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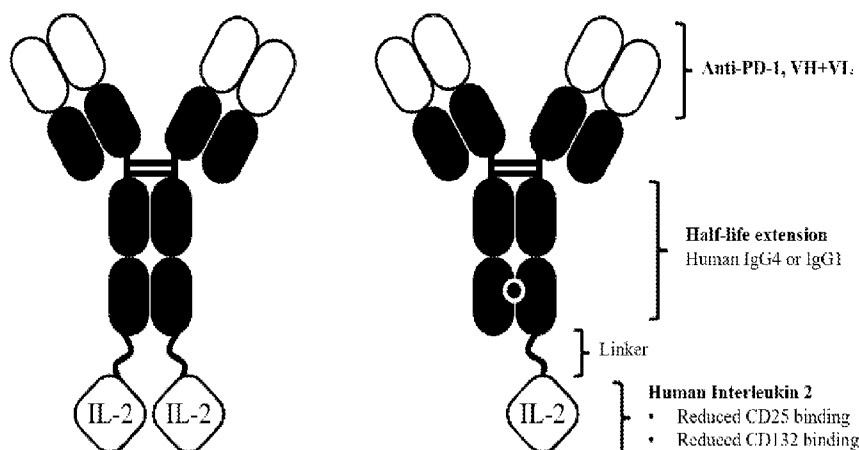
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FIG. 1



(57) Abstract: Provided herein are interleukin-2 muteins and fusion proteins comprising an interleukin-2 mutein and a half-life-extension domain. Also provided herein are their pharmaceutical compositions and methods of use for treating, preventing, or ameliorating one or more symptoms of a proliferative disease.

INTERLEUKIN-2 MUTEINS, FUSION PROTEINS, PHARMACEUTICAL COMPOSITIONS, AND THERAPEUTIC APPLICATIONS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of the priority of U.S. Provisional Application Nos. 63/226,178, filed July 28, 2021; and 63/364,718, filed May 13, 2021; the disclosure of each of which is incorporated herein by reference in its entirety.

FIELD

[0002] Provided herein are interleukin-2 muteins and fusion proteins comprising an interleukin-2 mutein and a half-life-extension domain. Also provided herein are their pharmaceutical compositions and methods of use for treating, preventing, or ameliorating one or more symptoms of a proliferative disease.

REFERENCE TO A SEQUENCE LISTING

[0003] The present specification is being filed with a Sequence Listing entitled 216A015WO01_SEQLIST_ST26.XML of 310,277 bytes in size and created July 26, 2022; the content of which is incorporated herein by reference in its entirety.

BACKGROUND

[0004] Dysregulation of the host immune system is one important immune resistance mechanism for cancer. Hanahan and Weinberg, *Cell* **2011**, *144*, 646-74; Pardoll, *Nat. Rev. Cancer* **2012**, *12*, 252-64. One class of immunotherapy is agents targeting specific checkpoint proteins that play critical roles in regulating T-cell activation and proliferation. Waldman *et al.*, *Nat. Rev. Immunol.* **2020**, *20*, 651-68. These proteins function as co-receptors on the surfaces of T-cells to help regulate T-cell responses following T-cell activation. Wolchok *et al.*, *Cancer J.* **2010**, *16*, 311-7. The two best characterized checkpoint proteins are cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death-1 (PD-1), both serve as negative regulators of T-cell activation. Waldman *et al.*, *Nat. Rev. Immunol.* **2020**, *20*, 651-68. T-cell activation induces expression of CTLA-4 and PD-1, thereby inhibits further T-cell activation and proliferation.

Pardoll, *Nat. Rev. Cancer* **2012**, 12, 252-64. Immune checkpoint blockade removes such inhibitory signals and unleashes antitumor immune responses. *Id.*; Sharma and Allison, *Science* **2015**, 348, 56-61. Ipilimumab, a CTLA-4 blocking antibody, was the first immune checkpoint inhibitor approved by the FDA for cancer treatment. *Id.* Several anti-PD-1 antibodies have since been approved for cancer treatment. Gong *et al.*, *J. Immunother. Cancer* **2018**, 6, 8. While immunotherapy has been a major advance in cancer treatment, up to 85 percent of patients whose cancer is treated with checkpoint inhibitors do not benefit. Haslam and Prasad, *JAMA Netw. Open* **2019**, 2, e192535.

[0005] An interleukin-2 (IL-2) is a pleiotropic cytokine that orchestrates the proliferation, survival, and function of both immune effector (Teff) cells and regulatory T (Treg) cells to maintain immune homeostasis. Bluestone, *N. Engl. J. Med.* **2011**, 365, 2129-31; Boyman *et al.*, *Nat. Rev. Immunol.* **2012**, 12, 180-90. The IL-2 drives T-cell growth, augments natural killer (NK) cytolytic activity, induces the differentiation of regulatory T (Treg) cells, and mediates activation-induced cell death. Liao *et al.*, *Curr. Opin. Immunol.* **2011**, 23, 598-604.

[0006] An interleukin-2 receptor (IL-2R) exists in three different forms generated from three different interleukin-2 receptor chains: α chain (IL-2R α or CD25), β chain (IL-2R β or CD122), and γ chain (IL-2R γ , γ_c , or CD132). Wang *et al.*, *Science* **2005**, 310, 1159-63. The IL-2 binds the IL-2R α with a low affinity ($K_d \approx 10$ nM). *Id.* From a crystal structure of a quaternary IL-2 signaling complex, fifteen amino acid residues (K35, T37, R38, T41, F42, K43, F44, Y45, E61, E62, K64, P65, E68, L72, and Y107) on the IL-2 are identified as interface residues between the IL-2 and IL-2R α . Stauber *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **2006**, 103, 2788-93. The IL-2 binds a heterodimeric complex of the IL-2R β and IL-2R γ , expressed on memory T cells and NK cells, with an intermediate affinity ($K_d \approx 1$ nM). Wang *et al.*, *Science* **2005**, 310, 1159-63. The IL-2 binds a heterotrimeric complex of the IL-2R α , IL-2R β , and IL-2R γ , expressed on Treg cells, with a high affinity ($K_d \approx 10$ pM). *Id.* The IL-2 binds the IL-2R β alone with a dissociation constant (K_d) of about 100 nM and has no detectable binding affinity for the IL-2R γ alone. *Id.* The IL-2R α by itself has no signal-transducing activity. *Id.* The IL-2 signals through the intermediate-affinity heterodimeric IL-2R β/γ complex or the high-affinity heterotrimeric IL-2R $\alpha/\beta/\gamma$ complex. Liao *et al.*, *Curr. Opin. Immunol.* **2011**, 23, 598-604. The binding of the IL-2 to the intermediate-affinity heterodimeric IL-2R β/γ complex leads to the

activation and proliferation of immunostimulatory Teff cells, while the binding of the IL-2 to the high-affinity heterotrimeric IL-2R α / β / γ complex results in the activation and proliferation of immunosuppressive Treg cells. Malek *et al.*, *Immunity* **2010**, 33, 153-65; Bluestone, *N. Engl. J. Med.* **2011**, 365, 2129-31; Boyman *et al.*, *Nat. Rev. Immunol.* **2012**, 12, 180-90; Spangler *et al.*, *Annu. Rev. Immunol.* **2015**, 33, 139-67. This dual opposing functions of immunostimulation and immunosuppression pose a major challenge in developing the IL-2 as a safe and effective therapeutic agent. Skrombolas *et al.*, *Expert Rev. Clin. Immunol.* **2014**, 10, 207-17; Abbas *et al.*, *Sci. Immunol.* **2018**, 3, eaat1482.

[0007] Aldesleukin, a recombinant human IL-2, was approved by the FDA for metastatic renal cell carcinoma in 1992 and for metastatic melanoma in 1998. Rosenberg, *J. Immunol.* **2014**, 192, 5451-8. Patients with metastatic melanoma or renal cancer experience a 5 to 10% rate of complete cancer regression, with an additional 10% experiencing a partial regression. Atkins *et al.*, *J. Clin. Oncol.* **1999**, 17, 2105-16; Klapper *et al.*, *Cancer* **2008**, 113, 293-301. Approximately 70% of complete responders to the IL-2 therapy do not recur. Rosenberg, *Sci. Transl. Med.* **2012**, 4, 127ps8. However, the success of the IL-2 as an immunotherapy for cancer has been hampered by its severe toxicities and limited efficacy. One major limiting factor for its efficacy as an anticancer agent is immunosuppression resulting from the IL-2–driven preferential expansion of Treg cells. Abbas *et al.*, *Sci. Immunol.* **2018**, 3, eaat1482. Moreover, for the IL-2 to be effective in cancer treatment, a high dose therapeutic schedule is required. Bluestone, *N. Engl. J. Med.* **2011**, 365, 2129-31; Abbas *et al.*, *Sci. Immunol.* **2018**, 3, eaat1482. This dosing regimen, however, causes vascular leak syndrome and results in the limited application of IL-2 in cancer treatment. Abbas *et al.*, *Sci. Immunol.* **2018**, 3, eaat1482.

[0008] Despite the advances in cancer treatment, cancer remains a major worldwide public health problem. It was estimated that there will be 1,898,160 new cancer cases diagnosed and 608,570 cancer deaths in the US alone in 2021. *Cancer Facts & Figures* 2021. Therefore, there is a need for an effective therapy for cancer treatment.

SUMMARY OF THE DISCLOSURE

[0009] Provided herein is an interleukin-2 (IL-2) mutein having attenuated binding affinities to an IL-2R α and a heterodimeric IL-2R β / γ complex as compared to a wild-type IL-2.

[0010] Also provided herein is an IL-2 mutein comprising (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a replacement of the amino acid residues from positions N29 to L40 with a peptide comprising an amino acid sequence of an IL-15 hinge or a fragment thereof; a disulfide bond formed between two amino acid residues from positions N30 to L80; or an amino acid substitution at position K8, K9, Q13, E15, H16, L19, D20, M23, K32, P34, K35, L36, T37, R38, M39, L40, T41, F42, K43, F44, Y45, M46, P47, E61, E62, L63, K64, P65, L66, E67, E68, V69, L70, N71, L72, A73, Q74, K76, H79, R81, D84, S87, N88, V91, I92, E95, Y107, D109, T111, or S127.

[0011] Additionally provided herein is an IL-2 mutein comprising (i) an amino acid substitution at position L18, Q126, or S130; (ii) a replacement of the amino acid residues from positions N29 to L40 with a peptide comprising an amino acid sequence of an IL-15 hinge or a fragment thereof; and (iii) optionally an amino acid substitution at position K8, K9, Q13, E15, H16, L19, D20, M23, K32, P34, K35, L36, T37, R38, M39, L40, T41, F42, K43, F44, Y45, M46, P47, E61, E62, L63, K64, P65, L66, E67, E68, V69, L70, N71, L72, A73, Q74, K76, H79, R81, D84, S87, N88, V91, I92, E95, Y107, D109, T111, or S127.

[0012] Furthermore, provided herein is a fusion protein comprising an IL-2 domain and a half-life-extension domain.

[0013] Provided herein is a fusion protein comprising an IL-2 domain and an albumin binding domain as a half-life-extension domain.

[0014] Provided herein is a fusion protein comprising an IL-2 domain, an albumin binding domain, and optionally a peptide linker; wherein a carboxyl terminus (*C*-terminus) of the albumin binding domain is connected to the amino terminus (*N*-terminus) of the IL-2 domain directly or *via* the peptide linker.

[0015] Provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and an albumin binding domain.

[0016] Provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and optionally first and second peptide linkers; wherein a *C*-terminus of the PD-1 binding domain is connected to an *N*-terminus of the albumin binding

domain directly or *via* the first peptide linker, and a *C*-terminus of the albumin binding domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the second peptide linker.

[0017] Provided herein is a fusion protein comprising an IL-2 domain, first and second PD-1 binding domains, an albumin binding domain, and optionally first, second, and third peptide linkers; wherein a *C*-terminus of the first PD-1 binding domain is connected to an *N*-terminus of the second PD-1 binding domain directly or *via* the first peptide linker, a *C*-terminus of the second PD-1 binding domain is connected to an *N*-terminus of the albumin binding domain directly or *via* the second peptide linker, and a *C*-terminus of the albumin binding domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the third peptide linker.

[0018] Provided herein is a fusion protein comprising an IL-2 domain and a fragment crystallizable (Fc) domain as a half-life-extension domain.

[0019] Provided herein is a fusion protein comprising an IL-2 domain, an Fc domain comprising first and second peptide chains, and optionally a peptide linker; wherein the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the peptide linker.

[0020] Provided herein is a fusion protein comprising first and second IL-2 domains, an Fc domain comprising first and second peptide chains, and optionally first and second peptide linkers; wherein the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the first IL-2 domain directly or *via* the first peptide linker, and the *C*-terminus of the second peptide chain of the Fc domain is connected to the *N*-terminus of the second IL-2 domain directly or *via* the second peptide linker.

[0021] Provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and an Fc domain.

[0022] Provided herein is a fusion protein comprising an IL-2 domain, first and second PD-1 binding domains, an Fc domain comprising first and second peptide chains, and optionally a peptide linker; wherein a *C*-terminus of the first PD-1 binding domain is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the IL-2

domain directly or *via* the peptide linker; and wherein a *C*-terminus of the second PD-1 binding domain is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker.

[0023] Provided herein is a fusion protein comprising first and second IL-2 domains, first and second PD-1 binding domains, an Fc domain comprising first and second peptide chains, and optionally first and second peptide linkers; wherein a *C*-terminus of the first PD-1 binding domain is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the peptide linker; and wherein a *C*-terminus of the second PD-1 binding domain is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the second peptide chain of the Fc domain is connected to the *N*-terminus of the second IL-2 domain directly or *via* the second peptide linker.

[0024] Provided herein is a fusion protein comprising an IL-2 domain and an anti-PD-1 antibody.

[0025] Provided herein is a fusion protein comprising an IL-2 domain, an anti-PD-1 antibody comprising first and second light chains and first and second heavy chains, and optionally a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD-1 antibody is connected to the *N*-terminus of the IL-2 domain directly or *via* the peptide linker.

[0026] Provided herein is a fusion protein comprising first and second IL-2 domains, an anti-PD-1 antibody comprising first and second light chains and first and second heavy chains, and optionally first and second peptide linkers; wherein the *C*-terminus of the first heavy chain of the anti-PD-1 antibody is connected to the *N*-terminus of the first IL-2 domain directly or *via* the first peptide linker; and wherein the *C*-terminus of the second heavy chain of the anti-PD-1 antibody is connected to the *N*-terminus of the second IL-2 domain directly or *via* the second peptide linker.

[0027] Provided herein is a pharmaceutical composition comprising an IL-2 mutein or fusion protein, and a pharmaceutically acceptable excipient.

[0028] Provided herein is a method of treating, preventing, or ameliorating one or more symptoms of a disorder, disease, or condition mediated by a PD-1 in a subject, comprising administering to the subject in need thereof a therapeutically effective amount of an IL-2 mutein or fusion protein.

[0029] Provided herein is a method of treating, preventing, or ameliorating one or more symptoms of a disorder, disease, or condition mediated by an IL-2 in a subject, comprising administering to the subject in need thereof a therapeutically effective amount of an IL-2 mutein or fusion protein.

[0030] Provided herein is a method of treating, preventing, or ameliorating one or more symptoms of a proliferative disease in a subject, comprising administering to the subject in need thereof a therapeutically effective amount of an IL-2 mutein or fusion protein.

[0031] Provided herein is a method of inhibiting the growth of a cell, comprising contacting the cell with an effective amount of an IL-2 mutein or fusion protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] FIG. 1 shows the configurations of exemplary fusion proteins: (i) an IL-2 fusion protein comprising first and second IL-2 domains, and a PD-L1 binding domain, *e.g.*, an intact anti-PD-1 antibody comprising first and second light chains and first and second heavy chains; wherein the *C*-terminus of the first heavy chain of the anti-PD-1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* a peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD-1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* a peptide linker; and (ii) an IL-2 fusion protein comprising an IL-2 domain, and a PD-L1 binding domain, *e.g.*, an intact anti-PD-1 antibody comprising first and second light chains and first and second heavy chains; wherein the *C*-terminus of the first heavy chain of the anti-PD-1 antibody is connected to the *N*-terminus of the IL-2 domain *via* a peptide linker; and wherein the anti-PD-1 antibody is in a knobs-into-holes configuration.

[0033] FIG. 2 shows the configurations of exemplary fusion proteins: (i) an IL-2 fusion protein comprising first and second IL-2 domains, first and second PD-1 binding domains, *e.g.*, anti-PD-1 V_HH single domain antibodies, and a Fc domain comprising first and second peptide

chains; wherein the C-terminus of the first anti-PD-1 V_HH single domain antibody is connected to the N-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the C-terminus of the first peptide chain of the Fc domain is connected to the N-terminus of the first IL-2 domain *via* a peptide linker; and wherein the C-terminus of the second anti-PD-1 V_HH single domain antibody is connected to the N-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker, and the C-terminus of the second peptide chain of the Fc domain is connected to the N-terminus of the second IL-2 domain *via* a peptide linker; and (ii) an IL-2 fusion protein comprising an IL-2 domain, first and second PD-1 binding domains, *e.g.*, anti-PD-1 V_HH single domain antibodies, and a Fc domain comprising first and second peptide chains; wherein the C-terminus of the first anti-PD-1 V_HH single domain antibody is connected to the N-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the C-terminus of the first peptide chain of the Fc domain is connected to the N-terminus of the first IL-2 domain *via* a peptide linker; wherein the C-terminus of the second anti-PD-1 V_HH single domain antibody is connected to the N-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker; and wherein the Fc domain is in a knobs-into-holes configuration.

[0034] FIG. 3 shows the configurations of exemplary fusion proteins: (i) an IL-2 fusion protein comprising an IL-2 domain, a PD-L1 binding domain, *e.g.*, an anti-PD-1 V_HH single domain antibody (sdAb), and an albumin binding domain, *e.g.*, an anti-HSA V_HH sdAb; wherein the C-terminus of the anti-PD-1 V_HH sdAb is connected to the N-terminus of the anti-HSA V_HH sdAb *via* a peptide linker, and the C-terminus of the anti-HSA V_HH sdAb *via* a peptide linker is connected to the N-terminus of the IL-2 domain *via* a peptide linker; (ii) an IL-2 fusion protein comprising an IL-2 domain, first and second PD-L1 binding domains, *e.g.*, anti-PD-1 V_HH sdAbs, and an albumin binding domain, *e.g.*, an anti-HSA V_HH sdAb; wherein the C-terminus of the first anti-PD-1 V_HH sdAb is connected to the N-terminus of the second anti-PD-1 V_HH sdAb *via* a peptide linker, the C-terminus of the second anti-PD-1 V_HH sdAb is connected to the N-terminus of the anti-HSA V_HH sdAb *via* a peptide linker, and the C-terminus of the anti-HSA V_HH sdAb *via* a peptide linker is connected to the N-terminus of the IL-2 domain *via* a peptide linker; and (iii) an IL-2 fusion protein comprising an IL-2 domain, a PD-L1 binding domain, *e.g.*, an anti-PD-1 single-chain fragment variable (scFv), and an albumin binding domain, *e.g.*, an anti-HSA V_HH sdAb; wherein the C-terminus of the anti-PD-1 scFv is connected to the N-

terminus of the anti-HSA V_HH sdAb *via* a peptide linker, and the C-terminus of the anti-HSA V_HH sdAb is connected to the N-terminus of the IL-2 domain *via* a peptide linker.

DETAILED DESCRIPTION

[0035] To facilitate understanding of the disclosure set forth herein, a number of terms are defined below.

[0036] Generally, the nomenclature used herein and the laboratory procedures in biochemistry, biology, cell biology, immunology, molecular biology, and pharmacology described herein are those well-known and commonly employed in the art. Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

[0037] The term “subject” refers to an animal, including, but not limited to, a primate (*e.g.*, human), cow, pig, sheep, goat, horse, dog, cat, rabbit, rat, or mouse. The terms “subject” and “patient” are used interchangeably herein in reference, for example, to a mammalian subject, such as a human subject. In one embodiment, the subject is a human.

[0038] The terms “treat,” “treating,” and “treatment” are meant to include alleviating or abrogating a disorder, disease, or condition, or one or more of the symptoms associated with the disorder, disease, or condition; or alleviating or eradicating the cause(s) of the disorder, disease, or condition itself.

[0039] The terms “prevent,” “preventing,” and “prevention” are meant to include a method of delaying and/or precluding the onset of a disorder, disease, or condition, and/or its attendant symptoms; barring a subject from acquiring a disorder, disease, or condition; or reducing a subject’s risk of acquiring a disorder, disease, or condition.

[0040] The terms “alleviate” and “alleviating” refer to easing or reducing one or more symptoms (*e.g.*, pain) of a disorder, disease, or condition. The terms can also refer to reducing adverse effects associated with an active ingredient. Sometimes, the beneficial effects that a subject derives from a prophylactic or therapeutic agent do not result in a cure of the disorder, disease, or condition.

