121 ylcgaislhp kakieespga elvvterile tstrypspsp kpegrfqgmv
igimsalvgi
      181 pvllllawal avfcstsmse argagskddt lkeepsaapv psvayeeldf
qgrektpelp
      241 tacvhteyat ivfteglgas amgrrgsadg lqgprppprhe dghcswpl
```

FIG. 3B
Homo sapiens PD-1 (SEQ ID NO:57)

```
        1 mqipqapwpv vwavlqlgwr pgwfldspdr pwnpptfspa llvvtegdna
tftcsfsnts
       61 esfvlnwyrm spsnqtdkla afpedrsqpg qdcrfrvtql pngrdfhmsv
vrarrndsgt
      121 ylcgaislap kaqikeslra elrvterrae vptahpspsp rpagqfqtlv
vgvvggllgs
      181 lvllvwvlav icsraargti garrtgqplk edpsavpvfs vdygeldfqw
rektpeppvp
      241 cvpeqteyat ivfpsgmgts sparrgsadg prsaqplrpe dghcswpl
```

FIG. 3C
Mus musculus B7-1 (SEQ ID NO:58)

1 **macncqlmqd tpllkfpcpr lillfvllir lsqvss**dvde qlsksvkdkv
llpcrynsph
61 edesedriyw qkhdkvvlsv iagklkvwpe yknrtlydnt tysliilglv
lsdrgtyscv
121 vqkkergtye vkhlalvkls ikadfstpni tesgnpsadt kritcfasgg
fpkprfswle
181 ngrelpgint tisqdpesel ytissqldfn ttrnhtikcl ikygdahvse
dftwekpped
241 ppdskntlvl fgagfgavit vvvivviikc fckhrscfrr neasretnns
ltfgpeeala
301 eqtvfl

FIG. 3D
Homo sapiens B7-1 (CD80) (SEQ ID NO:59)

1 **mghtrrqgts pskcpylnff qllvlaglsh fcsg**vihvtk evkevatlsc
ghnvsveela
61 qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd
egtyecvvlk
121 yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe
phlswlenge
181 elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn
wnttkqehfp
241 dnllpswait lisvngifvi ccltycfapr crerrrnerl rresvrpv

Figure 4A
GenBank 3S7G_A
Homo sapiens **IgG1** Fc (SEQ ID NO:60)
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:61)
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:62)
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:63)
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:64)
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:65)
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:66)
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:67)
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:68)

```
        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:69)

```
        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:70)

```
        1 mrvmaprall lllsgglalt 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
qepltlswep
      301 ssqptipimg ivaglavlvv lavlgavvta mmcrrkssgg kggscsqaac
snsaqgsdes
      361 litcka
```

FIG. 6

```
NP_004039.1          MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLL 60
NP_001090066.1       MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLL 60
NP_001040602.1       MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPPENGKPNFLNCYVSGFHPSDIEVDLL 60
NP_776318.1          MARFVALVLLGLLSLSGLDAIQRPPKIQVYSRHPPEDGKPNYLNCYVYGFHPPQIEIDLL 60
NP_033865.2          MARSVTLVFLVLVSLTGLYAIQKTPQIQVYSRHPPENGKPNILNCYVTQFHPPHIEIQML 60
                     *:** *::*..* *:***:** ****:.*:**********.*:**.* ***** ***..*****:**

NP_004039.1          KNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM 119
NP_001090066.1       KNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM 119
NP_001040602.1       KNGEKMGKVEHSDLSFSKDWSFYLLYYTEFTPNEKDEYACRVNHVTLSGPRTVKWDRDM 119
NP_776318.1          KNGEKI-KSEQSDLSFSKDWSFYLLSHAEFTENSKDQYSCRVKHVTLEQPRIVKWDRDL 118
NP_033865.2          KNGKKIPKVEMSDMSFSKDWSFYILAHTEFTPTETDTYACRVKHASMAEPKTVYWDRDM 119