[0041] The term “contacting” or “contact” is meant to refer to bringing together of a therapeutic agent and cell or tissue such that a physiological and/or chemical effect takes place as a result of such contact. Contacting can take place *in vitro*, *ex vivo*, or *in vivo*. In one embodiment, a therapeutic agent is contacted with a cell in cell culture (*in vitro*) to determine the effect of the therapeutic agent on the cell. In another embodiment, the contacting of a therapeutic agent with a cell or tissue includes the administration of a therapeutic agent to a subject having the cell or tissue to be contacted.

[0042] The term “therapeutically effective amount” or “effective amount” is meant to include the amount of a compound (*e.g.*, a polypeptide or fusion protein) that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the symptoms of the disorder, disease, or condition being treated. The term “therapeutically effective amount” or “effective amount” also refers to the amount of a compound that is sufficient to elicit a biological or medical response of a biological molecule (*e.g.*, a protein, enzyme, RNA, or DNA), cell, tissue, system, animal, or human, which is being sought by a researcher, veterinarian, medical doctor, or clinician.

[0043] The term “IC₅₀” or “EC₅₀” refers to an amount, concentration, or dosage of a compound (*e.g.*, a polypeptide or fusion protein) that is required for 50% inhibition of a maximal response in an assay that measures such a response.

[0044] The term “pharmaceutically acceptable carrier,” “pharmaceutically acceptable excipient,” “physiologically acceptable carrier,” or “physiologically acceptable excipient” refers to a pharmaceutically acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, solvent, or encapsulating material. In one embodiment, each component is “pharmaceutically acceptable” in the sense of being compatible with the other ingredients of a pharmaceutical formulation, and suitable for use in contact with the tissue or organ of a subject (*e.g.*, a human or an animal) without excessive toxicity, irritation, allergic response, immunogenicity, or other problems or complications, commensurate with a reasonable benefit/risk ratio. *See, e.g., Remington: The Science and Practice of Pharmacy*, 23rd ed.; Adejare Ed.; Academic Press, 2020; *Handbook of Pharmaceutical Excipients*, 9th ed.; Sheskey *et al.*, Eds.; Pharmaceutical Press, 2020; *Handbook of Pharmaceutical Additives*, 3rd ed.; Ash

and Ash Eds.; Synapse Information Resources, 2007; *Pharmaceutical Preformulation and Formulation*, 1st ed.; Gibson Ed.; CRC Press, 2015.

[0045] The term “about” or “approximately” means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined. In certain embodiments, the term “about” or “approximately” means within 1, 2, 3, or 4 standard deviations. In certain embodiments, the term “about” or “approximately” means within 50%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.05% of a given value or range.

[0046] The terms “substantially pure” and “substantially homogeneous,” when referring to a compound, mean sufficiently homogeneous to appear free of readily detectable impurities as determined by standard analytical methods used by one of ordinary skill in the art, including, but not limited to, gel electrophoresis, high performance liquid chromatography (HPLC), and mass spectrometry (MS); or sufficiently pure such that further purification would not detectably alter the physical, chemical, biological, and/or pharmacological properties, such as enzymatic and biological activities, of the compound. In certain embodiments, “substantially pure” or “substantially homogeneous” refers to a collection of molecules, wherein at least about 50%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or at least about 99.5% by weight of the molecules are a single compound as determined by a standard analytical method.

Interleukin-2 Muteins

[0047] In one embodiment, provided herein is an IL-2 mutein comprising, as set forth in SEQ ID NO: 179, 180, 181, 182, 183, or 184, (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a replacement of the amino acid residues from positions N29 to L40 with a peptide comprising an amino acid sequence of an IL-15 hinge or a fragment thereof (“an IL-15 hinge fragment-containing peptide”); a disulfide bond formed between two amino acid residues from positions N30 to L80; or an amino acid substitution at position K8, K9, Q13, E15, H16, L19, D20, M23, K32, P34, K35, L36, T37, R38, M39, L40, T41, F42, K43, F44, Y45, M46, P47, E61, E62, L63, K64, P65, L66, E67, E68, V69, L70, N71, L72, A73, Q74, K76, H79, R81, D84, S87, N88, V91, I92, E95, Y107, D109, T111, or S127.

[0048] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a replacement of the amino acid residues from positions N29 to L40 with an IL-15 hinge fragment-containing peptide; a disulfide bond formed between two amino acid residues from positions N30 to L80; an amino acid substitution at position E15, H16, D20, K32, K76, S87, N88, or I92; and/or an amino acid substitution at a position from R38 to Y45.

[0049] In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a replacement of the amino acid residues from positions N29 to L40 with an IL-15 hinge fragment-containing peptide; a disulfide bond formed between two amino acid residues from positions N30 to L80; or an amino acid substitution at position E15, H16, D20, K32, R38, L40, F42, K76, S87, N88, or I92.

[0050] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18 or Q126; and (ii) a replacement of the amino acid residues from positions N29 to L40 with an IL-15 hinge fragment-containing peptide; or an amino acid substitution at position I92.

[0051] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitutions at positions L18 and Q126; and (ii) a replacement of the amino acid residues from positions N29 to L40 with an IL-15 hinge fragment-containing peptide.

[0052] In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position Q126; and (ii) a replacement of the amino acid residues from positions N29 to L40 with an IL-15 hinge fragment-containing peptide; and an amino acid substitution at position I92.

[0053] In certain embodiments, the amino acid residue at position E15 is one of the twenty natural amino acids (*i.e.*, Ala (A), Cys (C), Asp (D), Glu (E), Phe (F), Gly (G), His (H), Ile (I), Lys (K), Leu (L), Met (M), Asn (N), Pro (P), Gln (Q), Arg (R), Ser (S), Thr (T), Val (V), Trp (W), and Tyr (Y)) other than E. In certain embodiments, the amino acid residue at position E15 is K.

[0054] In certain embodiments, the amino acid residue at position H16 is one of the

twenty natural amino acids other than H. In certain embodiments, the amino acid residue at position H16 is E, F, I, or V. In certain embodiments, the amino acid residue at position H16 is E, I, or V. In certain embodiments, the amino acid residue at position H16 is E. In certain embodiments, the amino acid residue at position H16 is F. In certain embodiments, the amino acid residue at position H16 is I. In certain embodiments, the amino acid residue at position H16 is V.

[0055] In certain embodiments, the amino acid residue at position L18 is one of the twenty natural amino acids other than L. In certain embodiments, the amino acid residue at position L18 is C or S. In certain embodiments, the amino acid residue at position L18 is C. In certain embodiments, the amino acid residue at position L18 is C and the amino acid residue at position C125 is C. In certain embodiments, the amino acid residue at position L18 is S.

[0056] In certain embodiments, the amino acid residue at position D20 is one of the twenty natural amino acids other than D. In certain embodiments, the amino acid residue at position D20 is A, E, K, or T. In certain embodiments, the amino acid residue at position D20 is A. In certain embodiments, the amino acid residue at position D20 is E. In certain embodiments, the amino acid residue at position D20 is K. In certain embodiments, the amino acid residue at position D20 is T.

[0057] In certain embodiments, the amino acid residue at position K32 is one of the twenty natural amino acids other than K. In certain embodiments, the amino acid residue at position K32 is D, E, or Q. In certain embodiments, the amino acid residue at position K32 is D. In certain embodiments, the amino acid residue at position K32 is E. In certain embodiments, the amino acid residue at position K32 is Q.

[0058] In certain embodiments, the amino acid residue at position R38 is one of the twenty natural amino acids other than R. In certain embodiments, the amino acid residue at position R38 is E or N. In certain embodiments, the amino acid residue at position R38 is E. In certain embodiments, the amino acid residue at position R38 is N.

[0059] In certain embodiments, the amino acid residue at position L40 is one of the twenty natural amino acids other than L. In certain embodiments, the amino acid residue at

position L40 is S or T. In certain embodiments, the amino acid residue at position L40 is S. In certain embodiments, the amino acid residue at position L40 is T.

[0060] In certain embodiments, the amino acid residue at position F42 is one of the twenty natural amino acids other than F. In certain embodiments, the amino acid residue at position F42 is A, C, K, or N. In certain embodiments, the amino acid residue at position F42 is A or K. In certain embodiments, the amino acid residue at position F42 is A. In certain embodiments, the amino acid residue at position F42 is C. In certain embodiments, the amino acid residue at position F42 is K. In certain embodiments, the amino acid residue at position F42 is N.

[0061] In certain embodiments, the amino acid residue at position K76 is one of the twenty natural amino acids other than K. In certain embodiments, the amino acid residue at position K76 is D, E, or Q. In certain embodiments, the amino acid residue at position K76 is D. In certain embodiments, the amino acid residue at position K76 is E. In certain embodiments, the amino acid residue at position K76 is Q.

[0062] In certain embodiments, the amino acid residue at position S87 is one of the twenty natural amino acids other than S. In certain embodiments, the amino acid residue at position S87 is D or E. In certain embodiments, the amino acid residue at position S87 is D. In certain embodiments, the amino acid residue at position S87 is E.

[0063] In certain embodiments, the amino acid residue at position N88 is one of the twenty natural amino acids other than N. In certain embodiments, the amino acid residue at position N88 is A.

[0064] In certain embodiments, the amino acid residue at position I92 is one of the twenty natural amino acids other than I. In certain embodiments, the amino acid residue at position I92 is A, D, E, or G. In certain embodiments, the amino acid residue at position I92 is A. In certain embodiments, the amino acid residue at position I92 is D. In certain embodiments, the amino acid residue at position I92 is E. In certain embodiments, the amino acid residue at position I92 is G.

[0065] In certain embodiments, the amino acid residue at position Q126 is one of the

twenty natural amino acids other than Q. In certain embodiments, the amino acid residue at position Q126 is E, K, R, S, or T. In certain embodiments, the amino acid residue at position Q126 is E, K, or R. In certain embodiments, the amino acid residue at position Q126 is E. In certain embodiments, the amino acid residue at position Q126 is K. In certain embodiments, the amino acid residue at position Q126 is R. In certain embodiments, the amino acid residue at position Q126 is S. In certain embodiments, the amino acid residue at position Q126 is T.

[0066] In certain embodiments, the amino acid residue at position S130 is one of the twenty natural amino acids other than S. In certain embodiments, the amino acid residue at position S130 is A, E, Q, or R. In certain embodiments, the amino acid residue at position S130 is A. In certain embodiments, the amino acid residue at position S130 is E. In certain embodiments, the amino acid residue at position S130 is Q. In certain embodiments, the amino acid residue at position S130 is R.

[0067] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; a disulfide bond formed between two different amino acid residues, each independently at position K35, R38, F42, Y45, E62, V69, or L72; or an amino acid substitution of K8D, K8E, K8Q, K9D, K9E, K9Q, Q13E, Q13N, E15K, E15Q, E15V, H16E, H16F, H16I, H16V, L19S, D20A, D20E, D20K, D20T, M23K, K32D, K32E, K32Q, P34N, K35N, L36S, L36T, T37N, R38E, R38N, M39N, L40S, L40T, T41N, F42A, F42C, F42K, F42N, K43N, F44N, Y45N, M46S, M46T, P47S, P47T, E61N, E62N, L63S, L63T, K64N, P65N, L66N, E67S, E67T, E68N, V69C, V69N, L70S, L70T, N71S, N71T, L72N, A73S, A73T, Q74S, Q74T, K76D, K76E, K76Q, H79D, H79E, H79Q, R81D, R81E, R81Q, D84T, S87D, S87E, N88A, V91I, I92A, I92D, I92E, I92G, I92L, E95K, E95N, E95Q, Y107N, D109N, T111S, S127A, S127E, S127F, or S127W.

[0068] In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; a

disulfide bond formed between two different amino acid residues, each independently at position K35, R38, F42, Y45, E62, V69, or L72; or an amino acid substitution of K8D, K8E, K8Q, K9D, K9E, K9Q, Q13E, Q13N, E15K, E15Q, E15V, H16E, H16F, H16I, H16V, L19S, D20A, D20E, D20K, D20T, M23K, K32D, K32E, K32Q, P34N, K35N, L36S, L36T, T37N, R38E, R38N, M39N, L40S, L40T, T41N, F42A, F42C, F42K, F42N, K43N, F44N, Y45N, M46S, M46T, P47S, P47T, E61N, E62N, L63S, L63T, K64N, P65N, L66N, E67S, E67T, E68N, V69C, V69N, L70S, L70T, N71S, N71T, L72N, A73S, A73T, Q74S, Q74T, K76D, K76E, K76Q, H79D, H79E, H79Q, R81D, R81E, R81Q, D84T, S87D, S87E, N88A, V91I, I92A, I92D, I92E, I92G, I92L, E95K, E95N, E95Q, Y107N, D109N, T111S, S127A, S127E, S127F, or S127W.

[0069] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; a disulfide bond formed between F42C and V69C; or an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40S, L40T, F42A, F42K, F42N, K76D, K76E, K76Q, S87D, S87E, N88A, I92A, I92D, I92E, or I92G.

[0070] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; a disulfide bond formed between F42C and V69C; or an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40S, L40T, F42A, F42K, F42N, K76D, K76E, K76Q, S87D, S87E, N88A, I92A, I92D, I92E, or I92G.

[0071] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, S130A, S130E, S130Q, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; a

disulfide bond formed between F42C and V69C; or an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40S, L40T, F42A, F42K, F42N, K76D, K76E, K76Q, S87D, S87E, N88A, I92A, I92D, I92E, or I92G.

[0072] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, S130A, S130E, S130Q, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; a disulfide bond formed between F42C and V69C; or an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40S, L40T, F42A, F42K, F42N, K76D, K76E, K76Q, S87D, S87E, N88A, I92A, I92D, I92E, or I92G.

[0073] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, S130A, S130E, S130Q, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; a disulfide bond formed between F42C and V69C; or an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40S, L40T, F42A, F42K, F42N, K76D, K76E, K76Q, S87D, S87E, N88A, I92A, I92D, I92E, or I92G.

[0074] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; a disulfide bond formed between F42C and V69C; or an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40S, L40T, F42A, F42K, F42N, K76D, K76E, K76Q, S87D, S87E, N88A, I92A, I92D, I92E, or I92G.

[0075] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18S or Q126E; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; or an amino acid substitution of I92A.

[0076] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitutions of L18S and Q126E; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide.

[0077] In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of Q126E; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; and an amino acid substitution of I92A.

[0078] In one embodiment, the IL-15 hinge fragment-containing peptide comprises an amino acid sequence of SEQ ID NO: 218 or 219. In another embodiment, the IL-15 hinge fragment-containing peptide comprises an amino acid sequence of SEQ ID NO: 218. In yet another embodiment, the IL-15 hinge fragment-containing peptide comprises an amino acid sequence of SEQ ID NO: 219.

[0079] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; a disulfide bond formed between F42C and V69C; or an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40T, F42A, F42K, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[0080] In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; a disulfide bond formed between F42C and V69C; or an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40T, F42A, F42K, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[0081] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an

amino acid substitution of L18C, L18S, Q126E, Q126K, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218; a disulfide bond formed between F42C and V69C; or an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40T, F42A, F42K, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[0082] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218; a disulfide bond formed between F42C and V69C; or an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40T, F42A, F42K, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[0083] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 219; a disulfide bond formed between F42C and V69C; or an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40T, F42A, F42K, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[0084] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, or S130R; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 219; a disulfide bond formed between F42C and V69C; or an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40T, F42A, F42K, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[0085] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18S or Q126E; and (ii) a replacement of the amino acid residues

from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218; or an amino acid substitution of I92A.

[0086] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18S or Q126E; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 219; or an amino acid substitution of I92A.

[0087] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitutions of L18S and Q126E; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218.

[0088] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitutions of L18S and Q126E; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 219.

[0089] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of Q126E; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218; and an amino acid substitution of I92A.

[0090] In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of Q126E; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 219; and an amino acid substitution of I92A.

[0091] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution at position K8,

K9, Q13, E15, H16, L19, D20, M23, K32, P34, K35, L36, T37, R38, M39, L40, T41, F42, K43, F44, Y45, M46, P47, E61, E62, L63, K64, P65, L66, E67, E68, V69, L70, N71, L72, A73, Q74, K76, H79, R81, D84, S87, N88, V91, I92, E95, Y107, D109, T111, or S127.

[0092] In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of K8D, K8E, K8Q, K9D, K9E, K9Q, Q13E, Q13N, E15K, E15Q, E15V, H16E, H16F, H16I, H16V, L19S, D20A, D20E, D20K, D20T, M23K, K32D, K32E, K32Q, P34N, K35N, L36S, L36T, T37N, R38E, R38N, M39N, L40S, L40T, T41N, F42A, F42C, F42K, F42N, K43N, F44N, Y45N, M46S, M46T, P47S, P47T, E61N, E62N, L63S, L63T, K64N, P65N, L66N, E67S, E67T, E68N, V69C, V69N, L70S, L70T, N71S, N71T, L72N, A73S, A73T, Q74S, Q74T, K76D, K76E, K76Q, H79D, H79E, H79Q, R81D, R81E, R81Q, D84T, S87D, S87E, N88A, V91I, I92A, I92D, I92E, I92G, I92L, E95K, E95N, E95Q, Y107N, D109N, T111S, S127A, S127E, S127F, or S127W.

[0093] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of K8D, K8E, K8Q, K9D, K9E, K9Q, Q13E, Q13N, E15K, E15Q, E15V, H16E, H16F, H16I, H16V, L19S, D20A, D20E, D20K, D20T, M23K, K32D, K32E, K32Q, P34N, K35N, L36S, L36T, T37N, R38E, R38N, M39N, L40S, L40T, T41N, F42A, F42C, F42K, F42N, K43N, F44N, Y45N, M46S, M46T, P47S, P47T, E61N, E62N, L63S, L63T, K64N, P65N, L66N, E67S, E67T, E68N, V69C, V69N, L70S, L70T, N71S, N71T, L72N, A73S, A73T, Q74S, Q74T, K76D, K76E, K76Q, H79D, H79E, H79Q, R81D, R81E, R81Q, D84T, S87D, S87E, N88A, V91I, I92A, I92D, I92E, I92G, I92L, E95K, E95N, E95Q, Y107N, D109N, T111S, S127A, S127E, S127F, or S127W.

[0094] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution at position E15, H16, D20, K32, R38, L40, F42, K76, S87, N88, or I92.

[0095] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of E15K, H16E, H16F, H16I,

H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40S, L40T, F42A, F42K, F42N, K76D, K76E, K76Q, S87D, S87E, N88A, I92A, I92D, I92E, or I92G.

[0096] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40S, L40T, F42A, F42K, F42N, K76D, K76E, K76Q, S87D, S87E, N88A, I92A, I92D, I92E, or I92G.

[0097] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40T, F42A, F42K, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[0098] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40T, F42A, F42K, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[0099] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, or S130R; and (ii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40T, F42A, F42K, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[00100] In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, or S130R; and (ii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, R38E, R38N, L40T, F42A, F42K, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[00101] In one embodiment, the IL-2 mutein provided herein comprises an amino acid substitution at position L18. In another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of L18C or L18S. In yet another embodiment, the IL-2 mutein

provided herein comprises an amino acid substitution of L18C. In still another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of L18S.

[00102] In one embodiment, the IL-2 mutein provided herein comprises an amino acid substitution at position Q126. In another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of Q126E, Q126K, Q126R, Q126S, or Q126T. In yet another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of Q126E. In yet another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of Q126K. In yet another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of Q126R. In yet another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of Q126S. In still another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of Q126T.

[00103] In one embodiment, the IL-2 mutein provided herein comprises an amino acid substitution at position S130. In another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of S130A, S130E, S130Q, or S130R. In yet another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of S130A. In yet another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of S130E. In yet another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of S130Q. In still another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of S130R.

[00104] In one embodiment, the IL-2 mutein provided herein comprises amino acid substitutions at positions L18 and S126. In another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of L18C or L18S and an amino acid substitution of Q126E, Q126K, Q126R, Q126S, or Q126T. In yet another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of L18C and an amino acid substitution of Q126E, Q126K, Q126R, Q126S, or Q126T. In yet another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of L18C and Q126E. In yet another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of L18C and Q126K. In yet another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of L18C and Q126R. In yet another embodiment, the IL-2 mutein provided herein

comprises amino acid substitutions of L18C and Q126S. In still another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of L18C and Q126T.

[00105] In one embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of L18S and an amino acid substitution of Q126E, Q126K, Q126R, Q126S, or Q126T. In another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of L18S and Q126E. In yet another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of L18S and Q126K. In yet another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of L18S and Q126R. In yet another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of L18S and Q126S. In still another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of L18S and Q126T.

[00106] In one embodiment, the IL-2 mutein provided herein comprises amino acid substitutions at positions S126 and S130. In another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of Q126E, Q126K, Q126R, Q126S, or Q126T, and an amino acid substitution of S130A, S130E, S130Q, or S130R. In yet another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of Q126E, Q126K, Q126R, Q126S, or Q126T, and an amino acid substitution of S130E or S130R.

[00107] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution at position E15. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of E15K. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of E15K.

[00108] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution at position H16. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of H16E, H16F, H16I, or H16V. In yet another

embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of H16E, H16F, H16I, or H16V. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of H16E, H16I, or H16V. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of H16E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of H16F. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of H16I. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of H16V.

[00109] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution at position D20. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of D20A, D20E, D20K, or D20T. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of D20A, D20E, D20K, or D20T. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of D20A. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of D20E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of D20K. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid

substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of D20T.

[00110] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution at position K32. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of K32E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of K32E.

[00111] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution at position R38. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of R38E or R38N. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of R38E or R38N. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of R38E. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of R38N.

[00112] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution at position L40. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of L40S or L40T. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of

L40S or L40T. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of L40S. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of L40T.

[00113] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution at position F42. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of F42A, F42C, or F42K. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of F42A, F42C, or F42K. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of F42A or F42K. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of F42A. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of F42C. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of F42K.

[00114] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution at position K76. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of K76E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R,

Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of K76E.

[00115] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution at position S87. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of S87D. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of S87D.

[00116] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution at position N88. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of N88A. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of N88A.

[00117] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution at position I92. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of I92A. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of I92D. In yet another embodiment, the IL-2 mutein

provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of I92E. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) an amino acid substitution of I92G.

[00118] In one embodiment, the IL-2 mutein provided herein comprises amino acid substitutions at positions I92 and S126. In another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of I92A, I92D, I92E, or I92G, and an amino acid substitution of Q126E, Q126K, Q126R, Q126S, or Q126T. In yet another embodiment, the IL-2 mutein provided herein comprises an amino acid substitution of I92A or I92G, and an amino acid substitution of Q126E, Q126K, Q126R, Q126S, or Q126T. In yet another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of I92A and Q126E. In yet another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of I92A and Q126K. In yet another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of I92A and Q126R. In yet another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of I92A and Q126S. In still another embodiment, the IL-2 mutein provided herein comprises amino acid substitutions of I92A and Q126T.

[00119] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions at positions R38 and L40. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions at positions E15, R38, and L40. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions at positions R38, L40, and K76. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions at positions E15, R38, L40, and K76.

[00120] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions of R38N and L40S or L40T. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid

substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N and L40S or L40T. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N and L40S or L40T. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of R38N, L40S or L40T, and K76E. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N, L40S or L40T, and K76E.

[00121] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions of R38N and L40S. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N and L40S. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N and L40S. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of R38N, L40S, and K76E. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N, L40S, and K76E.

[00122] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions of R38N and L40T. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N and L40T. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E,

Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N and L40T. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of R38N, L40T, and K76E. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N, L40T, and K76E.

[00123] In one embodiment, the IL-2 mutein provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 185 to 192, wherein the IL-2 mutein comprises an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; wherein the amino acid residue at position T3 is T or A, and the amino acid residue at position C125 is C or S; and wherein, when the IL-2 mutein comprises an amino acid substitution of L18C, the amino acid residue at position C125 is C.

[00124] In one embodiment, the IL-2 mutein provided herein is glycosylated. In another embodiment, the IL-2 mutein provided herein is *N*-glycosylated. In yet another embodiment, the IL-2 mutein provided herein is glycosylate at the nitrogen in the side chain of an asparagine residue. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) an amino acid substitution of R38N that is *N*-glycosylated.

[00125] In one embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions at positions R38 and L40. In another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution at position L18, Q126, or S130; (ii) amino acid substitutions at positions E15, R38, and L40; and (iii) optionally an amino acid substitution at position H16, D20, K76, S87, N88, or I92. In yet another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution of at position L18, Q126, or S130; (ii) amino acid substitutions at positions R38, L40, and K76; and (iii) optionally an amino acid substitution at position E15, H16, D20, S87, N88, or I92. In still another embodiment, the IL-2 mutein

provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution of at position L18, Q126, or S130; (ii) amino acid substitutions at positions E15, R38, L40, and K76; and (iii) optionally an amino acid substitution at position H16, D20, S87, N88, or I92.

[00126] In one embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions of R38N and L40S or L40T. In another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) comprising amino acid substitutions of E15K, R38N and L40S or L40T. In yet another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) comprising amino acid substitutions of E15K, R38N and L40S or L40T. In yet another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of R38N, L40S or L40T, and K76E. In still another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N, L40S or L40T, and K76E.

[00127] In one embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions of R38N and L40S. In another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N and L40S. In yet another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N and L40S. In yet another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid

substitutions of R38N, L40S, and K76E. In still another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N, L40S, and K76E.

[00128] In one embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions of R38N and L40T. In another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N and L40T. In yet another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N and L40T. In yet another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of R38N, L40T, and K76E. In still another embodiment, the IL-2 mutein provided herein is an *N*-glycosylated IL-2 mutein comprising: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38N, L40T, and K76E.

[00129] In one embodiment, the *N*-glycosylated IL-2 mutein provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 185 to 192, wherein the *N*-glycosylated IL-2 mutein comprises an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; wherein the amino acid residue at position T3 is T or A, and the amino acid residue at position C125 is C or S; and wherein, when the IL-2 mutein comprises an amino acid substitution of L18C, the amino acid residue at position C125 is C.

[00130] In one embodiment, the *N*-glycosylated IL-2 mutein provided herein comprises one glycan. In another embodiment, the *N*-glycosylated IL-2 mutein provided herein comprises one glycan attached to the nitrogen in the side chain of an asparagine residue. In yet another embodiment, the *N*-glycosylated IL-2 mutein provided herein comprises one glycan attached to

the nitrogen in the side chain of an asparagine residue at position R38N.

[00131] In one embodiment, the *N*-glycosylated IL-2 mutein provided herein comprises two glycans. In another embodiment, the *N*-glycosylated IL-2 mutein provided herein comprises two glycans, of which at least one glycan is attached to the nitrogen in the side chain of an asparagine residue. In yet another embodiment, the *N*-glycosylated IL-2 mutein provided herein comprises two glycans, each of which is attached to the nitrogen in the side chain of an asparagine residue.

[00132] In one embodiment, the *N*-glycosylated IL-2 mutein provided herein comprises three glycans. In one embodiment, the glycan is an *N*-glycan.

[00133] In one embodiment, the *N*-glycan on the *N*-glycosylated IL-2 mutein provided herein is oligomannose-type. In another embodiment, the *N*-glycan on the *N*-glycosylated IL-2 mutein provided herein is complex-type. In another embodiment, the *N*-glycan on the *N*-glycosylated IL-2 mutein provided herein is hydride-type.

[00134] In one embodiment, the *N*-glycan on the *N*-glycosylated IL-2 mutein provided herein is biantennary complex-type. In another embodiment, the *N*-glycan on the *N*-glycosylated IL-2 mutein provided herein is triantennary complex-type. In yet another embodiment, the *N*-glycan on the *N*-glycosylated IL-2 mutein provided herein is tetraantennary complex-type.

[00135] In one embodiment, the *N*-glycan on the *N*-glycosylated IL-2 mutein provided herein is one of the glycans described in Szabo *et al.*, *J. Proteome. Res.* **2018**, *17*, 1559-1574, the disclosure of which is incorporated herein by reference in its entirety.

[00136] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions at positions R38 and F42. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions at positions K32, R38, and F42. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions at positions K32, R38, and F42. In yet another embodiment, the IL-2 mutein

provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions at positions R38, F42, and K76. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions at positions E15, K32, R38, and F42. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions at positions E15, R38, F42, and K76.

[00137] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions of R38E and F42A or F42K. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of K32E, R38E, and F42A or F42K. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of K32E, R38E, and F42A or F42K. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of R38E, F42A or F42K, and K76E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, K32E, R38E, and F42A or F42K. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38E, F42A or F42K, and K76E.

[00138] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions at positions K32, R38, and F42. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of K32E, R38E, and F42A or F42K. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution

of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of K32E, R38E, and F42A or F42K. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of K32E, R38E, and F42A. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of K32E, R38E, and F42K.

[00139] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions at positions R38, F42, and K76. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of R38E, F42A or F42K, and K76E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of R38E, F42A or F42K, and K76E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of R38E, F42A, and K76E. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of R38E, F42K, and K76E.

[00140] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions at positions E15, K32, R38, and F42. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, K32E, R38E, and F42A or F42K. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, K32E, R38E, and F42A or F42K. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii)

amino acid substitutions of E15K, K32E, R38E, and F42A. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, K32E, R38E, and F42K.

[00141] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) amino acid substitutions at positions E15, R38, F42, and K76. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38E, F42A or F42K, and K76E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38E, F42A or F42K, and K76E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) amino acid substitutions of E15K, R38E, F42A, and K76E. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (b) amino acid substitutions of E15K, R38E, F42K, and K76E.

[00142] In one embodiment, the IL-2 mutein provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 193 to 204, wherein the IL-2 mutein comprises an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; wherein the amino acid residue at position T3 is T or A, and the amino acid residue at position C125 is C or S; and wherein, when the IL-2 mutein comprises an amino acid substitution of L18C, the amino acid residue at position C125 is C.

[00143] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a disulfide bond formed between two different amino acid residues from positions N30 to L80. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a disulfide bond formed between two different amino acid residues from positions K35

to L72. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a disulfide bond formed between two different amino acid residues from positions K35 to L69.

[00144] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a disulfide bond formed between two different amino acid residues, each independently at K35, R38, F42, Y45, E62, V69, or L72. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a disulfide bond formed between an amino acid residue at position K35, R38, F42, or Y45; and an amino acid residue at position E62, V69, or L72. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a disulfide bond formed between an amino acid residue at position F42 and an amino acid residue at position E62, V69, or L72. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a disulfide bond formed between an amino acid residue at position K35, R38, F42, or Y45, and an amino acid residue at position V69.

[00145] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) a disulfide bond formed between K35C and L72C, R38C and L72C, F42C and V69C, Y42C and L72C, or Y45C and E62C. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) a disulfide bond formed between K35C and L72C, R38C and L72C, F42C and V69C, Y42C and L72C, or Y45C and E62C. In yet another embodiment, the IL-2 domain in the fusion protein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) a disulfide bond formed between K35C and L72C. In yet another embodiment, the IL-2 domain in the fusion protein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) a disulfide bond formed between R38C and L72C. In yet another embodiment, the IL-2 domain in the fusion protein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) a disulfide bond formed between F42C and V69C. In yet another

embodiment, the IL-2 domain in the fusion protein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) a disulfide bond formed between Y42C and L72C. In still another embodiment, the IL-2 domain in the fusion protein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; and (ii) a disulfide bond formed between Y45C and E62C.

[00146] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a disulfide bond formed between two different amino acid residues from positions N30 to L80; and (iii) an amino acid substitution at position K8, K9, Q13, E15, H16, L19, D20, M23, K32, P34, K35, L36, T37, R38, M39, L40, T41, F42, K43, F44, Y45, M46, P47, E61, E62, L63, K64, P65, L66, E67, E68, V69, L70, N71, L72, A73, Q74, K76, H79, R81, D84, S87, N88, V91, I92, E95, Y107, D109, T111, or S127.

[00147] In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a disulfide bond formed between two different amino acid residues, each independently at position K35, R38, F42, Y45, E62, V69, or L72; and (iii) an amino acid substitution of K8D, K8E, K8Q, K9D, K9E, K9Q, Q13E, Q13N, E15K, E15Q, E15V, H16E, H16F, H16I, H16V, L19S, D20A, D20E, D20K, D20T, M23K, K32D, K32E, K32Q, P34N, K35N, L36S, L36T, T37N, R38E, R38N, M39N, L40S, L40T, T41N, F42A, F42C, F42K, F42N, K43N, F44N, Y45N, M46S, M46T, P47S, P47T, E61N, E62N, L63S, L63T, K64N, P65N, L66N, E67S, E67T, E68N, V69N, L70S, L70T, N71S, N71T, L72N, A73S, A73T, Q74S, Q74T, K76D, K76E, K76Q, H79D, H79E, H79Q, R81D, R81E, R81Q, D84T, S87D, S87E, N88A, V91I, I92A, I92D, I92E, I92G, E95K, E95N, E95Q, Y107N, D109N, T111S, S127A, S127E, S127F, or S127W.

[00148] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a disulfide bond formed between two different amino acid residues, each independently at position K35, R38, F42, Y45, E62, V69, or L72; and (iii) an amino acid substitution of K8D, K8E, K8Q, K9D, K9E, K9Q, Q13E, Q13N, E15K, E15Q, E15V, H16E,

H16F, H16I, H16V, L19S, D20A, D20E, D20K, D20T, M23K, K32D, K32E, K32Q, P34N, K35N, L36S, L36T, T37N, R38E, R38N, M39N, L40S, L40T, T41N, F42A, F42C, F42K, F42N, K43N, F44N, Y45N, M46S, M46T, P47S, P47T, E61N, E62N, L63S, L63T, K64N, P65N, L66N, E67S, E67T, E68N, V69N, L70S, L70T, N71S, N71T, L72N, A73S, A73T, Q74S, Q74T, K76D, K76E, K76Q, H79D, H79E, H79Q, R81D, R81E, R81Q, D84T, S87D, S87E, N88A, V91I, I92A, I92D, I92E, I92G, E95K, E95N, E95Q, Y107N, D109N, T111S, S127A, S127E, S127F, or S127W.

[00149] In yet another embodiment, the IL-2 mutein provided herein comprises (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a disulfide bond formed between F42C and V69C; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[00150] In yet another embodiment, the IL-2 mutein provided herein comprises (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a disulfide bond formed between F42C and V69C; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[00151] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a disulfide bond formed between F42C and V69C; and (iii) an amino acid substitution of E15K, H16E, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[00152] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a disulfide bond formed between F42C and V69C; and (iii) an amino acid substitution of E15K, H16E, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[00153] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an

amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a disulfide bond formed between F42C and V69C; and (iii) an amino acid substitution of E15K, K32E, or K76E.

[00154] In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a disulfide bond formed between F42C and V69C; and (iii) an amino acid substitution of E15K, K32E, or K76E.

[00155] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a disulfide bond formed between F42C and V69C. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; (ii) a disulfide bond formed between F42C and V69C; an amino acid substitution at position K32 or K76; and (iii) optionally an amino acid substitution at position E15. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a disulfide bond formed between F42C and V69C; and (iii) an amino acid substitution of K32E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a disulfide bond formed between F42C and V69C; and (iii) an amino acid substitution of K76E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a disulfide bond formed between F42C and V69C; and (iii) amino acid substitutions of E15K and K32E. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a disulfide bond formed between F42C and V69C; and (iii) amino acid substitutions of E15K and K76E.

[00156] In one embodiment, the IL-2 mutein provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 205 to 216, wherein the IL-2 mutein comprises an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; wherein the amino acid residue at position T3 is T or A, and the amino acid residue at

position C125 is C or S; and wherein, when the IL-2 mutein comprises an amino acid substitution of L18C, the amino acid residue at position C125 is C.

[00157] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; (ii) a replacement of the amino acid residues from positions N29 to L40 with an IL-15 hinge fragment-containing peptide. In another embodiment, the IL-2 mutein provided herein comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219. In yet another embodiment, the IL-2 mutein provided herein comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218. In still another embodiment, the IL-2 mutein provided herein comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 219.

[00158] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; (ii) a replacement of the amino acid residues from positions N29 to L40 with an IL-15 hinge fragment-containing peptide; and (iii) an amino acid substitution at position K8, K9, Q13, E15, H16, L19, D20, M23, E61, E62, L63, K64, P65, L66, E67, E68, V69, L70, N71, L72, A73, Q74, K76, H79, R81, D84, S87, N88, V91, I92, E95, Y107, D109, T111, or S127.

[00159] In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; and (iii) an amino acid substitution of K8D, K8E, K8Q, K9D, K9E, K9Q, Q13E, Q13N, E15K, E15Q, E15V, H16D, H16E, H16F, H16I, H16N, H16Q, H16V, L19S, D20A, D20E, D20K, D20T, M23K, E61N, E62N, L63S, L63T, K64N, P65N, L66N, E67S, E67T, E68N, V69N, L70S, L70T, N71S, N71T, L72N, A73S, A73T, Q74S, Q74T, K76D, K76E, K76Q,

H79D, H79E, H79Q, R81D, R81E, R81Q, D84T, S87D, S87E, N88A, V91I, I92A, I92D, I92E, I92G, I92L, E95K, E95N, E95Q, Y107N, D109N, T111S, S127A, S127E, S127F, or S127W.

[00160] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; and (iii) an amino acid substitution of K8D, K8E, K8Q, K9D, K9E, K9Q, Q13E, Q13N, E15K, E15Q, E15V, H16D, H16E, H16F, H16I, H16N, H16Q, H16V, L19S, D20A, D20E, D20K, D20T, M23K, E61N, E62N, L63S, L63T, K64N, P65N, L66N, E67S, E67T, E68N, V69N, L70S, L70T, N71S, N71T, L72N, A73S, A73T, Q74S, Q74T, K76D, K76E, K76Q, H79D, H79E, H79Q, R81D, R81E, R81Q, D84T, S87D, S87E, N88A, V91I, I92A, I92D, I92E, I92G, I92L, E95K, E95N, E95Q, Y107N, D109N, T111S, S127A, S127E, S127F, or S127W.

[00161] In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; and (iii) an amino acid substitution of K8D, K8E, K8Q, K9D, K9E, K9Q, Q13E, Q13N, E15K, E15Q, E15V, H16D, H16E, H16F, H16I, H16N, H16Q, H16V, L19S, D20A, D20E, D20K, D20T, M23K, E61N, E62N, L63S, L63T, K64N, P65N, L66N, E67S, E67T, E68N, V69N, L70S, L70T, N71S, N71T, L72N, A73S, A73T, Q74S, Q74T, K76D, K76E, K76Q, H79D, H79E, H79Q, R81D, R81E, R81Q, D84T, S87D, S87E, N88A, V91I, I92A, I92D, I92E, I92G, I92L, E95K, E95N, E95Q, Y107N, D109N, T111S, S127A, S127E, S127F, or S127W.

[00162] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; (ii) a replacement of the amino acid residues from positions N29 to L40 with an IL-15 hinge fragment-containing peptide; and (iii) an amino acid substitution at position E15, H16, D20, S87, N88, or I92.

[00163] In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40

having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00164] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00165] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00166] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00167] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00168] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00169] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00170] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00171] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00172] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an

amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 219; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00173] In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 219; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00174] In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 219; and (iii) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00175] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution at E15. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii)

an amino acid substitution of E15K. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of E15K.

[00176] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of H16. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of H16E, H16F, H16I, or H16V. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of H16E, H16F, H16I, or H16V.

[00177] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of D20. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii)

an amino acid substitution of D20A, D20E, D20K, or D20T. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of D20A, D20E, D20K, or D20T.

[00178] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of S87. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of S87D. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of S87D.

[00179] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of N88. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii)

an amino acid substitution of N88A. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of N88A.

[00180] In one embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution at position L18, Q126, or S130; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92. In another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92A. In

yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92A. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92A. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92D. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92D. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92D. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid

substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92E. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92G. In yet another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92G. In still another embodiment, the IL-2 mutein provided herein comprises: (i) an amino acid substitution of L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219; and (iii) an amino acid substitution of I92G.

[00181] In one embodiment, the IL-2 mutein provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 220 to 261, wherein the IL-2 mutein comprises an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; wherein the amino acid residue at position T3 is T or A, and the amino acid residue at position C125 is C or S; and wherein, when the IL-2 mutein comprises an amino acid substitution of L18C, the amino acid residue at position C125 is C. In another embodiment, the IL-2 mutein provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 220

to 261, wherein the IL-2 mutein comprises an amino acid substitution of L18C, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; wherein the amino acid residue at position T3 is T or A, and the amino acid residue at position C125 is C or S; and wherein, when the IL-2 mutein comprises an amino acid substitution of L18C, the amino acid residue at position C125 is C. In yet another embodiment, the IL-2 mutein provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 220 to 261, wherein the IL-2 mutein comprises an amino acid substitution of L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; wherein the amino acid residue at position T3 is T or A, and the amino acid residue at position C125 is C or S; and wherein, when the IL-2 mutein comprises an amino acid substitution of L18C, the amino acid residue at position C125 is C. In yet another embodiment, the IL-2 mutein provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 262 to 287. In still another embodiment, the IL-2 mutein provided herein comprises an amino acid sequence of SEQ ID NO: 301.

[00182] In certain embodiments, the IL-2 mutein provided herein has an attenuated binding affinity to an IL-2R α as compared to a wild-type IL-2. In certain embodiments, the binding affinity of the IL-2 mutein provided herein to an IL-2R α is measured by its K_a , which is the inverse of its K_d .

[00183] In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 2 times, no less than about 5 times, no less than about 10 times, no less than about 100 times, no less than about 200 times, no less than about 200 times, or no less than about 1,000 times higher than that of the wild-type IL-2 to the IL-2R α . In one embodiment, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 2 times higher than that of the wild-type IL-2 to the IL-2R α . In another embodiment, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 5 times higher than that of the wild-type IL-2 to the IL-2R α . In yet another embodiment, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 10 times higher than that of the wild-type IL-2 to the IL-2R α . In yet another embodiment, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 100 times higher than that of the wild-type IL-2 to the IL-2R α . In yet another embodiment, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 200 times higher than that of the wild-type IL-2 to the IL-2R α . In yet another embodiment, the IL-2 mutein provided herein

has a K_d to the IL-2R α of no less than about 500 times higher than that of the wild-type IL-2 to the IL-2R α . In still another embodiment, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 1,000 times higher than that of the wild-type IL-2 to the IL-2R α .