                     ***:::: * * **:**********:* ::*****...* *:****:*.::  :: * *****:
```

FIG. 7

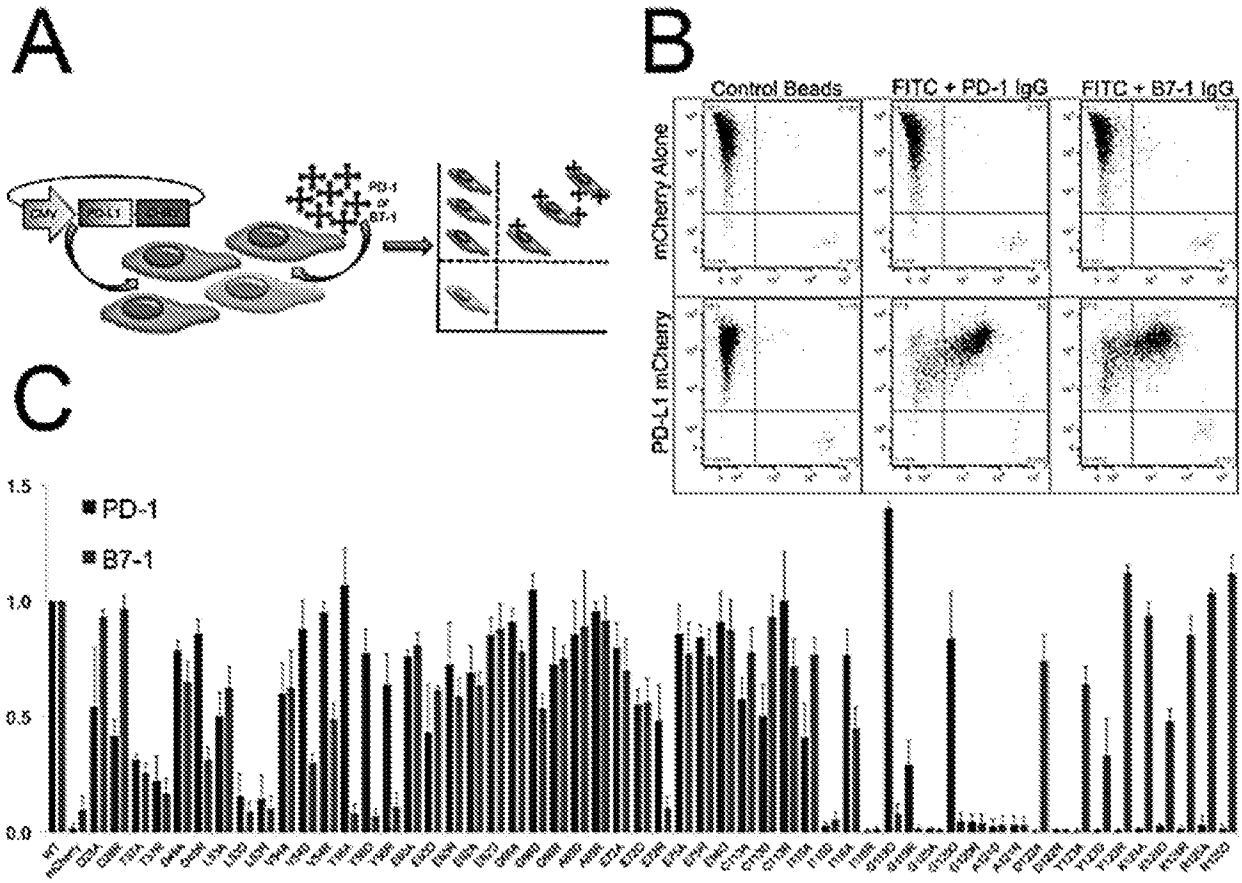
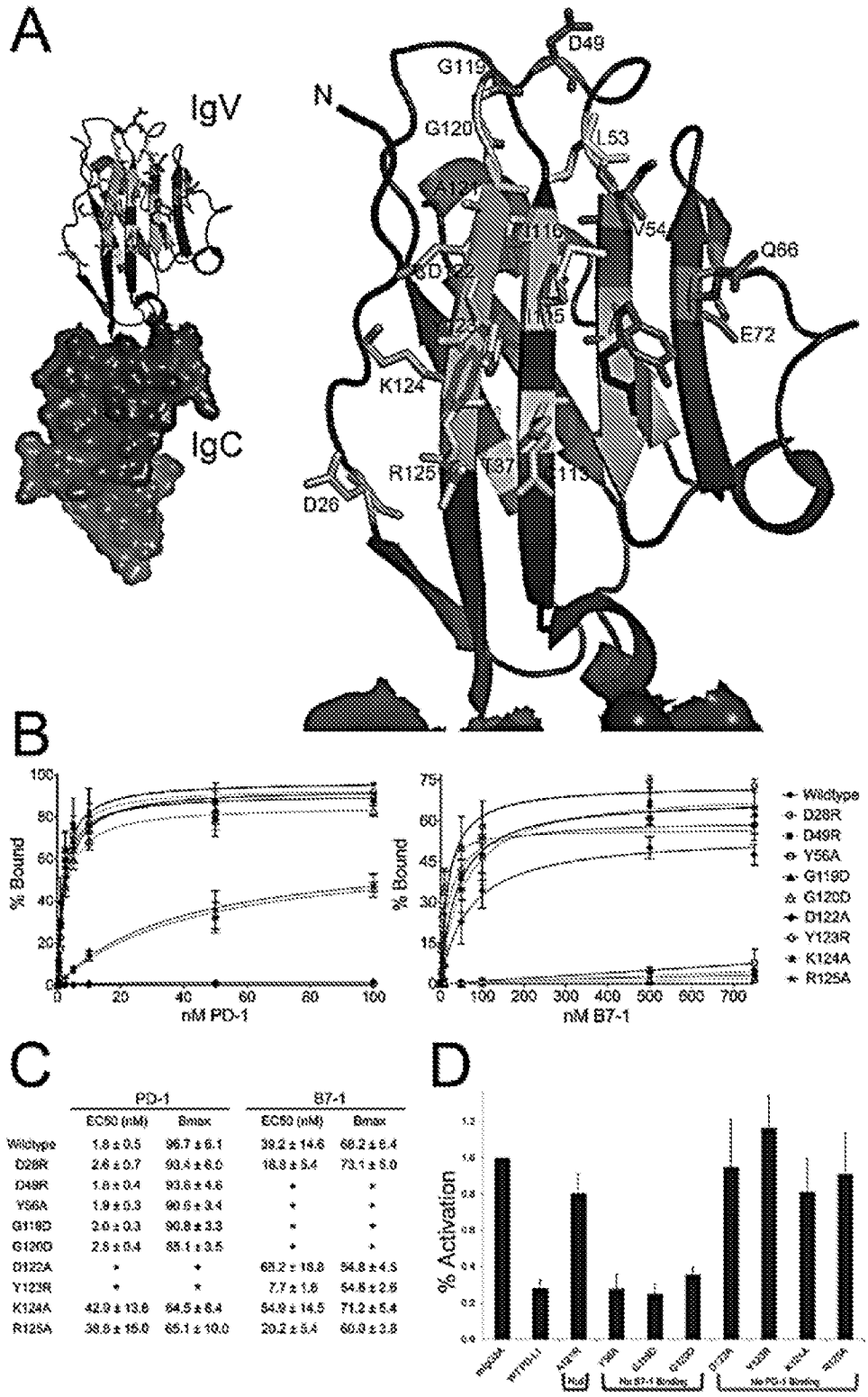

A
B
Control Beads
FITC + PD-1 IgG
FITC + B7-1 IgG
mCherry Alone
PD-L1 mCherry
C
PD-1
B7-1
1.5
1.0
0.5
0.0

FIG. 8



	PD-1		B7-1	
	EC50 (nM)	Bmax	EC50 (nM)	Bmax
Wildtype	1.8 ± 0.5	96.7 ± 6.1	39.2 ± 14.6	66.2 ± 6.4
D28R	2.6 ± 0.7	93.4 ± 6.0	16.8 ± 5.4	73.1 ± 5.0
D49R	1.6 ± 0.4	93.6 ± 4.6	*	*
Y56A	1.9 ± 0.3	90.6 ± 3.4	*	*
G119D	2.0 ± 0.3	90.8 ± 3.3	*	*
G120D	2.5 ± 0.4	85.1 ± 3.5	*	*
D122A	*	*	68.2 ± 18.8	94.8 ± 4.5
Y123R	*	*	7.7 ± 1.6	54.8 ± 2.6
K124A	42.9 ± 13.6	64.5 ± 6.4	54.8 ± 14.5	71.2 ± 5.4
R125A	38.8 ± 15.0	65.1 ± 10.0	20.2 ± 5.4	60.9 ± 3.8

FIG. 9

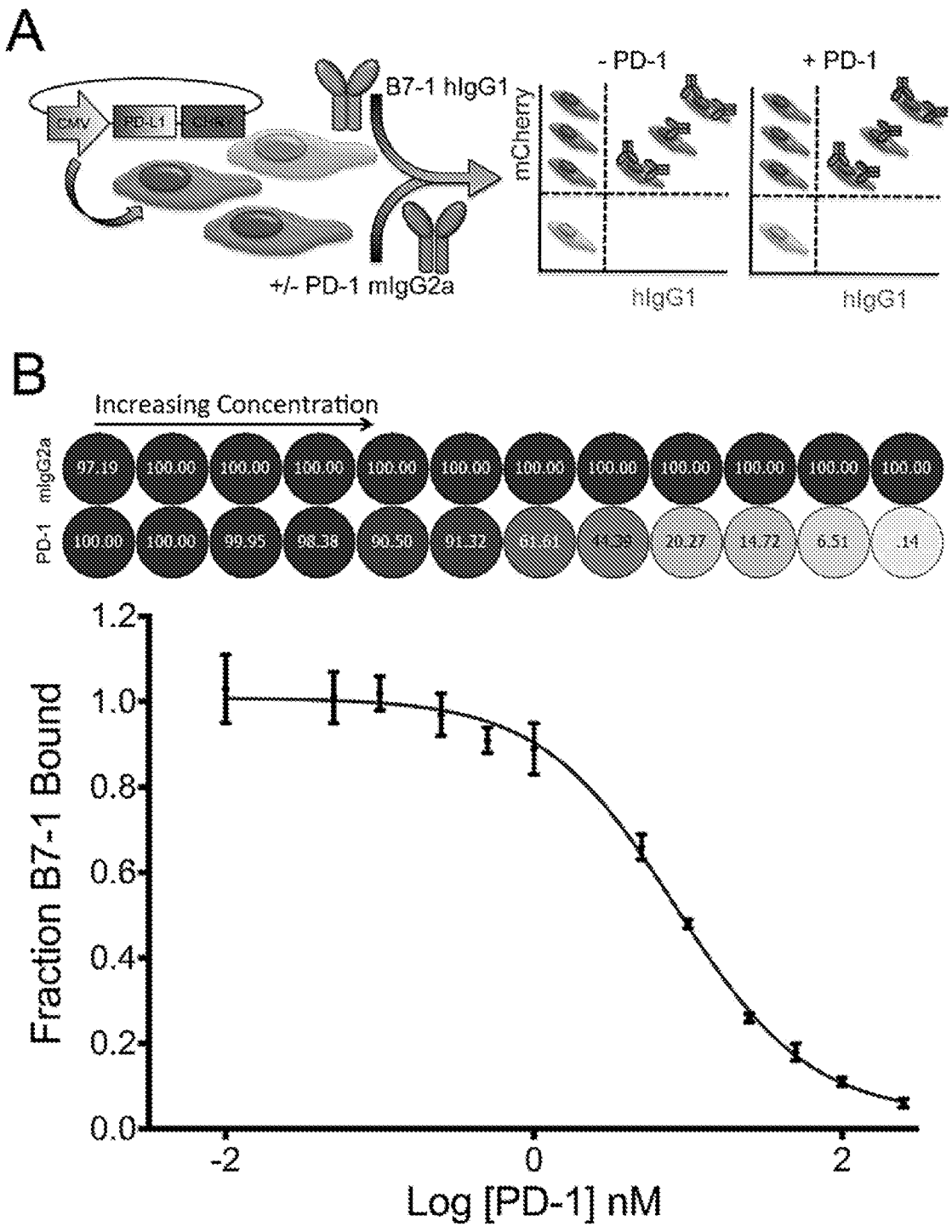

A
B7-1 hIgG1
+/- PD-1 mIgG2a
- PD-1
+ PD-1
mCherry
hIgG1
hIgG1
B
Increasing Concentration
mIgG2a
PD-1
97.19
100.00
100.00
100.00
100.00
100.00
100.00
100.00
100.00
100.00
100.00
100.00
100.00
100.00
99.55
98.38
90.50
91.32
83.61
71.23
20.27
14.72
6.51
.14
Fraction B7-1 Bound
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-2
0
2
Log [PD-1] nM

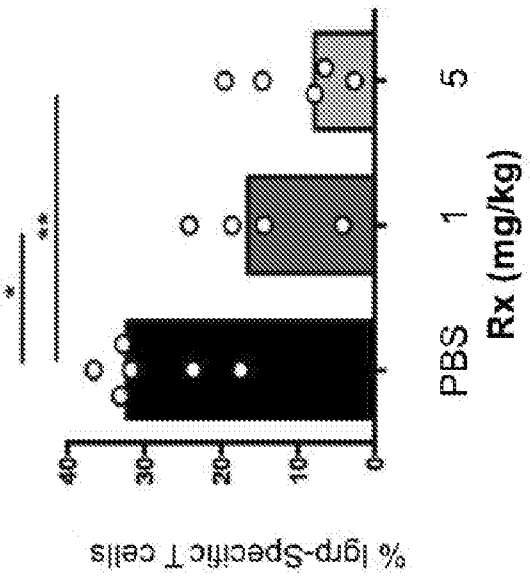
FIG. 10

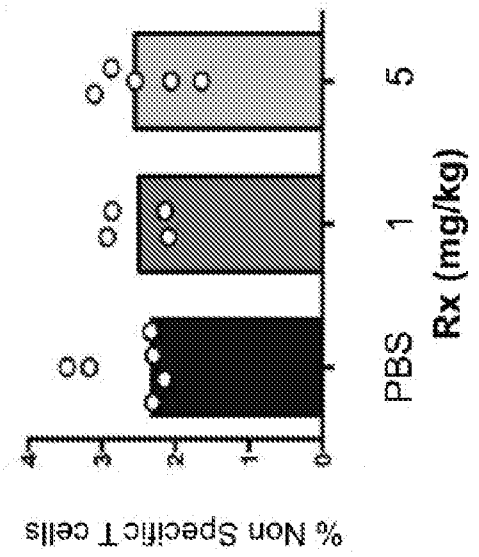
	PD-1	B7-1
WT	+	+
mCherry	-	-
D49A	+	+
D49R	+	R
L53R	-	-
V54D	R	R
V54A	+	+
Y56A	+	-
Y56D	+	-
Q66A	+	+
Q66D	+	R
E72A	+	+
E72R	+	-
G119D	+	-
G119R	-	-
G120D	+	-
A121R	-	-
D122A	-	+
Y123A	-	+
Y123R	-	+
K124A	-	+
K124D	-	+
R125A	R	+
R125D	-	+

FIG. 11

	PD-1	B7-1
WT	1.00	1.00
mCherry	0.02 ± 0.02	0.10 ± 0.06
D26A	0.54 ± 0.26	0.93 ± 0.03
D26R	0.42 ± 0.07	0.96 ± 0.06
T37A	0.31 ± 0.03	0.26 ± 0.04
T37R	0.22 ± 0.11	0.17 ± 0.07
D49R	0.86 ± 0.06	0.31 ± 0.06
L53D	0.16 ± 0.10	0.09 ± 0.04
L53R	0.14 ± 0.10	0.10 ± 0.05
V54D	0.88 ± 0.12	0.30 ± 0.04
V54R	0.95 ± 0.04	0.49 ± 0.07
Y56A	1.07 ± 0.16	0.08 ± 0.04
Y56D	0.77 ± 0.10	0.07 ± 0.03
Y56R	0.64 ± 0.13	0.11 ± 0.06
Q66D	1.05 ± 0.07	0.54 ± 0.06
E72D	0.55 ± 0.07	0.56 ± 0.10
E72R	0.48 ± 0.15	0.11 ± 0.05
I115D	0.02 ± 0.01	0.05 ± 0.03
I116R	0.01 ± 0.01	0.01 ± 0.01
G119D	1.40 ± 0.20	0.08 ± 0.04
G119R	0.29 ± 0.10	0.02 ± 0.01
G120A	0.02 ± 0.01	0.01 ± 0.01
G120D	0.84 ± 0.20	0.05 ± 0.04
G120R	0.05 ± 0.03	0.04 ± 0.04
A121D	0.03 ± 0.03	0.03 ± 0.03
A121R	0.03 ± 0.03	0.03 ± 0.03
D122A	0.01 ± 0.01	0.74 ± 0.12
D122R	0.01 ± 0.01	0.01 ± 0.01
Y123A	0.01 ± 0.01	0.65 ± 0.08
Y123D	0.01 ± 0.01	0.33 ± 0.16
Y123R	0.01 ± 0.01	1.12 ± 0.04
K124A	0.02 ± 0.01	0.94 ± 0.06
K124D	0.03 ± 0.01	0.48 ± 0.05
K124R	0.02 ± 0.01	0.85 ± 0.08
R125A	0.03 ± 0.04	1.03 ± 0.02
R125D	0.02 ± 0.01	1.12 ± 0.08

FIG. 12


% Igp-Specific T cells
40
30
20
10
0
*
**
PBS
1
5
Rx (mg/kg)


% Non Specific T cells
4
3
2
1
0
PBS
1
5
Rx (mg/kg)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/33042

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-6, 20-26, 29-86
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-Please See Supplemental Page-*-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Group 1+ claims 1, 2 (in-part), 3 (in-part), 7-15, 16 (in-part), 17 (in-part), 27 (in-part) and 28 (in-part)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/33042

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 39/395; C07K 14/705, 16/28 (2017.01)

CPC - A61K 39/3955; C07K 14/70539, 16/2827; G01N 33/6857