[00184] In certain embodiments, the IL-2 mutein provided herein has a K_d to an IL-2R α of no less than about 20 nM, no less than about 50 nM, no less than about 100 nM, no less than about 1 μ M, no less than about 10 μ M, no less than about 100 μ M, no less than about 200 μ M, no less than about 500 μ M, or no less than about 1 mM. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 20 nM. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 50 nM. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 100 nM. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 1 μ M. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 10 μ M. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 100 μ M. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 200 μ M. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 500 μ M. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R α of no less than about 1 mM. In certain embodiments, the IL-2 mutein provided herein has no measurable binding to the IL-2R α . In certain embodiments, the IL-2 mutein provided herein has no detectable binding to the IL-2R α as measured with a surface plasmon resonance (SPR) method. In certain embodiments, the IL-2 mutein provided herein has no detectable binding to the IL-2R α as measured with bio-layer interferometry (BLI).

[00185] In certain embodiments, the IL-2 mutein provided herein has a reduced binding affinity to an IL-2R β/γ complex as compared to a wild-type IL-2. In certain embodiments, the binding affinity of the IL-2 mutein provided herein to an IL-2R β/γ complex is measured by its K_a , which is the inverse of its K_d .

[00186] In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R β/γ complex of no less than about 5 times, no less than about 10 times, no less than about 20 times, no less than about 50 times, or no less than about 100 times higher than that of the wild-type IL-2

to the IL-2R β / γ complex. In one embodiment, the IL-2 mutein provided herein has a K_d to the IL-2R β / γ complex of no less than about 5 times higher than that of the wild-type IL-2 to the IL-2R β / γ complex. In another embodiment, the IL-2 mutein provided herein has a K_d to the IL-2R β / γ complex of no less than about 10 times higher than that of the wild-type IL-2 to the IL-2R β / γ complex. In yet another embodiment, the IL-2 mutein provided herein has a K_d to the IL-2R β / γ complex of no less than about 20 times higher than that of the wild-type IL-2 to the IL-2R β / γ complex. In yet another embodiment, the IL-2 mutein provided herein has a K_d to the IL-2R β / γ complex of no less than about 50 times higher than that of the wild-type IL-2 to the IL-2R β / γ complex. In still another embodiment, the IL-2 mutein provided herein has a K_d to the IL-2R β / γ complex of no less than about 100 times higher than that of the wild-type IL-2 to the IL-2R β / γ complex.

[00187] In certain embodiments, the IL-2 mutein provided herein has a K_d to an IL-2R β / γ complex of no less than about 5 nM, no less than about 10 nM, no less than about 20 nM, no less than about 50 nM, no less than about 100 nM, or no less than about 200 nM. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R β / γ complex of no less than about 5 nM. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R β / γ complex of no less than about 10 nM. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R β / γ complex of no less than about 20 nM. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R β / γ complex of no less than about 50 nM. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R β / γ complex of no less than about 100 nM. In certain embodiments, the IL-2 mutein provided herein has a K_d to the IL-2R β / γ complex of no less than about 200 nM.

[00188] In one embodiment, the IL-2R α is a human IL-2R α . In another embodiment, the human IL-2R α has an amino acid sequence of SEQ ID NO: 289.

[00189] In one embodiment, the IL-2R β is a human IL-2R β . In another embodiment, the human IL-2R β has an amino acid sequence of SEQ ID NO: 290.

[00190] In one embodiment, the IL-2R γ is a human IL-2R γ . In another embodiment, the human IL-2R γ has an amino acid sequence of SEQ ID NO: 291.

[00191] In one embodiment, a K_d of an IL-2 to an IL-2R α is determined with a surface plasmon resonance (SPR) method. In another embodiment, a K_d of an IL-2 to an IL-2R α is determined with a BIACORE® assay. In yet another embodiment, a K_d of an IL-2 to an IL-2R α is determined with bio-layer interferometry (BLI). In still another embodiment, a K_d of an IL-2 to an IL-2R α is determined with an OCTET® assay.

[00192] In one embodiment, a K_d of an IL-2 to an IL-2R β is determined with an SPR method. In another embodiment, a K_d of an IL-2 to an IL-2R β is determined with a BIACORE® assay. In yet another embodiment, a K_d of an IL-2 to an IL-2R β is determined with BLI. In still another embodiment, a K_d of an IL-2 to an IL-2R β is determined with an OCTET® assay.

[00193] In one embodiment, a K_d of an IL-2 to an IL-2R β/γ complex is determined with an SPR method. In another embodiment, a K_d of an IL-2 to an IL-2R β/γ complex is determined with a BIACORE® assay. In yet another embodiment, a K_d of an IL-2 to an IL-2R β/γ complex is determined with BLI. In still another embodiment, a K_d of an IL-2 to an IL-2R β/γ complex is determined with an OCTET® assay.

[00194] In one embodiment, a K_d of an IL-2 to an IL-2R $\alpha/\beta/\gamma$ complex is determined with an SPR method. In another embodiment, a K_d of an IL-2 to an IL-2R $\alpha/\beta/\gamma$ complex is determined with a BIACORE® assay. In yet another embodiment, a K_d of an IL-2 to an IL-2R $\alpha/\beta/\gamma$ complex is determined with BLI. In still another embodiment, a K_d of an IL-2 to an IL-2R $\alpha/\beta/\gamma$ complex is determined with an OCTET® assay.

[00195] In certain embodiments, the IL-2 mutein provided herein comprises an amino acid sequence that is no less than about 80%, no less than about 85%, no less than about 90%, no less than about 91%, no less than about 92%, no less than about 93%, no less than about 94%, no less than about 95%, no less than about 96%, no less than about 97%, no less than about 98%, or no less than about 99% identical to the amino acid sequence of SEQ ID NO: 179, 180, 181, 182, 183, or 184. In certain embodiments, the IL-2 mutein provided herein comprises an amino acid sequence that is no less than about 80% identical to the amino acid sequence of SEQ ID NO: 179, 180, 181, 182, 183, or 184. In certain embodiments, the IL-2 mutein provided herein comprises an amino acid sequence that is no less than about 85% identical to the amino acid sequence of SEQ ID NO: 179, 180, 181, 182, 183, or 184. In certain embodiments, the IL-2

mutein provided herein comprises an amino acid sequence that is no less than about 90% identical to the amino acid sequence of SEQ ID NO: 179, 180, 181, 182, 183, or 184. In certain embodiments, the IL-2 mutein provided herein comprises an amino acid sequence that is no less than about 91% identical to the amino acid sequence of SEQ ID NO: 179, 180, 181, 182, 183, or 184. In certain embodiments, the IL-2 mutein provided herein comprises an amino acid sequence that is no less than about 92% identical to the amino acid sequence of SEQ ID NO: 179, 180, 181, 182, 183, or 184. In certain embodiments, the IL-2 mutein provided herein comprises an amino acid sequence that is no less than about 93% identical to the amino acid sequence of SEQ ID NO: 179, 180, 181, 182, 183, or 184. In certain embodiments, the IL-2 mutein provided herein comprises an amino acid sequence that is no less than about 94% identical to the amino acid sequence of SEQ ID NO: 179, 180, 181, 182, 183, or 184. In certain embodiments, the IL-2 mutein provided herein comprises an amino acid sequence that is no less than about 95% identical to the amino acid sequence of SEQ ID NO: 179, 180, 181, 182, 183, or 184. In certain embodiments, the IL-2 mutein provided herein comprises an amino acid sequence that is no less than about 96% identical to the amino acid sequence of SEQ ID NO: 179, 180, 181, 182, 183, or 184. In certain embodiments, the IL-2 mutein provided herein comprises an amino acid sequence that is no less than about 97% identical to the amino acid sequence of SEQ ID NO: 179, 180, 181, 182, 183, or 184. In certain embodiments, the IL-2 mutein provided herein comprises an amino acid sequence that is no less than about 98% identical to the amino acid sequence of SEQ ID NO: 179, 180, 181, 182, 183, or 184. In certain embodiments, the IL-2 mutein provided herein comprises an amino acid sequence that is no less than about 99% identical to the amino acid sequence of SEQ ID NO: 179, 180, 181, 182, 183, or 184.

[00196] In certain embodiments, the IL-2 mutein provided herein comprises one of the amino acid sequences of interleukin-2 variants and muteins described in CN 111018961 A, US 2018/0326010 A1, and WO 2020/005819 A1, the disclosure of each of which is incorporated herein by reference in its entirety; wherein the IL-2 mutein comprises an amino acid substitution at position L18, Q126, or S130.

[00197] In certain embodiments, the IL-2 mutein provided herein further includes one or more additional substitutions, deletions, and/or insertions; and/or one or more additional post-

translational modifications.

Fusion Proteins

[00198] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain and a half-life-extension domain.

[00199] In one embodiment, the half-life-extension domain comprises an albumin binding domain, a constant region of an antibody, a constant region of a heavy chain of an antibody, a fragment crystallizable (Fc) domain, a serum albumin, a polyethylene glycol (PEG) group, or a fatty acyl group. In another embodiment, the half-life-extension domain is an albumin binding domain. In yet another embodiment, the half-life-extension domain is a constant region of an antibody, comprising two light chain constant domains (C_L) and two heavy chain constant domains (C_{H1}, C_{H2}, and C_{H3}). In yet another embodiment, the half-life-extension domain is a constant region (C_{H1}, C_{H2}, and C_{H3}) of a heavy chain of an antibody. In yet another embodiment, the half-life-extension domain is an Fc domain. In yet another embodiment, the half-life-extension domain is an Fc domain comprising first and second peptide chains. In yet another embodiment, the half-life-extension domain is a serum albumin. In yet another embodiment, the half-life-extension domain is a PEG group. In still another embodiment, the half-life-extension domain is a fatty acyl group.

[00200] In certain embodiments, the half-life-extension domain extends the half-life of the IL-2 domain *in vivo* as compared to the corresponding free IL-2. In certain embodiments, the half-life-extension domain extends the half-life of the IL-2 domain *in vivo* as compared to a wild-type IL-2 of SEQ ID NO: 179, 180, 181, 182, 183, or 184. In certain embodiments, the half-life-extension domain extends the half-life of the IL-2 domain *in vivo* as compared to the corresponding free IL-2.

[00201] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain and an albumin binding domain as a half-life-extension domain.

[00202] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, an albumin binding domain, and optionally a peptide linker; wherein the carboxy-terminus (C-terminus) of the IL-2 domain is connected to an amino-terminus (N-terminus) of the albumin

binding domain directly or *via* the peptide linker; or wherein a *C*-terminus of the albumin binding domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the peptide linker.

[00203] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, an albumin binding domain, and optionally a peptide linker; wherein the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the albumin binding domain directly or *via* the peptide linker.

[00204] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, an albumin binding domain, and optionally a peptide linker; wherein a *C*-terminus of the albumin binding domain is connected to a *N*-terminus of the IL-2 domain directly or *via* the peptide linker.

[00205] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and an albumin binding domain as a half-life-extension domain.

[00206] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and optionally first and second peptide linkers; wherein a *C*-terminus of the PD-1 binding domain is connected to an *N*-terminus of the albumin binding domain directly or *via* the first peptide linker, and a *C*-terminus of the albumin binding domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the second peptide linker.

[00207] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and optionally first and second peptide linkers; wherein the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the albumin binding domain directly or *via* the first peptide linker, and a *C*-terminus of the albumin binding domain is connected to an *N*-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

[00208] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and optionally first and second peptide linkers; wherein a *C*-terminus of the albumin binding domain is connected to an *N*-

terminus of the PD-1 binding domain directly or *via* the first peptide linker, and an *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the second peptide linker.

[00209] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and optionally first and second peptide linkers; wherein the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the PD-1 binding domain directly or *via* the first peptide linker, and a *C*-terminus of the PD-1 binding domain is connected to an *N*-terminus of the albumin binding domain directly or *via* the second peptide linker.

[00210] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and optionally first and second peptide linkers; wherein a *C*-terminus of the albumin binding domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the first peptide linker, and the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

[00211] In still another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and optionally first and second peptide linkers; wherein a *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the first peptide linker, and the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the albumin binding domain directly or *via* the second peptide linker.

[00212] In one embodiment, the albumin binding domain is an antibody or a fragment thereof that binds to an albumin. In another embodiment, the albumin binding domain is an antibody or a fragment thereof that binds to a human serum albumin (HSA). In yet another embodiment, the albumin binding domain is an antibody or a fragment thereof that binds to an HSA specifically.

[00213] In certain embodiments, the anti-HSA antibody binds to an HSA with a K_d ranging from about 10 pM to about 1,000 nM, from about 1 to about 500 nM, from about 1 to

about 200 nM, or from about 1 to about 100 nM at a pH of about 7. In certain embodiments, the anti-HSA antibody binds to an HSA with a K_d ranging from about 10 pM to about 1,000 nM at a pH of about 7. In certain embodiments, the anti-HSA antibody binds to an HSA with a K_d ranging from about 1 nM to about 500 nM at a pH of about 7. In certain embodiments, the anti-HSA antibody binds to an HSA with a K_d ranging from about 1 nM to about 200 nM at a pH of about 7. In certain embodiments, the anti-HSA antibody binds to an HSA with a K_d ranging from about 1 nM to about 100 nM at a pH of about 7.

[00214] In one embodiment, the albumin binding domain is an anti-HSA antibody or a fragment thereof, comprising (i) a complementarity determining region 1 (CDR1) of SEQ ID NO: 1, a complementarity determining region 2 (CDR2) of SEQ ID NO: 2, and a complementarity determining region 3 (CDR3) of SEQ ID NO: 3; or (ii) a CDR1 of SEQ ID NO: 9, a CDR2 of SEQ ID NO: 10, and a CDR3 of SEQ ID NO: 11. In another embodiment, the albumin binding domain is an anti-HSA antibody or a fragment thereof, comprising a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2, and a CDR3 of SEQ ID NO: 3. In yet another embodiment, the albumin binding domain comprises a CDR1 of SEQ ID NO: 9, a CDR2 of SEQ ID NO: 10, and a CDR3 of SEQ ID NO: 11.

[00215] In yet another embodiment, the albumin binding domain is an anti-HSA antibody or a fragment thereof, comprising an amino acid sequence of SEQ ID NO: 8 or 15. In yet another embodiment, the albumin binding domain is an anti-HSA antibody or a fragment thereof, comprising an amino acid sequence of SEQ ID NO: 8. In still another embodiment, the albumin binding domain comprises an amino acid sequence of SEQ ID NO: 15.

[00216] In certain embodiments, the albumin binding domain is an anti-HSA antibody disclosed in WO 2019/246004 A1 or WO 2020/172528 A1, the disclosure of each of which is incorporated herein by reference in its entirety.

[00217] In certain embodiments, the antibody is a human antibody. In certain embodiments, the antibody is a humanized antibody.

[00218] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain and an anti-HSA antibody.

[00219] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, an anti-HSA antibody, and optionally a peptide linker; wherein the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the anti-HSA antibody directly or *via* the peptide linker; or wherein a *C*-terminus of the anti-HSA antibody is connected to the *N*-terminus of the IL-2 domain directly or *via* the peptide linker.

[00220] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, an anti-HSA antibody, and optionally a peptide linker; wherein the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the anti-HSA antibody directly or *via* the peptide linker.

[00221] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, an anti-HSA antibody, and optionally a peptide linker; wherein a *C*-terminus of the anti-HSA antibody is connected to a *N*-terminus of the IL-2 domain directly or *via* the peptide linker.

[00222] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and an anti-HSA antibody.

[00223] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA antibody, and optionally first and second peptide linkers; wherein a *C*-terminus of the PD-1 binding domain is connected to an *N*-terminus of the anti-HSA antibody directly or *via* the first peptide linker, and a *C*-terminus of the anti-HSA antibody is connected to the *N*-terminus of the IL-2 domain directly or *via* the second peptide linker.

[00224] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA antibody, and optionally first and second peptide linkers; wherein the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the anti-HSA antibody directly or *via* the first peptide linker, and a *C*-terminus of the anti-HSA antibody is connected to an *N*-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

[00225] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA antibody, and optionally first and second peptide linkers; wherein a *C*-terminus of the anti-HSA antibody is connected to an *N*-terminus of the PD-

1 binding domain directly or *via* the first peptide linker, and an *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the second peptide linker.

[00226] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA antibody, and optionally first and second peptide linkers; wherein the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the PD-1 binding domain directly or *via* the first peptide linker, and a *C*-terminus of the PD-1 binding domain is connected to an *N*-terminus of the anti-HSA antibody directly or *via* the second peptide linker.

[00227] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA antibody, and optionally first and second peptide linkers; wherein a *C*-terminus of the anti-HSA antibody is connected to the *N*-terminus of the IL-2 domain directly or *via* the first peptide linker, and the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

[00228] In still another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA antibody, and optionally first and second peptide linkers; wherein a *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the first peptide linker, and the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the anti-HSA antibody directly or *via* the second peptide linker.

[00229] In certain embodiments, the anti-HSA antibody is an anti-HSA single domain antibody (sdAb).

[00230] In certain embodiments, the anti-HSA sdAb binds to an HSA with a K_d ranging from about 10 pM to about 1,000 nM, from about 1 to about 500 nM, from about 1 to about 200 nM, or from about 1 to about 100 nM at a pH of about 7. In certain embodiments, the anti-HSA sdAb binds to an HSA with a K_d ranging from about 10 pM to about 1,000 nM at a pH of about 7. In certain embodiments, the anti-HSA sdAb binds to an HSA with a K_d ranging from about 1 to about 500 nM at a pH of about 7. In certain embodiments, the anti-HSA sdAb binds to an HSA with a K_d ranging from about 1 to about 200 nM at a pH of about 7. In certain

embodiments, the anti-HSA sdAb binds to an HSA with a K_d ranging from about 1 to about 100 nM at a pH of about 7.

[00231] In one embodiment, the anti-HSA sdAb comprises (i) a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2, and a CDR3 of SEQ ID NO: 3; or (ii) a CDR1 of SEQ ID NO: 9, a CDR2 of SEQ ID NO: 10, and a CDR3 of SEQ ID NO: 11. In another embodiment, the anti-HSA sdAb comprises a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2, and a CDR3 of SEQ ID NO: 3. In yet another embodiment, the anti-HSA sdAb comprises a CDR1 of SEQ ID NO: 9, a CDR2 of SEQ ID NO: 10, and a CDR3 of SEQ ID NO: 11.

[00232] In one embodiment, the anti-HSA sdAb has the structure of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

CDR1, CDR2, and CDR3 are:

- (i) a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2, and a CDR3 of SEQ ID NO: 3; or
- (ii) a CDR1 of SEQ ID NO: 9, a CDR2 of SEQ ID NO: 10, and a CDR3 of SEQ ID NO: 11;

FR1 is an amino acid sequence of SEQ ID NO: 4 or 12;

FR2 is an amino acid sequence of SEQ ID NO: 5 or 13;

FR3 is an amino acid sequence of SEQ ID NO: 6; and

FR4 is an amino acid sequence of SEQ ID NO: 7 or 14.

[00233] In another embodiment, the anti-HSA sdAb has the structure of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

CDR1, CDR2, and CDR3 are:

- (i) a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2, and a CDR3 of SEQ ID NO: 3; or
- (ii) a CDR1 of SEQ ID NO: 9, a CDR2 of SEQ ID NO: 10, and a CDR3 of SEQ ID NO: 11;

FR1 is an amino acid sequence of SEQ ID NO: 4;

FR2 is an amino acid sequence of SEQ ID NO: 5;

FR3 is an amino acid sequence of SEQ ID NO: 6; and

FR3 is an amino acid sequence of SEQ ID NO: 7.

[00234] In yet another embodiment, the anti-HSA sdAb has the structure of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

CDR1, CDR2, and CDR3 are:

- (i) a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2, and a CDR3 of SEQ ID NO: 3; or
- (ii) a CDR1 of SEQ ID NO: 9, a CDR2 of SEQ ID NO: 10, and a CDR3 of SEQ ID NO: 11;

FR1 is an amino acid sequence of SEQ ID NO: 12;

FR2 is an amino acid sequence of SEQ ID NO: 13;

FR3 is an amino acid sequence of SEQ ID NO: 6; and

FR3 is an amino acid sequence of SEQ ID NO: 14.

[00235] In one embodiment, the anti-HSA sdAb has an amino acid sequence of SEQ ID NO: 8 or 15. In another embodiment, the anti-HSA sdAb has an amino acid sequence of SEQ ID NO: 8. In yet another embodiment, the anti-HSA sdAb has an amino acid sequence of SEQ ID NO: 15.

[00236] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain and an anti-HSA sdAb.

[00237] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, an anti-HSA sdAb, and optionally a peptide linker; wherein the C-terminus of the IL-2 domain is connected to the N-terminus of the anti-HSA sdAb directly or *via* the peptide linker; or wherein the C-terminus of the anti-HSA sdAb is connected to the N-terminus of the IL-2 domain directly or *via* the peptide linker.