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016/000619 A1 (BEIGENE, LTD) 07 January 2016; paragraphs [0112], [0149], [0156], [0159]	1-2, 3/1-2, 7-15, 16/7-15, 17/16/7-15, 27/7-14, 28/7-14
A	US 2013/0017199 A1 (LANGERMANN, S) 17 January 2013; paragraphs [0078]-[0080], [0085], [0093]	1-2, 3/1-2, 7-15, 16/7-15, 17/16/7-15, 27/7-14, 28/7-14
A	US 2016/0011204 A1 (ALBERT EINSTEIN COLLEGE OF MEDICINE OF YESHIVA UNIVERSITY) 14 January 2016; figures 12, 18; paragraphs [0031], [0038]	1-2, 3/1-2, 7-15, 16/7-15, 17/16/7-15, 27/7-14, 28/7-14
A	WO 2015/195531 A2 (ALBERT EINSTEIN COLLEGE OF MEDICINE, INC.) 23 December 2015; paragraphs [0033]-[0034], [0049]; figures 19F, 26A	7-15, 16/7-15, 17/16/7-15, 27/7-14, 28/7-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 September 2017 (26.09.2017)	10 OCT 2017
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US17/33042

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-3, 7-19, 27, 28; a D26 mutation; NP_004039 (SEQ ID NO: 3) and SEQ ID NO: 68 are directed toward a variant PD-L1 immunomodulatory polypeptide and a multimeric polypeptide comprising the variant PD-L1 immunomodulatory polypeptide.

The immunomodulatory peptide and multimeric polypeptide will be searched to the extent they encompass a D26 mutation (first exemplary PD-L1 mutation), a B2-microglobulin polypeptide encompassing NP_004039 (SEQ ID NO: 3) (first exemplary B2-microglobulin sequence) and a MHC polypeptide encompassing SEQ ID NO: 68 (first exemplary MHC polypeptide). Applicant is invited to elect additional mutation site(s), and/or B2-microglobulin sequence(s), with specified SEQ ID NO: for each; and/or MHC polypeptide sequence(s), with specified SEQ ID NO for each, to be