[00238] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, an anti-HSA sdAb, and optionally a peptide linker; wherein the C-terminus of the IL-2 domain is connected to the N-terminus of the anti-HSA sdAb directly or *via* the peptide linker.

[00239] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, an anti-HSA sdAb, and optionally a peptide linker; wherein the C-terminus of the anti-

HSA sdAb is connected to a *N*-terminus of the IL-2 domain directly or *via* the peptide linker.

[00240] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and an anti-HSA sdAb.

[00241] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA sdAb, and optionally first and second peptide linkers; wherein a *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the anti-HSA sdAb directly or *via* the first peptide linker, and the *C*-terminus of the anti-HSA sdAb is connected to the *N*-terminus of the IL-2 domain directly or *via* the second peptide linker.

[00242] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA sdAb, and optionally first and second peptide linkers; wherein the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the anti-HSA sdAb directly or *via* the first peptide linker, and the *C*-terminus of the anti-HSA sdAb is connected to an *N*-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

[00243] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA sdAb, and optionally first and second peptide linkers; wherein the *C*-terminus of the anti-HSA sdAb is connected to a *N*-terminus of the PD-1 binding domain directly or *via* the first peptide linker, and an *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the second peptide linker.

[00244] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA sdAb, and optionally first and second peptide linkers; wherein the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the PD-1 binding domain directly or *via* the first peptide linker, and a *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the anti-HSA sdAb directly or *via* the second peptide linker.

[00245] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA sdAb, and optionally first and second peptide linkers; wherein the *C*-terminus of the anti-HSA sdAb is connected to the *N*-terminus of the IL-2

domain directly or *via* the first peptide linker, and the C-terminus of the IL-2 domain is connected to an N-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

[00246] In still another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA sdAb, and optionally first and second peptide linkers; wherein a C-terminus of the PD-1 binding domain is connected to the N-terminus of the IL-2 domain directly or *via* the first peptide linker, and the C-terminus of the IL-2 domain is connected to the N-terminus of the anti-HSA sdAb directly or *via* the second peptide linker.

[00247] In certain embodiments, the anti-HSA antibody is an anti-HSA V_HH sdAb.

[00248] In certain embodiments, the anti-HSA V_HH sdAb binds to an HSA with a K_d ranging from about 10 pM to about 1,000 nM, from about 1 to about 500 nM, from about 1 to about 200 nM, or from about 1 to about 100 nM at a pH of about 7. In certain embodiments, the anti-HSA V_HH sdAb binds to an HSA with a K_d ranging from about 10 pM to about 1,000 nM at a pH of about 7. In certain embodiments, the anti-HSA V_HH sdAb binds to an HSA with a K_d ranging from about 1 to about 500 nM at a pH of about 7. In certain embodiments, the anti-HSA V_HH sdAb binds to an HSA with a K_d ranging from about 1 to about 200 nM at a pH of about 7. In certain embodiments, the anti-HSA V_HH sdAb binds to an HSA with a K_d ranging from about 1 to about 100 nM at a pH of about 7.

[00249] In one embodiment, the anti-HSA V_HH sdAb comprises (i) a heavy chain CDR1 of SEQ ID NO: 1, a heavy chain CDR2 of SEQ ID NO: 2, and a heavy chain CDR3 of SEQ ID NO: 3; or (ii) a heavy chain CDR1 of SEQ ID NO: 9, a heavy chain CDR2 of SEQ ID NO: 10, and a heavy chain CDR3 of SEQ ID NO: 11. In another embodiment, the anti-HSA V_HH sdAb comprises a heavy chain CDR1 of SEQ ID NO: 1, a heavy chain CDR2 of SEQ ID NO: 2, and a heavy chain CDR3 of SEQ ID NO: 3. In yet another embodiment, the anti-HSA V_HH sdAb comprises a heavy chain CDR1 of SEQ ID NO: 9, a heavy chain CDR2 of SEQ ID NO: 10, and a heavy chain CDR3 of SEQ ID NO: 11.

[00250] In one embodiment, the anti-HSA V_HH sdAb has the structure of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

CDR1, CDR2, and CDR3 are:

- (i) a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2, and a CDR3 of SEQ ID NO: 3; or
- (ii) a CDR1 of SEQ ID NO: 9, a CDR2 of SEQ ID NO: 10, and a CDR3 of SEQ ID NO: 11;

FR1 is an amino acid sequence of SEQ ID NO: 4 or 12;

FR2 is an amino acid sequence of SEQ ID NO: 5 or 13;

FR3 is an amino acid sequence of SEQ ID NO: 6; and

FR4 is an amino acid sequence of SEQ ID NO: 7 or 14.

[00251] In another embodiment, the anti-HSA V_HH sdAb has the structure of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

CDR1, CDR2, and CDR3 are:

- (i) a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2, and a CDR3 of SEQ ID NO: 3; or
- (ii) a CDR1 of SEQ ID NO: 9, a CDR2 of SEQ ID NO: 10, and a CDR3 of SEQ ID NO: 11;

FR1 is an amino acid sequence of SEQ ID NO: 4;

FR2 is an amino acid sequence of SEQ ID NO: 5;

FR3 is an amino acid sequence of SEQ ID NO: 6; and

FR3 is an amino acid sequence of SEQ ID NO: 7.

[00252] In yet another embodiment, the anti-HSA V_HH sdAb has the structure of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

CDR1, CDR2, and CDR3 are:

- (i) a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2, and a CDR3 of SEQ ID NO: 3; or
- (ii) a CDR1 of SEQ ID NO: 9, a CDR2 of SEQ ID NO: 10, and a CDR3 of SEQ ID NO: 11;

FR1 is an amino acid sequence of SEQ ID NO: 12;

FR2 is an amino acid sequence of SEQ ID NO: 13;

FR3 is an amino acid sequence of SEQ ID NO: 6; and

FR3 is an amino acid sequence of SEQ ID NO: 14.

[00253] In one embodiment, the anti-HSA V_HH sdAb has an amino acid sequence of SEQ ID NO: 8 or 15. In another embodiment, the anti-HSA V_HH sdAb has an amino acid sequence of SEQ ID NO: 8. In yet another embodiment, the anti-HSA V_HH sdAb has an amino acid sequence of SEQ ID NO: 15.

[00254] In certain embodiments, the anti-HSA V_HH sdAb is a human antibody. In certain embodiments, the anti-HSA V_HH sdAb is a humanized antibody.

[00255] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain and an anti-HSA V_HH sdAb.

[00256] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, an anti-HSA V_HH sdAb, and optionally a peptide linker; wherein the C-terminus of the IL-2 domain is connected to the N-terminus of the anti-HSA V_HH sdAb directly or *via* the peptide linker; or wherein the C-terminus of the anti-HSA V_HH sdAb is connected to the N-terminus of the IL-2 domain directly or *via* the peptide linker.

[00257] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, an anti-HSA V_HH sdAb, and optionally a peptide linker; wherein the C-terminus of the IL-2 domain is connected to the N-terminus of the anti-HSA V_HH sdAb directly or *via* the peptide linker.

[00258] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, an anti-HSA V_HH sdAb, and optionally a peptide linker; wherein the C-terminus of the anti-HSA V_HH sdAb is connected to a N-terminus of the IL-2 domain directly or *via* the peptide linker.

[00259] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and an anti-HSA V_HH sdAb.

[00260] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA V_HH sdAb, and optionally first and second peptide linkers; wherein a C-terminus of the PD-1 binding domain is connected to the N-terminus of the anti-HSA V_HH sdAb directly or *via* the first peptide linker, and the C-terminus of the anti-HSA V_HH

sdAb is connected to the *N*-terminus of the IL-2 domain directly or *via* the second peptide linker.

[00261] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA V_HH sdAb, and optionally first and second peptide linkers; wherein the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the anti-HSA V_HH sdAb directly or *via* the first peptide linker, and the *C*-terminus of the anti-HSA V_HH sdAb is connected to an *N*-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

[00262] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA V_HH sdAb, and optionally first and second peptide linkers; wherein the *C*-terminus of the anti-HSA V_HH sdAb is connected to a *N*-terminus of the PD-1 binding domain directly or *via* the first peptide linker, and an *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the second peptide linker.

[00263] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA V_HH sdAb, and optionally first and second peptide linkers; wherein the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the PD-1 binding domain directly or *via* the first peptide linker, and a *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the anti-HSA V_HH sdAb directly or *via* the second peptide linker.

[00264] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA V_HH sdAb, and optionally first and second peptide linkers; wherein the *C*-terminus of the anti-HSA V_HH sdAb is connected to the *N*-terminus of the IL-2 domain directly or *via* the first peptide linker, and the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

[00265] In still another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an anti-HSA V_HH sdAb, and optionally first and second peptide linkers; wherein a *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the first peptide linker, and the *C*-terminus of the IL-2 domain is

connected to the *N*-terminus of the anti-HSA V_HH sdAb directly or *via* the second peptide linker.

[00266] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain and an Fc domain as a half-life-extension domain.

[00267] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, an Fc domain comprising first and second peptide chains, and optionally a peptide linker; wherein the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* the peptide linker, or the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the peptide linker.

[00268] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, an Fc domain comprising first and second peptide chains, and optionally a peptide linker; wherein the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* the peptide linker.

[00269] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, an Fc domain comprising first and second peptide chains, and optionally a peptide linker; wherein the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the peptide linker.

[00270] In yet another embodiment, provided herein is a fusion protein comprising two IL-2 domains and an Fc domain.

[00271] In one embodiment, the fusion protein provided herein comprises first and second IL-2 domains, an Fc domain comprising first and second peptide chains, and optionally first and second peptide linkers; wherein the *C*-terminus of the first IL-2 domain is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* the first peptide linker, and the *C*-terminus of the second IL-2 domain is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* the second peptide linker.

[00272] In another embodiment, the fusion protein provided herein comprises first and second IL-2 domains, an Fc domain comprising first and second peptide chains, and optionally

first and second peptide linkers; wherein the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the first IL-2 domain directly or *via* the first peptide linker, and the *C*-terminus of the second IL-2 domain is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* the second peptide linker.

[00273] In yet another embodiment, the fusion protein provided herein comprises first and second IL-2 domains, an Fc domain comprising first and second peptide chains, and optionally first and second peptide linkers; wherein the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the first IL-2 domain directly or *via* the first peptide linker, and the *C*-terminus of the second peptide chain of the Fc domain is connected to the *N*-terminus of the second IL-2 domain directly or *via* the second peptide linker.

[00274] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and an Fc domain as a half-life-extension domain.

[00275] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, two PD-1 binding domains, and an Fc domain as a half-life-extension domain.

[00276] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, first and second PD-1 binding domains, an Fc domain comprising first and second peptide chains, and optionally a peptide linker; wherein a *C*-terminus of the first PD-1 binding domain is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the peptide linker; and wherein a *C*-terminus of the second PD-1 binding domain is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker.

[00277] In yet another embodiment, provided herein is a fusion protein comprising two IL-2 domain, two PD-1 binding domains, and an Fc domain as a half-life-extension domain.

[00278] In one embodiment, the fusion protein provided herein comprises first and second IL-2 domains, first and second PD-1 binding domains, an Fc domain comprising first and second peptide chains, and optionally a peptide linker; wherein a *C*-terminus of the first PD-1 binding domain is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via*

a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the first IL-2 domain directly or *via* the first peptide linker; and wherein a *C*-terminus of the second PD-1 binding domain is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the second peptide chain of the Fc domain is connected to the *N*-terminus of the second IL-2 domain directly or *via* the second peptide linker.

[00279] In one embodiment, the PD-1 binding domain is a single-chain variable fragment (scFv), Fab, Fab', F(ab)₂, F(ab')₂, Fv, diabody, triabody, tetrabody, minibody, or a V_HH sdAb. In another embodiment, the PD-1 binding domain is an anti-PD-1 scFv. In yet another embodiment, the PD-1 binding domain is an anti-PD-1 Fab. In yet another embodiment, the PD-1 binding domain is an anti-PD-1 Fab'. In yet another embodiment, the PD-1 binding domain is an anti-PD-1 F(ab)₂. In yet another embodiment, the PD-1 binding domain is an anti-PD-1 F(ab')₂. In yet another embodiment, the PD-1 binding domain is an anti-PD-1 Fv. In yet another embodiment, the PD-1 binding domain is an anti-PD-1 diabody. In yet another embodiment, the PD-1 binding domain is an anti-PD-1 triabody. In yet another embodiment, the PD-1 binding domain is an anti-PD-1 tetrabody. In yet another embodiment, the PD-1 binding domain is an anti-PD-1 minibody. In yet another embodiment, the PD-1 binding domain is an anti-PD-1 sdAb. In still another embodiment, the PD-1 binding domain is an anti-PD-1 V_HH sdAb.

[00280] In one embodiment, the PD-1 binding domain is an anti-PD-1 sdAb.

[00281] Thus, in one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an anti-PD-1 sdAb, and an Fc domain. In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, two anti-PD-1 sdAbs, and an Fc domain.

[00282] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, first and second anti-PD-1 sdAbs, an Fc domain comprising a first and second peptide chains, and optionally a peptide linker; wherein the *C*-terminus of the first anti-PD-1 sdAb is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the peptide linker; and wherein the *C*-terminus of the second anti-PD-1 sdAb is connected to the *N*-terminus of the second peptide chain of the Fc domain directly

or *via* a peptide linker.

[00283] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, first and second anti-PD-1 sdAbs, an Fc domain comprising a first and second peptide chains, and optionally a peptide linker; wherein the *C*-terminus of the first anti-PD-1 sdAb is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the *C*-terminus of the second anti-PD-1 sdAb is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker.

[00284] In another embodiment, provided herein is a fusion protein comprising two IL-2 domains, two anti-PD-1 sdAbs, and an Fc domain.

[00285] In one embodiment, the fusion protein provided herein comprises first and second IL-2 domains, first and second anti-PD-1 sdAbs, an Fc domain comprising a first and second peptide chains, and optionally first and second peptide linker; wherein the *C*-terminus of the first anti-PD-1 sdAb is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the first IL-2 domain directly or *via* the first peptide linker; and wherein the *C*-terminus of the second anti-PD-1 sdAb is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the second peptide chain of the Fc domain is connected to the *N*-terminus of the second IL-2 domain directly or *via* the second peptide linker.

[00286] In another embodiment, the fusion protein provided herein comprises first and second IL-2 domains, first and second anti-PD-1 sdAbs, an Fc domain comprising a first and second peptide chains, and optionally first and second peptide linker; wherein the *C*-terminus of the first anti-PD-1 sdAb is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker; and wherein the *C*-terminus of the second anti-PD-1 sdAb is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the

second peptide chain of the Fc domain is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker.

[00287] In another embodiment, the PD-1 binding domain is an anti-PD-1 V_HH sdAb.

[00288] Thus, in one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an anti-PD-1 V_HH sdAb, and an Fc domain. In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, two anti-PD-1 V_HH sdAbs, and an Fc domain.

[00289] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, first and second anti-PD-1 V_HH sdAbs, an Fc domain comprising a first and second peptide chains, and optionally a peptide linker; wherein the *C*-terminus of the first anti-PD-1 V_HH sdAb is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the peptide linker; and wherein the *C*-terminus of the second anti-PD-1 V_HH sdAb is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker.

[00290] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, first and second anti-PD-1 V_HH sdAbs, an Fc domain comprising a first and second peptide chains, and optionally a peptide linker; wherein the *C*-terminus of the first anti-PD-1 V_HH sdAb is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the *C*-terminus of the second anti-PD-1 V_HH sdAb is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker.

[00291] In another embodiment, provided herein is a fusion protein comprising two IL-2 domains, two anti-PD-1 V_HH sdAbs, and an Fc domain.

[00292] In one embodiment, the fusion protein provided herein comprises first and second IL-2 domains, first and second anti-PD-1 V_HH sdAbs, an Fc domain comprising a first and second peptide chains, and optionally first and second peptide linker; wherein the *C*-terminus of the first anti-PD-1 V_HH sdAb is connected to the *N*-terminus of the first peptide chain of the Fc

domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the first IL-2 domain directly or *via* the first peptide linker; and wherein the *C*-terminus of the second anti-PD-1 V_HH sdAb is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the second peptide chain of the Fc domain is connected to the *N*-terminus of the second IL-2 domain directly or *via* the second peptide linker.

[00293] In another embodiment, the fusion protein provided herein comprises first and second IL-2 domains, first and second anti-PD-1 V_HH sdAbs, an Fc domain comprising a first and second peptide chains, and optionally first and second peptide linker; wherein the *C*-terminus of the first anti-PD-1 V_HH sdAb is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker; and wherein the *C*-terminus of the second anti-PD-1 V_HH sdAb is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the second peptide chain of the Fc domain is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker.

[00294] In one embodiment, each anti-PD-1 V_HH sdAb independently comprises (i) a CDR1 of SEQ ID NO: 119, a CDR2 of SEQ ID NO: 120, and a CDR3 of SEQ ID NO: 121; (ii) a CDR1 of SEQ ID NO: 123, a CDR2 of SEQ ID NO: 124, and a CDR3 of SEQ ID NO: 125; (iii) a CDR1 of SEQ ID NO: 127, a CDR2 of SEQ ID NO: 128, and a CDR3 of SEQ ID NO: 129; (iv) a CDR1 of SEQ ID NO: 131, a CDR2 of SEQ ID NO: 132, and a CDR3 of SEQ ID NO: 133; or (v) a CDR1 of SEQ ID NO: 135, a CDR2 of SEQ ID NO: 136, and a CDR3 of SEQ ID NO: 137. In another embodiment, each anti-PD-1 V_HH sdAb comprises a CDR1 of SEQ ID NO: 119, a CDR2 of SEQ ID NO: 120, and a CDR3 of SEQ ID NO: 121. In yet another embodiment, each anti-PD-1 V_HH single domain antibody comprises a CDR1 of SEQ ID NO: 123, a CDR2 of SEQ ID NO: 124, and a CDR3 of SEQ ID NO: 125. In yet another embodiment, each anti-PD-1 V_HH single domain antibody comprises a CDR1 of SEQ ID NO: 127, a CDR2 of SEQ ID NO: 128, and a CDR3 of SEQ ID NO: 129. In yet another embodiment, each anti-PD-1 V_HH single domain antibody comprises a CDR1 of SEQ ID NO: 131, a CDR2 of SEQ ID NO: 132, and a CDR3 of SEQ ID NO: 133. In still another embodiment, each anti-PD-1 V_HH single domain

antibody comprises a CDR1 of SEQ ID NO: 135, a CDR2 of SEQ ID NO: 136, and a CDR3 of SEQ ID NO: 137.

[00295] In yet another embodiment, the PD-1 binding domain is an anti-PD-1 scFv.

[00296] Thus, in one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an anti-PD-1 scFv, and an Fc domain. In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, two anti-PD-1 scFvs, and an Fc domain.

[00297] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, first and second anti-PD-1 scFvs, an Fc domain comprising a first and second peptide chains, and optionally a peptide linker; wherein the *C*-terminus of the first anti-PD-1 scFv is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the IL-2 domain directly or *via* the peptide linker; and wherein the *C*-terminus of the second anti-PD-1 scFv is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker.

[00298] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, first and second anti-PD-1 scFvs, an Fc domain comprising a first and second peptide chains, and optionally a peptide linker; wherein the *C*-terminus of the first anti-PD-1 scFv is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the *C*-terminus of the second anti-PD-1 scFv is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker.

[00299] In another embodiment, provided herein is a fusion protein comprising two IL-2 domains, two anti-PD-1 scFvs, and an Fc domain.

[00300] In one embodiment, the fusion protein provided herein comprises first and second IL-2 domains, first and second anti-PD-1 scFvs, an Fc domain comprising a first and second peptide chains, and optionally first and second peptide linker; wherein the *C*-terminus of the first anti-PD-1 scFv is connected to the *N*-terminus of the first peptide chain of the Fc domain directly

or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the first IL-2 domain directly or *via* the first peptide linker; and wherein the *C*-terminus of the second anti-PD-1 scFv is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the second peptide chain of the Fc domain is connected to the *N*-terminus of the second IL-2 domain directly or *via* the second peptide linker.

[00301] In another embodiment, the fusion protein provided herein comprises first and second IL-2 domains, first and second anti-PD-1 scFvs, an Fc domain comprising a first and second peptide chains, and optionally first and second peptide linker; wherein the *C*-terminus of the first anti-PD-1 scFv is connected to the *N*-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the first peptide chain of the Fc domain is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker; and wherein the *C*-terminus of the second anti-PD-1 scFv is connected to the *N*-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker, and the *C*-terminus of the second peptide chain of the Fc domain is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker.