searched. Additional mutation(s) and/or B2-microglobulin and/or MHC polypeptide sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1, 2 (in-part), 3 (in-part), 7-15, 16 (in-part), 17 (in-part), 27 (in-part) and 28 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass a D26 mutation (PD-L1 mutation), NP_004039 (SEQ ID NO: 3) (B2-microglobulin sequence) and SEQ ID NO: 68 (MHC polypeptide). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a PD-L1 mutation encompassing a T37 mutation (first exemplary elected PD-L1 mutation).

No technical features are shared between the B2-microglobulin and/or MHC polypeptide sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: 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 at least one of 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 comprises an amino acid sequence having at least 85% amino acid sequence identity to the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, to the PD-L1 amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2, wherein the variant PD-L1 immunomodulatory polypeptide has one or more amino acid substitutions relative to the PD-L1 amino acid sequence depicted in FIG. 2A or to the PD-L1 amino acid sequence set forth in SEQ ID NO: 1; and wherein the variant PD-L1 immunomodulatory polypeptide exhibits: a) reduced binding affinity to a PD1 polypeptide having an amino acid sequence depicted in FIG. 3A or 3B, compared to the binding affinity of the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, or compared to the binding affinity of the PD-L1 amino acid sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 2, for the PD1 polypeptide.