[00302] In one embodiment, each anti-PD-1 scFv independently comprises:

- (i) a light chain complementarity determining region 1 (CDRL1) of SEQ ID NO: 29, a light chain complementarity determining region 2 (CDRL2) of DAS, a light chain complementarity determining region 3 (CDRL3) of SEQ ID NO: 30, a heavy chain complementarity determining region 1 (CDRH1) of SEQ ID NO: 31, a heavy chain complementarity determining region 2 (CDRH2) of SEQ ID NO: 32, and a heavy chain complementarity determining region 3 (CDRH3) of SEQ ID NO: 33;
- (ii) a CDRL1 of SEQ ID NO: 38, a CDRL2 of LAS, a CDRL3 of SEQ ID NO: 39, a CDRH1 of SEQ ID NO: 40, a CDRH2 of SEQ ID NO: 41, and a CDRH3 of SEQ ID NO: 42;
- (iii) a CDRL1 of SEQ ID NO: 47, a CDRL2 of KVS, a CDRL3 of SEQ ID NO: 48, a CDRH1 of SEQ ID NO: 49, a CDRH2 of SEQ ID NO: 50, and a CDRH3 of SEQ ID NO: 51;
- (iv) a CDRL1 of SEQ ID NO: 56, a CDRL2 of TAT, a CDRL3 of SEQ ID NO: 57, a CDRH1 of SEQ ID NO: 58, a CDRH2 of SEQ ID NO: 59, and a CDRH3 of SEQ ID NO: 60;
- (v) a CDRL1 of SEQ ID NO: 65, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 66, a CDRH1

- of SEQ ID NO: 67, a CDRH2 of SEQ ID NO: 68, and a CDRH3 of SEQ ID NO: 69;
- (vi) a CDRL1 of SEQ ID NO: 74, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 75, a CDRH1 of SEQ ID NO: 76, a CDRH2 of SEQ ID NO: 77, and a CDRH3 of SEQ ID NO: 78;
 - (vii) a CDRL1 of SEQ ID NO: 83, a CDRL2 of YAF, a CDRL3 of SEQ ID NO: 84, a CDRH1 of SEQ ID NO: 85, a CDRH2 of SEQ ID NO: 86, and a CDRH3 of SEQ ID NO: 87;
 - (viii) a CDRL1 of SEQ ID NO: 92, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 93, a CDRH1 of SEQ ID NO: 94, a CDRH2 of SEQ ID NO: 95, and a CDRH3 of SEQ ID NO: 96;
 - (ix) a CDRL1 of SEQ ID NO: 101, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 102, a CDRH1 of SEQ ID NO: 103, a CDRH2 of SEQ ID NO: 104, and a CDRH3 of SEQ ID NO: 105; or
 - (x) a CDRL1 of SEQ ID NO: 110, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 111, a CDRH1 of SEQ ID NO: 112, a CDRH2 of SEQ ID NO: 113, and a CDRH3 of SEQ ID NO: 114.

[00303] In one embodiment, each anti-PD-1 scFv comprises a CDRL1 of SEQ ID NO: 29, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 30, a CDRH1 of SEQ ID NO: 31, a CDRH2 of SEQ ID NO: 32, and a CDRH3 of SEQ ID NO: 33. In another embodiment, each anti-PD-1 scFv comprises a CDRL1 of SEQ ID NO: 38, a CDRL2 of LAS, a CDRL3 of SEQ ID NO: 39, a CDRH1 of SEQ ID NO: 40, a CDRH2 of SEQ ID NO: 41, and a CDRH3 of SEQ ID NO: 42. In yet another embodiment, each anti-PD-1 scFv comprises a CDRL1 of SEQ ID NO: 47, a CDRL2 of KVS, a CDRL3 of SEQ ID NO: 48, a CDRH1 of SEQ ID NO: 49, a CDRH2 of SEQ ID NO: 50, and a CDRH3 of SEQ ID NO: 51. In yet another embodiment, each anti-PD-1 scFv comprises a CDRL1 of SEQ ID NO: 56, a CDRL2 of TAT, a CDRL3 of SEQ ID NO: 57, a CDRH1 of SEQ ID NO: 58, a CDRH2 of SEQ ID NO: 59, and a CDRH3 of SEQ ID NO: 60. In yet another embodiment, each anti-PD-1 scFv comprises a CDRL1 of SEQ ID NO: 65, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 66, a CDRH1 of SEQ ID NO: 67, a CDRH2 of SEQ ID NO: 68, and a CDRH3 of SEQ ID NO: 69. In yet another embodiment, each anti-PD-1 scFv comprises a CDRL1 of SEQ ID NO: 74, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 75, a CDRH1 of SEQ ID NO: 76, a CDRH2 of SEQ ID NO: 77, and a CDRH3 of SEQ ID NO: 78. In yet another embodiment, each anti-PD-1 scFv comprises a CDRL1 of SEQ ID NO: 83, a CDRL2 of YAF, a CDRL3 of SEQ ID NO: 84, a CDRH1 of SEQ ID NO: 85, a CDRH2 of SEQ ID NO:

86, and a CDRH3 of SEQ ID NO: 87. In yet another embodiment, each anti-PD-1 scFv comprises a CDRL1 of SEQ ID NO: 92, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 93, a CDRH1 of SEQ ID NO: 94, a CDRH2 of SEQ ID NO: 95, and a CDRH3 of SEQ ID NO: 96. In yet another embodiment, each anti-PD-1 scFv comprises a CDRL1 of SEQ ID NO: 101, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 102, a CDRH1 of SEQ ID NO: 103, a CDRH2 of SEQ ID NO: 104, and a CDRH3 of SEQ ID NO: 105. In still another embodiment, each anti-PD-1 scFv comprises a CDRL1 of SEQ ID NO: 110, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 111, a CDRH1 of SEQ ID NO: 112, a CDRH2 of SEQ ID NO: 113, and a CDRH3 of SEQ ID NO: 114.

[00304] In another embodiment, each anti-PD-1 scFv comprises:

- (i) a light chain variable region of SEQ ID NO: 34 and a heavy chain variable region of SEQ ID NO: 35;
- (ii) a light chain variable region of SEQ ID NO: 43 and a heavy chain variable region of SEQ ID NO: 44;
- (iii) a light chain variable region of SEQ ID NO: 52 and a heavy chain variable region of SEQ ID NO: 53;
- (iv) a light chain variable region of SEQ ID NO: 61 and a heavy chain variable region of SEQ ID NO: 62;
- (v) a light chain variable region of SEQ ID NO: 70 and a heavy chain variable region of SEQ ID NO: 71;
- (vi) a light chain variable region of SEQ ID NO: 79 and a heavy chain variable region of SEQ ID NO: 80;
- (vii) a light chain variable region of SEQ ID NO: 88 and a heavy chain variable region of SEQ ID NO: 89;
- (viii) a light chain variable region of SEQ ID NO: 97 and a heavy chain variable region of SEQ ID NO: 98;
- (ix) a light chain variable region of SEQ ID NO: 106 and a heavy chain variable region of SEQ ID NO: 107; or
- (xi) a light chain variable region of SEQ ID NO: 115 and a heavy chain variable region of SEQ ID NO: 116.

[00305] In one embodiment, each anti-PD-1 scFv comprises a light chain variable region of SEQ ID NO: 34 and a heavy chain variable region of SEQ ID NO: 35. In another embodiment, each anti-PD-1 scFv comprises a light chain variable region of SEQ ID NO: 43 and a heavy chain variable region of SEQ ID NO: 44. In yet another embodiment, each anti-PD-1 scFv comprises a light chain variable region of SEQ ID NO: 52 and a heavy chain variable region of SEQ ID NO: 53. In yet another embodiment, each anti-PD-1 scFv comprises a light chain variable region of SEQ ID NO: 61 and a heavy chain variable region of SEQ ID NO: 62. In yet another embodiment, each anti-PD-1 scFv comprises a light chain variable region of SEQ ID NO: 70 and a heavy chain variable region of SEQ ID NO: 71. In yet another embodiment, each anti-PD-1 scFv comprises a light chain variable region of SEQ ID NO: 79 and a heavy chain variable region of SEQ ID NO: 80. In yet another embodiment, each anti-PD-1 scFv comprises a light chain variable region of SEQ ID NO: 88 and a heavy chain variable region of SEQ ID NO: 89. In yet another embodiment, each anti-PD-1 scFv comprises a light chain variable region of SEQ ID NO: 97 and a heavy chain variable region of SEQ ID NO: 98. In yet another embodiment, each anti-PD-1 scFv comprises a light chain variable region of SEQ ID NO: 106 and a heavy chain variable region of SEQ ID NO: 107. In still another embodiment, each anti-PD-1 scFv comprises a light chain variable region of SEQ ID NO: 115 and a heavy chain variable region of SEQ ID NO: 116.

[00306] In one embodiment, each anti-PD-1 scFv comprising a light chain (V_L), a heavy chain (V_H), and optionally a peptide linker, wherein the C-terminal of the light chain is connected to the N-terminal of the heavy chain directly or *via* the peptide linker, or wherein the C-terminal of the heavy chain is connected to the N-terminal of the light chain directly or *via* the peptide linker. In another embodiment, each anti-PD-1 scFv comprising a light chain (V_L), a heavy chain (V_H), and optionally a peptide linker, wherein the C-terminal of the light chain is connected to the N-terminal of the heavy chain *via* the peptide linker, or wherein the C-terminal of the heavy chain is connected to the N-terminal of the light chain *via* the peptide linker. In yet another embodiment, each anti-PD-1 scFv comprising a light chain (V_L), a heavy chain (V_H), and optionally a peptide linker, wherein the C-terminal of the light chain is connected to the N-terminal of the heavy chain *via* the peptide linker. In still another embodiment, each anti-PD-1 scFv comprising a light chain (V_L), a heavy chain (V_H), and optionally a peptide linker, wherein the C-terminal of the heavy chain is connected to the N-terminal of the light chain *via* the peptide

linker.

[00307] In one embodiment, the Fc domain is a hIgG1 Fc domain or a mutein thereof, or a fragment thereof. In another embodiment, the Fc domain is a hIgG1 Fc having an amino acid substitution of N297A. In yet another embodiment, the Fc domain is a hIgG2 Fc domain or a mutein thereof, or a fragment thereof. In still another embodiment, the Fc domain is a hIgG4 Fc domain or a mutein thereof, or a fragment thereof.

[00308] In one embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27. Thus, in one embodiment, the FC domain comprises an amino acid sequence of SEQ ID NO: 16. In yet another embodiment, the FC domain comprises an amino acid sequence of SEQ ID NO: 17. In yet another embodiment, the FC domain comprises an amino acid sequence of SEQ ID NO: 18. In yet another embodiment, the FC domain comprises an amino acid sequence of SEQ ID NO: 19. In yet another embodiment, the FC domain comprises an amino acid sequence of SEQ ID NO: 20. In yet another embodiment, the FC domain comprises an amino acid sequence of SEQ ID NO: 21. In yet another embodiment, the FC domain comprises an amino acid sequence of SEQ ID NO: 22. In yet another embodiment, the FC domain comprises an amino acid sequence of SEQ ID NO: 23. In yet another embodiment, the FC domain comprises an amino acid sequence of SEQ ID NO: 24. In yet another embodiment, the FC domain comprises an amino acid sequence of SEQ ID NO: 25. In yet another embodiment, the FC domain comprises an amino acid sequence of SEQ ID NO: 26. In still another embodiment, the FC domain comprises an amino acid sequence of SEQ ID NO: 27.

[00309] In one embodiment, the FC domain as a half-life-extension domain comprises a pair of peptide chains in a knobs-in-holes (KIH) configuration. Thus, in one embodiment, the FC domain comprises an amino acid sequence pair of SEQ ID NOs: 19 and 20, 21 and 22, 23 and 24, or 26 and 27 in a knobs-in-holes configuration. In another embodiment, the FC domain comprises an amino acid sequence pair of SEQ ID NOs: 19 and 20 in a knobs-in-holes configuration. In yet another embodiment, the FC domain comprises an amino acid sequence pair of SEQ ID NOs: 21 and 22 in a knobs-in-holes configuration. In yet another embodiment, the Fc domain comprises an amino acid sequence pair of SEQ ID NOs: 23 and 24 in a knobs-in-

holes configuration. In still another embodiment, the FC domain comprises an amino acid sequence pair of SEQ ID NOs: 26 and 27 in a knobs-in-holes configuration.

[00310] In one embodiment, the PD-1 binding domain and the Fc domain in a fusion protein provided herein are parts of an intact anti-PD-1 antibody comprising two light chains and two heavy chains.

[00311] Thus, in one embodiment, provided herein is a fusion protein comprising an intact anti-PD-1 antibody comprising two light chains and two heavy chains, an IL-2 domain, and optionally a peptide linker.

[00312] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, an intact anti-PD-1 antibody comprising first and second light chains and first and second heavy chains, and optionally a peptide linker; wherein the *C*-terminus of the first heavy chain of the intact anti-PD-1 antibody is connected to the *N*-terminus of the IL-2 domain directly or *via* the peptide linker.

[00313] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, an intact anti-PD-1 antibody comprising first and second light chains and first and second heavy chains, and a peptide linker; wherein the *C*-terminus of the first heavy chain of the intact anti-PD-1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker.

[00314] In another embodiment, provided herein is a fusion protein comprising two IL-2 domains, and an intact anti-PD-1 antibody comprising two light chains and two heavy chains.

[00315] In one embodiment, the fusion protein provided herein comprises first and second IL-2 domains, an intact anti-PD-1 antibody comprising first and second light chains and first and second heavy chains, and optionally first and second peptide linkers; wherein the *C*-terminus of the first heavy chain of the intact anti-PD-1 antibody is connected to the *N*-terminus of the first IL-2 domain directly or *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the intact anti-PD-1 antibody is connected to the *N*-terminus of the second IL-2 domain directly or *via* the second peptide linker.

[00316] In another embodiment, the fusion protein provided herein comprises first and second IL-2 domains, an intact anti-PD-1 antibody comprising first and second light chains and first and second heavy chains, and first and second peptide linkers; wherein the *C*-terminus of the first heavy chain of the intact anti-PD-1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the intact anti-PD-1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker.

[00317] In one embodiment, the intact anti-PD-1 antibody comprises:

- (i) a light chain complementarity determining region 1 (CDRL1) of SEQ ID NO: 29, a light chain complementarity determining region 2 (CDRL2) of DAS, a light chain complementarity determining region 3 (CDRL3) of SEQ ID NO: 30, a heavy chain complementarity determining region 1 (CDRH1) of SEQ ID NO: 31, a heavy chain complementarity determining region 2 (CDRH2) of SEQ ID NO: 32, and a heavy chain complementarity determining region 3 (CDRH3) of SEQ ID NO: 33;
- (ii) a CDRL1 of SEQ ID NO: 38, a CDRL2 of LAS, a CDRL3 of SEQ ID NO: 39, a CDRH1 of SEQ ID NO: 40, a CDRH2 of SEQ ID NO: 41, and a CDRH3 of SEQ ID NO: 42;
- (iii) a CDRL1 of SEQ ID NO: 47, a CDRL2 of KVS, a CDRL3 of SEQ ID NO: 48, a CDRH1 of SEQ ID NO: 49, a CDRH2 of SEQ ID NO: 50, and a CDRH3 of SEQ ID NO: 51;
- (iv) a CDRL1 of SEQ ID NO: 56, a CDRL2 of TAT, a CDRL3 of SEQ ID NO: 57, a CDRH1 of SEQ ID NO: 58, a CDRH2 of SEQ ID NO: 59, and a CDRH3 of SEQ ID NO: 60;
- (v) a CDRL1 of SEQ ID NO: 65, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 66, a CDRH1 of SEQ ID NO: 67, a CDRH2 of SEQ ID NO: 68, and a CDRH3 of SEQ ID NO: 69;
- (vi) a CDRL1 of SEQ ID NO: 74, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 75, a CDRH1 of SEQ ID NO: 76, a CDRH2 of SEQ ID NO: 77, and a CDRH3 of SEQ ID NO: 78;
- (vii) a CDRL1 of SEQ ID NO: 83, a CDRL2 of YAF, a CDRL3 of SEQ ID NO: 84, a CDRH1 of SEQ ID NO: 85, a CDRH2 of SEQ ID NO: 86, and a CDRH3 of SEQ ID NO: 87;
- (viii) a CDRL1 of SEQ ID NO: 92, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 93, a CDRH1 of SEQ ID NO: 94, a CDRH2 of SEQ ID NO: 95, and a CDRH3 of SEQ ID NO: 96;
- (ix) a CDRL1 of SEQ ID NO: 101, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 102, a CDRH1 of SEQ ID NO: 103, a CDRH2 of SEQ ID NO: 104, and a CDRH3 of SEQ ID NO: 105;

NO: 105; or

- (x) a CDRL1 of SEQ ID NO: 110, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 111, a CDRH1 of SEQ ID NO: 112, a CDRH2 of SEQ ID NO: 113, and a CDRH3 of SEQ ID NO: 114.

[00318] In one embodiment, the intact anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 29, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 30, a CDRH1 of SEQ ID NO: 31, a CDRH2 of SEQ ID NO: 32, and a CDRH3 of SEQ ID NO: 33. In another embodiment, the intact anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 38, a CDRL2 of LAS, a CDRL3 of SEQ ID NO: 39, a CDRH1 of SEQ ID NO: 40, a CDRH2 of SEQ ID NO: 41, and a CDRH3 of SEQ ID NO: 42. In yet another embodiment, the intact anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 47, a CDRL2 of KVS, a CDRL3 of SEQ ID NO: 48, a CDRH1 of SEQ ID NO: 49, a CDRH2 of SEQ ID NO: 50, and a CDRH3 of SEQ ID NO: 51. In yet another embodiment, the intact anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 56, a CDRL2 of TAT, a CDRL3 of SEQ ID NO: 57, a CDRH1 of SEQ ID NO: 58, a CDRH2 of SEQ ID NO: 59, and a CDRH3 of SEQ ID NO: 60. In yet another embodiment, the intact anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 65, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 66, a CDRH1 of SEQ ID NO: 67, a CDRH2 of SEQ ID NO: 68, and a CDRH3 of SEQ ID NO: 69. In yet another embodiment, the intact anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 74, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 75, a CDRH1 of SEQ ID NO: 76, a CDRH2 of SEQ ID NO: 77, and a CDRH3 of SEQ ID NO: 78. In yet another embodiment, the intact anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 83, a CDRL2 of YAF, a CDRL3 of SEQ ID NO: 84, a CDRH1 of SEQ ID NO: 85, a CDRH2 of SEQ ID NO: 86, and a CDRH3 of SEQ ID NO: 87. In yet another embodiment, the intact anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 92, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 93, a CDRH1 of SEQ ID NO: 94, a CDRH2 of SEQ ID NO: 95, and a CDRH3 of SEQ ID NO: 96. In yet another embodiment, the intact anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 101, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 102, a CDRH1 of SEQ ID NO: 103, a CDRH2 of SEQ ID NO: 104, and a CDRH3 of SEQ ID NO: 105. In still another embodiment, the intact anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 110, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 111, a CDRH1 of SEQ ID NO: 112, a CDRH2 of SEQ ID NO: 113, and a CDRH3 of SEQ ID NO: 114.

[00319] In another embodiment, the intact anti-PD-1 antibody comprises:

- (i) a light chain variable region of SEQ ID NO: 34 and a heavy chain variable region of SEQ ID NO: 35;
- (ii) a light chain variable region of SEQ ID NO: 43 and a heavy chain variable region of SEQ ID NO: 44;
- (iii) a light chain variable region of SEQ ID NO: 52 and a heavy chain variable region of SEQ ID NO: 53;
- (iv) a light chain variable region of SEQ ID NO: 61 and a heavy chain variable region of SEQ ID NO: 62;
- (v) a light chain variable region of SEQ ID NO: 70 and a heavy chain variable region of SEQ ID NO: 71;
- (vi) a light chain variable region of SEQ ID NO: 79 and a heavy chain variable region of SEQ ID NO: 80;
- (vii) a light chain variable region of SEQ ID NO: 88 and a heavy chain variable region of SEQ ID NO: 89;
- (viii) a light chain variable region of SEQ ID NO: 97 and a heavy chain variable region of SEQ ID NO: 98;
- (ix) a light chain variable region of SEQ ID NO: 106 and a heavy chain variable region of SEQ ID NO: 107; or
- (x) a light chain variable region of SEQ ID NO: 115 and a heavy chain variable region of SEQ ID NO: 116.

[00320] In one embodiment, the intact anti-PD-1 antibody comprises a light chain variable region of SEQ ID NO: 34 and a heavy chain variable region of SEQ ID NO: 35. In another embodiment, the intact anti-PD-1 antibody comprises a light chain variable region of SEQ ID NO: 43 and a heavy chain variable region of SEQ ID NO: 44. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain variable region of SEQ ID NO: 52 and a heavy chain variable region of SEQ ID NO: 53. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain variable region of SEQ ID NO: 61 and a heavy chain variable region of SEQ ID NO: 62. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain variable region of SEQ ID NO: 70 and a heavy chain variable region of SEQ ID NO: 71. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain variable

region of SEQ ID NO: 79 and a heavy chain variable region of SEQ ID NO: 80. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain variable region of SEQ ID NO: 88 and a heavy chain variable region of SEQ ID NO: 89. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain variable region of SEQ ID NO: 97 and a heavy chain variable region of SEQ ID NO: 98. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain variable region of SEQ ID NO: 106 and a heavy chain variable region of SEQ ID NO: 107. In still another embodiment, the intact anti-PD-1 antibody comprises a light chain variable region of SEQ ID NO: 115 and a heavy chain variable region of SEQ ID NO: 116.

[00321] In one embodiment, the intact anti-PD-1 antibody is an IgA1, IgA2, IgG1, IgG2, IgG3, or IgG4 antibody. In another embodiment, the intact anti-PD-1 antibody is an IgA1 or IgA2. In yet another embodiment, the intact anti-PD-1 antibody is an IgA1. In yet another embodiment, the anti-PD-1 intact antibody is an IgA2. In yet another embodiment, the intact anti-PD-1 antibody is an IgG1, IgG2, IgG3, or IgG4 antibody. In yet another embodiment, the intact anti-PD-1 antibody is an IgG1 antibody. In yet another embodiment, the intact anti-PD-1 antibody is an IgG1 antibody with an N297A mutation. In yet another embodiment, the intact anti-PD-1 antibody is an IgG2 antibody. In yet another embodiment, the intact anti-PD-1 antibody is an IgG3 antibody. In still another embodiment, the intact anti-PD-1 antibody is an IgG4 antibody.

[00322] In one embodiment, the light chain of the intact anti-PD-1 antibody is a kappa or lambda chain. In another embodiment, the light chain of the intact anti-PD-1 antibody is a kappa chain. In yet another embodiment, the light chain of the intact anti-PD-1 antibody is a lambda chain.

[00323] In one embodiment, the intact anti-PD-1 antibody is in a knobs-in-holes configuration. In another embodiment, the intact anti-PD-1 antibody is an IgG1 antibody comprising in a knobs-in-holes configuration. In yet another embodiment, the intact anti-PD-1 antibody is an IgG4 antibody in a knobs-in-holes configuration.

[00324] In yet another embodiment, the intact anti-PD-1 antibody comprises:

- (i) a light chain of SEQ ID NO: 36 and a heavy chain of SEQ ID NO: 37;

- (ii) a light chain of SEQ ID NO: 45 and a heavy chain of SEQ ID NO: 46;
- (iii) a light chain of SEQ ID NO: 54 and a heavy chain of SEQ ID NO: 55;
- (iv) a light chain of SEQ ID NO: 63 and a heavy chain of SEQ ID NO: 64;
- (v) a light chain of SEQ ID NO: 72 and a heavy chain of SEQ ID NO: 73;
- (vi) a light chain of SEQ ID NO: 81 and a heavy chain of SEQ ID NO: 82;
- (vii) a light chain of SEQ ID NO: 90 and a heavy chain of SEQ ID NO: 91;
- (viii) a light chain of SEQ ID NO: 99 and a heavy chain of SEQ ID NO: 100;
- (ix) a light chain of SEQ ID NO: 108 and a heavy chain of SEQ ID NO: 109; or
- (x) a light chain of SEQ ID NO: 117 and a heavy chain of SEQ ID NO: 118.

[00325] In one embodiment, the intact anti-PD-1 antibody comprises a light chain of SEQ ID NO: 36 and a heavy chain of SEQ ID NO: 37. In another embodiment, the intact anti-PD-1 antibody comprises a light chain of SEQ ID NO: 45 and a heavy chain of SEQ ID NO: 46. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain of SEQ ID NO: 54 and a heavy chain of SEQ ID NO: 55. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain of SEQ ID NO: 63 and a heavy chain of SEQ ID NO: 64. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain of SEQ ID NO: 72 and a heavy chain of SEQ ID NO: 73. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain of SEQ ID NO: 81 and a heavy chain of SEQ ID NO: 82. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain of SEQ ID NO: 90 and a heavy chain of SEQ ID NO: 91. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain of SEQ ID NO: 99 and a heavy chain of SEQ ID NO: 100. In yet another embodiment, the intact anti-PD-1 antibody comprises a light chain of SEQ ID NO: 108 and a heavy chain of SEQ ID NO: 109. In still another embodiment, the intact anti-PD-1 antibody comprises a light chain of SEQ ID NO: 117 and a heavy chain of SEQ ID NO: 118.

[00326] In one embodiment, each IL-2 domain in a fusion protein provided herein independently comprises an amino acid sequence of an IL-2 mutein provided herein. In another embodiment, each first IL-2 domain in a fusion protein provided herein independently comprises an amino acid sequence of an IL-2 mutein provided herein. In yet another embodiment, each second IL-2 domain in a fusion protein provided herein independently comprises an amino acid sequence of an IL-2 mutein provided herein.

[00327] In one embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of GSG or one of SEQ ID NOs: 139 to 178. In another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of GSG or SEQ ID NO: 139, 140, or 141. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 142, 143, 144, or 145. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 146, 147, 148, or 149. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 150, 151, 152, or 153. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 154, 155, 156, or 157. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 158, 159, 160, or 161. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 162, 163, 164, or 165. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 166, 167, 168, or 169. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 170. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 171 or 172. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 173 or 174. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 175 or 176. In still another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 177 or 178.

[00328] In one embodiment, each first peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of GSG or one of SEQ ID NOs: 139 to 178. In another embodiment, each first peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of GSG or SEQ ID NO: 139, 140, or 141. In yet another embodiment, each first peptide linker in a fusion protein provided herein independently

comprises an amino acid sequence of SEQ ID NO: 142, 143, 144, or 145. In yet another embodiment, each first peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 146, 147, 148, or 149. In yet another embodiment, each first peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 150, 151, 152, or 153. In yet another embodiment, each first peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 154, 155, 156, or 157. In yet another embodiment, each first peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 158, 159, 160, or 161. In yet another embodiment, each first peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 162, 163, 164, or 165. In yet another embodiment, each first peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 166, 167, 168, or 169. In yet another embodiment, each first peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 170. In yet another embodiment, each first peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 171 or 172. In yet another embodiment, each first peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 173 or 174. In yet another embodiment, each first peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 175 or 176. In still another embodiment, each first peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 177 or 178.

[00329] In one embodiment, each second peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of GSG or one of SEQ ID NOs: 139 to 178. In another embodiment, each second peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of GSG or SEQ ID NO: 139, 140, or 141. In yet another embodiment, each second peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 142, 143, 144, or 145. In yet another embodiment, each second peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 146, 147, 148, or 149. In yet another embodiment, each second peptide linker in a fusion protein provided herein

independently comprises an amino acid sequence of SEQ ID NO: 150, 151, 152, or 153. In yet another embodiment, each second peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 154, 155, 156, or 157. In yet another embodiment, each second peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 158, 159, 160, or 161. In yet another embodiment, each second peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 162, 163, 164, or 165. In yet another embodiment, each second peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 166, 167, 168, or 169. In yet another embodiment, each second peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 170. In yet another embodiment, each second peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 171 or 172. In yet another embodiment, each second peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 173 or 174. In yet another embodiment, each second peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 175 or 176. In still another embodiment, each second peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 177 or 178.

[00330] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 29, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 30, a CDRH1 of SEQ ID NO: 31, a CDRH2 of SEQ ID NO: 32, and a CDRH3 of SEQ ID NO: 33.

[00331] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein

the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 34 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 35.

[00332] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 36 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 37.

[00333] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 38, a CDRL2 of LAS, a CDRL3 of SEQ ID NO: 39, a CDRH1 of SEQ ID NO: 40, a CDRH2 of SEQ ID NO: 41, and a CDRH3 of SEQ ID NO: 42.

[00334] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 43 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 44.

[00335] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein

the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 45 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 46.

[00336] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 47, a CDRL2 of KVS, a CDRL3 of SEQ ID NO: 48, a CDRH1 of SEQ ID NO: 49, a CDRH2 of SEQ ID NO: 50, and a CDRH3 of SEQ ID NO: 51.

[00337] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 52 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 53.

[00338] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 54 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 55.

[00339] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein

the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 56, a CDRL2 of TAT, a CDRL3 of SEQ ID NO: 57, a CDRH1 of SEQ ID NO: 58, a CDRH2 of SEQ ID NO: 59, and a CDRH3 of SEQ ID NO: 60.

[00340] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 61 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 62.

[00341] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 63 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 64.

[00342] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 65, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 66, a CDRH1 of SEQ ID NO: 67, a CDRH2 of SEQ ID NO: 68, and a CDRH3 of SEQ ID NO: 69.

[00343] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1

antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 70 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 71.

[00344] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 72 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 73.

[00345] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 74, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 75, a CDRH1 of SEQ ID NO: 76, a CDRH2 of SEQ ID NO: 77, and a CDRH3 of SEQ ID NO: 78.

[00346] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 79 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 80.

[00347] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1

antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 81 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 82.

[00348] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 83, a CDRL2 of YAF, a CDRL3 of SEQ ID NO: 84, a CDRH1 of SEQ ID NO: 85, a CDRH2 of SEQ ID NO: 86, and a CDRH3 of SEQ ID NO: 87.

[00349] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 88 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 89.

[00350] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 90 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 91.

[00351] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1

antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 92, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 93, a CDRH1 of SEQ ID NO: 94, a CDRH2 of SEQ ID NO: 95, and a CDRH3 of SEQ ID NO: 96.

[00352] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 97 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 98.

[00353] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 99 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 100.

[00354] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 101, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 102, a CDRH1 of SEQ ID NO: 103, a CDRH2 of SEQ ID NO: 104, and a CDRH3 of SEQ ID NO: 105.

[00355] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy

chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 106 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 107.

[00356] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 108 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 109.

[00357] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 110, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 111, a CDRH1 of SEQ ID NO: 112, a CDRH2 of SEQ ID NO: 113, and a CDRH3 of SEQ ID NO: 114.

[00358] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 115 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 116.

[00359] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy

chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 117 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 118.

[00360] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 29, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 30, a CDRH1 of SEQ ID NO: 31, a CDRH2 of SEQ ID NO: 32, and a CDRH3 of SEQ ID NO: 33.

[00361] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 34 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 35.

[00362] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is

connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 36 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 37.

[00363] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 38, a CDRL2 of LAS, a CDRL3 of SEQ ID NO: 39, a CDRH1 of SEQ ID NO: 40, a CDRH2 of SEQ ID NO: 41, and a CDRH3 of SEQ ID NO: 42.

[00364] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 43 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 44.

[00365] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and

wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 45 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 46.

[00366] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 47, a CDRL2 of KVS, a CDRL3 of SEQ ID NO: 48, a CDRH1 of SEQ ID NO: 49, a CDRH2 of SEQ ID NO: 50, and a CDRH3 of SEQ ID NO: 51.

[00367] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 52 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 53.

[00368] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to

287; and each light chain comprises the amino acid sequence of SEQ ID NO: 54 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 55.

[00369] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 56, a CDRL2 of TAT, a CDRL3 of SEQ ID NO: 57, a CDRH1 of SEQ ID NO: 58, a CDRH2 of SEQ ID NO: 59, and a CDRH3 of SEQ ID NO: 60.

[00370] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 61 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 62.

[00371] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 63 and each heavy

chain comprises the amino acid sequence of SEQ ID NO: 64.

[00372] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 65, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 66, a CDRH1 of SEQ ID NO: 67, a CDRH2 of SEQ ID NO: 68, and a CDRH3 of SEQ ID NO: 69.

[00373] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 70 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 71.

[00374] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 72 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 73.

[00375] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 74, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 75, a CDRH1 of SEQ ID NO: 76, a CDRH2 of SEQ ID NO: 77, and a CDRH3 of SEQ ID NO: 78.

[00376] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 79 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 80.

[00377] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 81 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 82.

[00378] In one embodiment, provided herein is a fusion protein comprising first and

second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 83, a CDRL2 of YAF, a CDRL3 of SEQ ID NO: 84, a CDRH1 of SEQ ID NO: 85, a CDRH2 of SEQ ID NO: 86, and a CDRH3 of SEQ ID NO: 87.

[00379] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 88 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 89.

[00380] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 90 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 91.

[00381] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and

second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 92, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 93, a CDRH1 of SEQ ID NO: 94, a CDRH2 of SEQ ID NO: 95, and a CDRH3 of SEQ ID NO: 96.

[00382] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 97 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 98.

[00383] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 99 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 100.

[00384] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first

heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 101, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 102, a CDRH1 of SEQ ID NO: 103, a CDRH2 of SEQ ID NO: 104, and a CDRH3 of SEQ ID NO: 105.

[00385] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 106 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 107.

[00386] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 108 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 109.

[00387] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via*

the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 110, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 111, a CDRH1 of SEQ ID NO: 112, a CDRH2 of SEQ ID NO: 113, and a CDRH3 of SEQ ID NO: 114.

[00388] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 115 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 116.

[00389] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of any one of SEQ ID NOs: 262 to 287; and each light chain comprises the amino acid sequence of SEQ ID NO: 117 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 118.

[00390] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the

anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 29, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 30, a CDRH1 of SEQ ID NO: 31, a CDRH2 of SEQ ID NO: 32, and a CDRH3 of SEQ ID NO: 33.

[00391] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 34 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 35.

[00392] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 36 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 37.

[00393] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 38, a CDRL2 of LAS, a CDRL3 of SEQ ID NO: 39, a CDRH1 of SEQ ID NO: 40, a CDRH2 of SEQ ID NO: 41, and a CDRH3 of SEQ ID NO: 42.

[00394] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein

the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 43 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 44.

[00395] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 45 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 46.

[00396] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 47, a CDRL2 of KVS, a CDRL3 of SEQ ID NO: 48, a CDRH1 of SEQ ID NO: 49, a CDRH2 of SEQ ID NO: 50, and a CDRH3 of SEQ ID NO: 51.

[00397] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 52 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 53.

[00398] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein

the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 54 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 55.

[00399] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 56, a CDRL2 of TAT, a CDRL3 of SEQ ID NO: 57, a CDRH1 of SEQ ID NO: 58, a CDRH2 of SEQ ID NO: 59, and a CDRH3 of SEQ ID NO: 60.

[00400] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 61 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 62.

[00401] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 63 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 64.

[00402] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein

the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 65, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 66, a CDRH1 of SEQ ID NO: 67, a CDRH2 of SEQ ID NO: 68, and a CDRH3 of SEQ ID NO: 69.

[00403] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 70 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 71.

[00404] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 72 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 73.

[00405] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 74, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 75, a CDRH1 of SEQ ID NO: 76, a CDRH2 of SEQ ID NO: 77, and a CDRH3 of SEQ ID NO: 78.

[00406] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1

antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 79 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 80.

[00407] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 81 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 82.

[00408] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 83, a CDRL2 of YAF, a CDRL3 of SEQ ID NO: 84, a CDRH1 of SEQ ID NO: 85, a CDRH2 of SEQ ID NO: 86, and a CDRH3 of SEQ ID NO: 87.

[00409] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 88 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 89.

[00410] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1

antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 90 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 91.

[00411] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 92, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 93, a CDRH1 of SEQ ID NO: 94, a CDRH2 of SEQ ID NO: 95, and a CDRH3 of SEQ ID NO: 96.

[00412] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 97 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 98.

[00413] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 99 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 100.

[00414] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1

antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 101, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 102, a CDRH1 of SEQ ID NO: 103, a CDRH2 of SEQ ID NO: 104, and a CDRH3 of SEQ ID NO: 105.

[00415] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 106 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 107.

[00416] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 108 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 109.

[00417] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 110, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 111, a CDRH1 of SEQ ID NO: 112, a CDRH2 of SEQ ID NO: 113, and a CDRH3 of SEQ ID NO: 114.

[00418] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy

chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 115 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 116.

[00419] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 117 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 118.

[00420] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 29, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 30, a CDRH1 of SEQ ID NO: 31, a CDRH2 of SEQ ID NO: 32, and a CDRH3 of SEQ ID NO: 33.

[00421] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301;

and each light chain comprises the amino acid sequence of SEQ ID NO: 34 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 35.

[00422] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 36 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 37.

[00423] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 38, a CDRL2 of LAS, a CDRL3 of SEQ ID NO: 39, a CDRH1 of SEQ ID NO: 40, a CDRH2 of SEQ ID NO: 41, and a CDRH3 of SEQ ID NO: 42.

[00424] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 43 and each heavy chain

comprises the amino acid sequence of SEQ ID NO: 44.

[00425] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 45 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 46.

[00426] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 47, a CDRL2 of KVS, a CDRL3 of SEQ ID NO: 48, a CDRH1 of SEQ ID NO: 49, a CDRH2 of SEQ ID NO: 50, and a CDRH3 of SEQ ID NO: 51.

[00427] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 52 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 53.

[00428] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 54 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 55.

[00429] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 56, a CDRL2 of TAT, a CDRL3 of SEQ ID NO: 57, a CDRH1 of SEQ ID NO: 58, a CDRH2 of SEQ ID NO: 59, and a CDRH3 of SEQ ID NO: 60.

[00430] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 61 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 62.

[00431] In yet another embodiment, provided herein is a fusion protein comprising first

and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 63 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 64.

[00432] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 65, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 66, a CDRH1 of SEQ ID NO: 67, a CDRH2 of SEQ ID NO: 68, and a CDRH3 of SEQ ID NO: 69.

[00433] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 70 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 71.

[00434] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and

second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 72 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 73.

[00435] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 74, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 75, a CDRH1 of SEQ ID NO: 76, a CDRH2 of SEQ ID NO: 77, and a CDRH3 of SEQ ID NO: 78.

[00436] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 79 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 80.

[00437] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first

heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 81 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 82.

[00438] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 83, a CDRL2 of YAF, a CDRL3 of SEQ ID NO: 84, a CDRH1 of SEQ ID NO: 85, a CDRH2 of SEQ ID NO: 86, and a CDRH3 of SEQ ID NO: 87.

[00439] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 88 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 89.

[00440] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via*

the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 90 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 91.

[00441] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 92, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 93, a CDRH1 of SEQ ID NO: 94, a CDRH2 of SEQ ID NO: 95, and a CDRH3 of SEQ ID NO: 96.

[00442] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 97 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 98.

[00443] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is

connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 99 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 100.

[00444] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 101, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 102, a CDRH1 of SEQ ID NO: 103, a CDRH2 of SEQ ID NO: 104, and a CDRH3 of SEQ ID NO: 105.

[00445] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 106 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 107.

[00446] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and

wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 108 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 109.

[00447] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 110, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 111, a CDRH1 of SEQ ID NO: 112, a CDRH2 of SEQ ID NO: 113, and a CDRH3 of SEQ ID NO: 114.

[00448] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301; and each light chain comprises the amino acid sequence of SEQ ID NO: 115 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 116.

[00449] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers, wherein the *C*-terminus of the first heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the first IL-2 domain *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the anti-PD1 antibody is connected to the *N*-terminus of the second IL-2 domain *via* the second peptide linker; and wherein the IL-2 domain comprises the amino acid sequence of SEQ ID NO: 284, 285, or 301;

and each light chain comprises the amino acid sequence of SEQ ID NO: 117 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 118.

[00450] In certain embodiments, the PD-1 binding domain does not block the binding interaction between a PD-1 and a PD-L1. In certain embodiments, the PD-1 binding domain blocks the binding interaction between a PD-1 and a PD-L1.

[00451] In certain embodiments, the anti-PD-1 antibody is a non-blocking antibody, that is, an antibody does not block the binding interaction between a PD-1 and a PD-L1. In certain embodiments, the anti-PD-1 antibody is a blocking antibody, that is, an antibody blocks the binding interaction between a PD-1 and a PD-L1.

[00452] In one embodiment, provided herein is:

PD-L1-IL-2 fusion protein **A1** comprising amino acid sequences of SEQ ID NOs: 36, 292, and 300;

PD-L1-IL-2 fusion protein **A2** comprising amino acid sequences of SEQ ID NOs: 36, 293, and 300;

PD-L1-IL-2 fusion protein **A3** comprising amino acid sequences of SEQ ID NOs: 36, 294, and 300;

PD-L1-IL-2 fusion protein **A4** comprising amino acid sequences of SEQ ID NOs: 36, 295, and 300;

PD-L1-IL-2 fusion protein **A5** comprising amino acid sequences of SEQ ID NOs: 36, 296, and 300;

PD-L1-IL-2 fusion protein **A6** comprising amino acid sequences of SEQ ID NOs: 36, 297, and 300; or

PD-L1-IL-2 fusion protein **A7** comprising amino acid sequences of SEQ ID NOs: 36, 298, and 300.

[00453] In another embodiment, provided herein is PD-L1-IL-2 fusion protein **A8** comprising amino acid sequences of SEQ ID NOs: 36, 299, and 300.

Pharmaceutical Compositions

[00454] In one embodiment, provided herein is a pharmaceutical composition comprising

an IL-2 mutein or fusion protein provided herein, and a pharmaceutically acceptable excipient.

[00455] In one embodiment, the pharmaceutical composition is formulated as single dosage form.

[00456] In one embodiment, the pharmaceutical composition provided herein is a solid formulation. In another embodiment, the pharmaceutical composition provided herein is a lyophilized solid formulation. In yet another embodiment, the pharmaceutical composition provided herein is a solution. In still another embodiment, the pharmaceutical composition provided herein is an aqueous solution.