However, these shared technical features are previously disclosed by WO 2015/195531 A2 to Albert Einstein College of Medicine, Inc. (hereinafter 'Einstein') in view of US 2016/0011204 A1 to Albert Einstein College of Medicine of Yeshiva University (hereinafter 'Yeshiva') and US 2013/0017199 A1 (LANGERMANN).

Einstein discloses a multimeric polypeptide (a multimeric polypeptide; paragraph [0033]) comprising: a) a first polypeptide (comprising: a) a first polypeptide; paragraph [0033]) comprising, in order from N-terminus to C-terminus (comprising, in order from N-terminus to C-terminus; paragraph [0033]): i) an epitope (an epitope; paragraph [0033]); ii) a first major histocompatibility complex (MHC) polypeptide (a first major histocompatibility complex (MHC) polypeptide; paragraph [0033]); and b) a second polypeptide (and b) a second polypeptide; paragraph [0033]) comprising, in order from N-terminus to C-terminus (comprising, in order from N-terminus to C-terminus; paragraph [0033]): i) a second MHC polypeptide (a second MHC polypeptide; paragraph [0033]); wherein the multimeric polypeptide comprises one or more immunomodulatory domains (wherein the multimeric polypeptide comprises one or more immunomodulatory domains; paragraph [0033]), wherein at least one of the one or more immunomodulatory domain is: A) at the C-terminus of the first polypeptide (wherein at least one of the one or more immunomodulatory domain is: A) at the C-terminus of the first polypeptide; paragraph [0033]), wherein the immunomodulatory domain comprises a PD-L1 polypeptide or modified form thereof (wherein the immunomodulatory domain comprises a PD-L1 polypeptide or modified form thereof; paragraph [0034], [0049], Figure 19F); as well as a reference PD-L1 sequence comprising SEQ ID NO: 1 (a reference PD-L1 sequence comprising SEQ ID NO: 94 (SEQ ID NO: 1); paragraph [0069], Figure 26A, SEQ ID NO: 94; wherein SEQ ID NO: 94 is 100% identical to Applicants' SEQ ID NO: 1).

Einstein does not disclose wherein the immunomodulatory domain comprises an amino acid sequence having at least 85% amino acid sequence identity to the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, to the PD-L1 amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2, wherein the variant PD-L1 immunomodulatory polypeptide has one or more amino acid substitutions relative to the PD-L1 amino acid sequence depicted in FIG. 2A or to the PD-L1 amino acid sequence set forth in SEQ ID NO: 1; and wherein the variant PD-L1 immunomodulatory polypeptide exhibits: a) reduced binding affinity to a PD1 polypeptide having an amino acid sequence depicted in FIG. 3A or 3B, compared to the binding affinity of the PD-L1 amino acid sequence depicted in FIG. 2A or 2B, or compared to the binding affinity of the PD-L1 amino acid sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 2, for the PD1 polypeptide.

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from Previous Supplemental Page:

Yeshiva discloses PD-L1 single amino acid mutants (PD-L1 single amino acid mutants; Figure 12; paragraph [0031]); wherein the mutants demonstrated impaired binding to PD-1 or B7-1 or both (wherein the mutants demonstrated impaired binding to PD-1 or B7-1 or both; paragraph [0031]).

Langermann discloses inhibiting PD-1 (inhibiting PD-1; abstract), including a murine PD-1 sequence comprising SEQ ID NO: 56 (a Murine PD-1 sequence comprising SEQ ID NO: 17 (SEQ ID NO: 56); paragraph [0093]); SEQ ID NO: 17; wherein SEQ ID NO: 17 is 100% identical to Applicants' SEQ ID NO: 56).

It would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of Einstein to have used a reference sequence for an interacting receptor, such as a murine PD-1 reference sequence as disclosed by Langermann, in order to enable a practitioner of skill in the art to express the receptor for use in assessing binding to the constructs disclosed by Einstein. It further would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of Einstein include multimeric constructs including of single amino acid PD-L1 mutants (having at least 85% identity to the PD-L1 sequence disclosed by Einstein), wherein the mutants have reduced binding affinity for a cognate receptor, such as PD-1, as disclosed by Yeshiva, in order to have assessed the immunomodulatory capabilities of the mutant PD-L1 molecules.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Einstein, Yeshiva and Langermann references, unity of invention is lacking.