[00457] In one embodiment, the pharmaceutical composition provided herein is formulated in a dosage form for parenteral administration. In another embodiment, the pharmaceutical composition provided herein is formulated in a dosage form for intravenous administration. In yet another embodiment, the pharmaceutical composition provided herein is formulated in a dosage form for intramuscular administration. In yet another embodiment, the pharmaceutical composition provided herein is formulated in a dosage form for subcutaneous administration. In still another embodiment, the pharmaceutical composition provided herein is formulated in a dosage form for intratumoral administration.

Methods of Use

[00458] In one embodiment, provided herein is a method of treating, preventing, or ameliorating one or more symptoms of a disorder, disease, or condition mediated by a PD-1 in a subject, comprising administering to the subject in need thereof a therapeutically effective amount of an IL-2 mutein or fusion protein provided herein.

[00459] In certain embodiments, the disorder, disease, or condition mediated by a PD-1 is a proliferative disease.

[00460] In another embodiment, provided herein is a method of treating, preventing, or ameliorating one or more symptoms of a disorder, disease, or condition mediated by an IL-2 in a subject, comprising administering to the subject in need thereof a therapeutically effective amount of an IL-2 mutein or fusion protein provided herein.

[00461] In certain embodiments, the disorder, disease, or condition mediated by an IL-2 is a proliferative disease.

[00462] In yet another embodiment, provided herein is a method for treating, preventing, or ameliorating a proliferative disease in a subject, comprising administering to the subject in need thereof a therapeutically effective amount of an IL-2 mutein or fusion protein provided herein.

[00463] In one embodiment, the proliferative disease is cancer. In another embodiment, the proliferative disease is colon cancer or colorectal cancer.

[00464] In certain embodiments, the cancer is refractory and/or relapsed. In certain embodiments, the cancer is refractory. In certain embodiments, the cancer is relapsed. In certain embodiments, the cancer is metastatic. In certain embodiments, the cancer is resectable. In certain embodiments, the cancer is unresectable. In certain embodiments, the cancer is metastatic.

[00465] In certain embodiments, the cancer is drug-resistant. In certain embodiments, the cancer is multidrug-resistant. In certain embodiments, the cancer is resistant to a chemotherapy. In certain embodiments, the cancer is resistant to an immunotherapy. In certain embodiments, the cancer is resistant to a standard therapy for the cancer.

[00466] In certain embodiments, the subject is a mammal. In certain embodiments, the subject is a human.

[00467] In another embodiment, provided herein is a method of inhibiting the growth of a cell, comprising contacting the cell with an effective amount of an IL-2 mutein or fusion protein provided herein. In certain embodiments, the cell is a cancerous cell. In certain embodiments, the cell is a human cancerous cell. In certain embodiments, the cell is a metastatic cancerous cell.

[00468] In certain embodiments, the therapeutically effective amount is ranging from about 0.001 mg per kg subject body weight every month (mg/kg per month) to 100 mg per kg subject body weight per day (mg/kg per day), from about 0.01 mg/kg per month to about 75

mg/kg per day, from about 0.1 mg/kg per month to about 50 mg/kg per day, from about 0.5 mg/kg per month to about 25 mg/kg per day, or from about 1 mg/kg per month to about 20 mg/kg per day, which can be administered in single or multiple doses. Within this range, the dosage can be ranging from about 0.005 mg/kg per month to about 0.05 mg/kg per day, from about 0.05 mg/kg per month to about 0.5 mg/kg per day, from about 0.5 mg/kg per month to about 5.0 mg/kg per day, from about 1 mg/kg per month to about 15 mg/kg per day, from about 1 mg/kg per month to about 20 mg/kg per day, or from about 1 mg/kg per month to about 50 mg/kg per day.

[00469] The disclosure will be further understood by the following non-limiting examples.

EXAMPLES

Example 1

Cloning, Expression, and Purification of Fusion Proteins

[00470] The amino acid sequence of human IL-2 (hIL-2) was obtained from UNIPROT (hIL-2: P60568, 21-153 aa). A mutant hIL-2 was generated by introducing a mutation to attenuating CD25 and CD122/CD132 bindings. The amino acid sequences of a human anti-PD-1 antibody (VH & VL) were obtained from the Therapeutic Antibody Database (TABS). The deoxyoligonucleotide (DNA) sequences encoding the mutant hIL-2 and human anti-PD-1 antibody were codon optimized for CHO cell expression.

[00471] The DNA sequences encoding (i) the hIL-2 mutein, (ii) the human anti-PD-1 antibody or a fragment thereof, and (iii) a peptide linker were seamlessly assembled together by homology assembly cloning with commercially available kits. The oligonucleotides of each fusion protein were inserted into a UCOE® expression vector CET1019-AS-Puro for CHO cell expression.

[00472] Each fusion protein produced in the CHO cells was purified by a two-step purification process: protein A affinity chromatography using protein A (*e.g.*, AMSPHERE™ A3 or MABSELECT™ SURE™) resin, and ion exchange chromatography (*e.g.*, CAPTO™ Q IMPRES or CAPTO™ S IMPACT) or hydrophobic interaction chromatography (*e.g.*, Phenyl HP).

Example 2

Binding Studies of IL-2 Muteins to CD122 and CD122/CD132

[00473] OCTET® RED96 was used to characterize the interactions of wild-type hIL-2 and hIL-2 muteins with CD25, CD122, or CD122/CD132 heterodimer. AVT-TAG CD122/CD132 heterodimer was purchased from ACROBIO. The protein samples were diluted in the OCTET® kinetic buffer. Briefly, hIL-2 receptors were loaded onto a biosensor. The biosensor was then dipped into a solution containing a wild-type hIL-2 and IL-2 muteins at 10 to 2,000 nM at 24 °C. The association and dissociation sensorgraph was fitted by global or local fitting using the OCTET® Data Analysis HT software. The results are summarized in Table 1. All the hIL-2 muteins tested were found to have abolished or significantly reduced CD25 binding.

Table 1. Interactions of Wild-type hIL-2 and hIL-2 Muteins with CD122 or CD122/CD132

Fusion Protein	SEQ ID NO:	K _d (nM)	
		CD122	CD122/CD132
WT IL-2	179		1.9
hIL-2 Mutein (T3A, R38E, F42A, K76E, C125S)	200		1
IL-2-IL-15 Chimera 2 (T3A, C125S)	221	104	11
IL-2-IL-15 Chimera 29 (I92A, C125S)	248		3.5
IL-2-IL-15 Chimera 42 (E15K, N88A, C125S)	261		53
IL-2-IL-15 Chimera 44 (T3A, L18C)	263		12
IL-2-IL-15 Chimera 46 (T3A, C125S, Q126E)	265		219
IL-2-IL-15 Chimera 54 (T3A, C125S, Q126T)	273	650	30
IL-2-IL-15 Chimera 56 (T3A, C125S, S130R)	275	808	12
IL-2-IL-15 Chimera 58 (T3A, L18C, Q126E)	277	188	229
IL-2-IL-15 Chimera 64 (T3A, L18C, Q126S)	283		382

Fusion Protein	SEQ ID NO:	K _d (nM)	
		CD122	CD122/CD132
IL-2-IL-15 Chimera 65 (T3A, I92A, C125S, Q126E)	285		242
IL-2-IL-15 Chimera 68 (T3A, C125S, Q126T, S130R)	287	466	32
hIL-2 Mutein (T3A, F42A, Y45A, L72G, C125S)	288	550	6.8
IL-2-IL-15 Chimera 69 (T3A, L18S, C125S, Q126E)	301		322

Example 3

Antitumor Activity of Anti-PD-1/IL-2 Fusion Protein in a Xenograft Mouse Model

[00474] MC38 cells are cultured and maintained in DMEM media supplemented with 10% fetal bovine serum, GLUTAMAX™, non-essential amino acids (NEAA), sodium pyruvate, and penicillin/streptomycin. The cells are trypsinized, washed with the media, and counted. The cells are diluted with PBS and 5 x 10⁵ cells in PBS (50 µL) are injected subcutaneously into anesthetized C57BL/6 mice using an 18-gauge needle. A stock solution of an anti-PD-1/IL-2 fusion protein is diluted in PBS on the day of dosing and the mice are dosed intraperitoneally with PBS (control) or the anti-PD-1/IL-2 fusion protein in PBS twice a week for two weeks. Tumor sizes (length (L) and width (W)) are measured twice per week using a digital caliper, and the tumor volume is calculated (L x W x W)/2.

Example 4. Antitumor Activity of Anti-PD-1/IL-2 Fusion Protein in a Xenograft Model using PD-1 Knock-in Mice

[00475] MC38 cells or B16F10 cells are cultured and maintained in DMEM media supplemented with 10% fetal bovine serum, GLUTAMAX™, non-essential amino acids (NEAA), sodium pyruvate, and penicillin/streptomycin. The cells are trypsinized, washed with the media, and counted. The cells are diluted with PBS and 5 x 10⁵ cells in PBS (50 µL) are injected subcutaneously into anesthetized C57BL/6 mice with human PD-1 knock-in using an 18-gauge needle. A stock solution of an anti-PD-1/IL-2 fusion protein is diluted in PBS on the day of dosing and the mice are dosed intraperitoneally with PBS (control) or the anti-PD-1/IL-2 fusion protein in PBS twice a week for two weeks. Tumor sizes (length (L) and width (W)) are

measured twice per week using a digital caliper, and the tumor volume is calculated $(L \times W \times W)/2$.

Example 5

Antitumor Activity of Anti-PD-1/IL-2 Fusion Protein in a Xenograft Mouse Model

[00476] CT26 mouse cells are cultured and maintained in RPMI media supplemented with 10% fetal bovine serum, GLUTAMAX™, and penicillin/streptomycin. The cells are trypsinized, washed with media, and counted. The cells are diluted with PBS and 1×10^6 cells in PBS (100 μ L) are injected subcutaneously into anesthetized BALB/c mice using an 18-gauge needle. A stock solution of an anti-PD-1/IL-2 fusion protein is diluted in PBS on the day of dosing and the mice are dosed intraperitoneally with PBS (control) or the anti-PD-1/IL-2 fusion protein in PBS twice a week for two weeks. Tumor sizes (length (L) and width (W)) are measured twice per week using a digital caliper, and the tumor volume is calculated $(L \times W \times W)/2$.

Example 6

Antitumor Activity of Anti-PD-1/IL-2 Fusion Protein in a Xenograft Mouse Model

[00477] HT-29 cells are cultured and maintained in McCoy's 5a media supplemented with 10% fetal bovine serum and penicillin/streptomycin. The cells are trypsinized, washed with media, counted, and washed with PBS. The cell suspension (1×10^6 cells in PBS (100 μ L)) is injected subcutaneously into anesthetized NCG mice using a 27-gauge needle. After 6 days, human PBMCs (1×10^7 cells in PBS (100 μ L)) are injected into the tail vein of each mouse. A stock solution of an anti-PD-1/IL-2 fusion protein is diluted in PBS on the day of dosing and the mice are dosed intraperitoneally with PBS (control) or the anti-PD-1/IL-2 fusion protein in PBS twice a week for two weeks. Tumor sizes (length (L) and width (W)) are measured twice per week using a digital caliper, and the tumor volume is calculated $(L \times W \times W)/2$.

Example 7

Effect of Anti-PD-1/IL-2 Fusion Proteins on STAT5 Signaling

[00478] Anti-PD-1/IL-2 fusion proteins are evaluated for their effect on pSTAT5 signaling *via* its IL-2 domain in CD3 T-cells. Briefly, CD3 T-cells (100,000) are treated with an anti-PD-

1/IL-2 fusion protein for 30 min at 37 °C and 5% CO₂ in Hanks balanced salt solution containing 10 mM HEPES. Phospho-STAT5 is measured using a phosphor-STAT5 (Tyr694) HTRF assay. The signal ratio at 665 nm/620 nm is multiplied by 1,000 and the data is analyzed with global fitting to determine EC₅₀ values.

[00479] Sequences described herein are provided in the sequence table below.

SEQUENCE TABLE

SEQ ID NO:	Description	Amino Acid Sequence
1	Anti-HSA-1 CDR1	GSTWSINT
2	Anti-HSA-1 CDR2	ISSGGST
3	Anti-HSA-1 CDR3	YAQSTWYPPS
4	Anti-HSA-1 FR1	EVQLVESGGGLVQPGGSLRLSCAAS
5	Anti-HSA-1 FR2	LAWYRQAPGKQRDLVAR
6	Anti-HSA-1 FR3	YYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYC
7	Anti-HSA-1 FR4	WGQGTLLTVSS
8	Anti-HSA-1	EVQLVESGGGLVQPGGSLRLSCAASGSTWSINTLA WYRQAPGKQRDLVARISSGGSTYYADSVKGRFTIS RDN SKNTLYLQMNSLRAEDTAVYYCYAQSTWYPP SWGQGTLLTVSS
9	Anti-HSA-2 CDR1	GFAFRGFG
10	Anti-HSA-2 CDR2	INNGGSDT
11	Anti-HSA-2 CDR3	AIGGPGASP
12	Anti-HSA-2 FR1	QVQLVESGGGVVQPGGSLRLSCAAS
13	Anti-HSA-2 FR2	MSWVRQAPGKGLEWVSS
14	Anti-HSA-2 FR4	SGQGTQVTVSS
15	Anti-HSA-2	QVQLVESGGGVVQPGGSLRLSCAASGFAFRGFGM SWVRQAPGKGLEWVSSINNGGSDTYYADSVKGRF

SEQ ID NO:	Description	Amino Acid Sequence
		TISRDNSKNTLYLQMNSLRAEDTAVYYCAIGGPGA SPSGQGTQVTVSS
16	hIgG1 C _H	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEA LHNHYTQKSLSLSPGK
17	hIgG1 C _H Mutein (N297A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEA LHNHYTQKSLSLSPGK
18	hIgG1 C _H Mutein (LALA, N297A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEA LHNHYTQKSLSLSPGK
19	hIgG1 C _H Mutein (S354C, T366W)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQ VSLWCLVKGFYPSDIAVEWESNGQPENNYKTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEA LHNHYTQKSLSLSPGK
20	hIgG1 C _H Mutein (Y349C, T366S, L368A, Y407V)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK

SEQ ID NO:	Description	Amino Acid Sequence
		ALPAPIEKTISKAKGQPREPQVCTLPSSREEMTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
21	hIgG1 C _H Mutein (S354C, T366W, N297A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVSVSLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQ VSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
22	hIgG1 C _H Mutein (Y349C, T366S, L368A, Y407V, N297A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVSVSLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVCTLPSSREEMTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
23	hIgG1 C _H Mutein (S354C, T366W, LALA, N297A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVSVSLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQ VSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
24	hIgG1 C _H Mutein (Y349C, T366S, L368A, Y407V, LALA, N297A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVSVSLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVCTLPSSREEMTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
25	hIgG4 C _H	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYTCNVDHKPSNTKVDKRVESKYGPCC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV

SEQ ID NO:	Description	Amino Acid Sequence
		VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL VKGIFYPSDIAVEWESNGQPENNYKTTTPVLDSDGS FFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYT QKSLSLSLGK
26	hIgG4 C _H Mutein (T366W)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTKITYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHY TQKSLSLSLGK
27	hIgG4 C _H Mutein (T366S, T368A)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTKITYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLSC AVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHY TQKSLSLSLGK
28	hKappa C _L	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC
29	Anti-hPD-1-A CDRL1 (IMGT)	QSVSSY
	Anti-hPD-1-A CDRL2 (IMGT)	DAS
30	Anti-hPD-1-A CDRL3 (IMGT)	QQSSNWPRT
31	Anti-hPD-1-A CDRH1 (IMGT)	GITFSNSG
32	Anti-hPD-1-A CDRH2 (IMGT)	IWYDGSKR
33	Anti-hPD-1-A CDRH3 (IMGT)	ATNDDY

SEQ ID NO:	Description	Amino Acid Sequence
34	Anti-hPD-1-A Light Chain Variable	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWY QQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFT LTISSELPEDFAVYYCQQSSNWPRTFGQGTKVEIK
35	Anti-hPD-1-A Heavy Chain Variable	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF TISRDN SKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGT LTVSS
36	Anti-hPD-1-A Light Chain	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWY QQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFT LTISSELPEDFAVYYCQQSSNWPRTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVCLLNFPYREA KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTL TLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
37	Anti-hPD-1-A Heavy Chain	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF TISRDN SKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGT LTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVPSSSLGKTKYTCNV DHKPSNTKVD KRVESKYGPCCPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSC SVMHEALHNHYTQKSLSLGLK
38	Anti-hPD-1-B CDRL1 (IMGT)	KGVSTSGYSY
	Anti-hPD-1-B CDRL2 (IMGT)	LAS
39	Anti-hPD-1-B CDRL3 (IMGT)	QHSRDLPLT
40	Anti-hPD-1-B CDRH1 (IMGT)	GYTFTNYY
41	Anti-hPD-1-B CDRH2 (IMGT)	INPSNGGT
42	Anti-hPD-1-B CDRH3 (IMGT)	ARRDYRFDMGFDY
43	Anti-hPD-1-B Light Chain Variable	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYL HWYQQKPGQAPRLLIYLASYLESVGPARGSGSGS TDFTLTISSELPEDFAVYYCQHSRDLPLTFGGGTKV EIK

SEQ ID NO:	Description	Amino Acid Sequence
44	Anti-hPD-1-B Heavy Chain Variable	QVQLVQSGVEVKKPGASVKVSKASGYTFTNYYM YWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNR VTLTDSSTTTAYMELKSLQFDDTAVYYCARRDY RFDMGFDYWGQGTTVTVSS
45	Anti-hPD-1-B Light Chain	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYL HWYQQKPGQAPRLLIYLASYLESQVPAFSGSGSG TDFTLTISLEPEDFAVYYCQHSRDLPFTFGGGTKV EIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSYSTSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC
46	Anti-hPD-1-B Heavy Chain	QVQLVQSGVEVKKPGASVKVSKASGYTFTNYYM YWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNR VTLTDSSTTTAYMELKSLQFDDTAVYYCARRDY RFDMGFDYWGQGTTVTVSSASTKGPSVFPLAPCSR STSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDH KPSNTKVKDKRVESKYGPPCPPCPAPEFLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMEALHNHYTQKSLSLSLGK
47	Anti-hPD-1-C CDRL1 (IMGT)	QSIVHSNGNTY
	Anti-hPD-1-C CDRL2 (IMGT)	KVS
48	Anti-hPD-1-C CDRL3 (IMGT)	FQGS HVPLT
49	Anti-hPD-1-C CDRH1 (IMGT)	GYTFTDYE
50	Anti-hPD-1-C CDRH2 (IMGT)	IESETGGT
51	Anti-hPD-1-C CDRH3 (IMGT)	AREGITT VATTYYWYFDV
52	Anti-hPD-1-C Light Chain Variable	DVVM TQSPLSLPVT LGQPASISCRSSQSIVHSNGNT YLEWYLQKPGQSPQLLIYKVS NRFSQV PDRFSGSG SGTDFTLTKISRVEAEDVGVYYCFQGS HVPLTFGQG TKLEIK
53	Anti-hPD-1-C Heavy Chain Variable	QGQLVQSGAEVKKPGASVKVSKASGYTFTDYEM HWVRQAPIHG LEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSED TAVYYCAREGITTVA

SEQ ID NO:	Description	Amino Acid Sequence
		TTYWYFDVWGQGTTVTVSS
54	Anti-hPD-1-C Light Chain (Kappa)	DVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNT YLEWYLQKPGQSPQLLIYKVSNRFSQVDPDRFSGSG SGTDFTLKISRVEAEDVGVYYCFQGSHTVPLTFGQG TKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC
55	Anti-hPD-1-C Heavy Chain	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQFKGRVT ITADKSTSTAYMELSSLRSEDVAVYYCAREGITTVA TTYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTKAKGQPREP QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMEALHNHYTQKSLSLGLK
56	Anti-hPD-1-D CDRL1 (IMGT)	QTIGTW
	Anti-hPD-1-D CDRL2 (IMGT)	TAT
57	Anti-hPD-1-D CDRL3 (IMGT)	QQVYSIPWT
58	Anti-hPD-1-D CDRH1 (IMGT)	GFTFSSYM
59	Anti-hPD-1-D CDRH2 (IMGT)	ISGGGANT
60	Anti-hPD-1-D CDRH3 (IMGT)	ARQLYYFDY
61	Anti-hPD-1-D Light Chain Variable	DIQMTQSPSSLSASVGDRVITITCLASQTIGTWLTWY QQKPGKAPKLLIYTATSLADGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQVYSIPWTFGGGTKVEIK
62	Anti-hPD-1-D Heavy Chain Variable	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYMM WVRQAPGKGLEWVATISGGGANTYYPDVSKGRFT ISRDNANKNSLYLQMNSLRAEDTAVYYCARQLYYF DYWGQGTTVTVSS
63	Anti-hPD-1-D Light Chain	DIQMTQSPSSLSASVGDRVITITCLASQTIGTWLTWY QQKPGKAPKLLIYTATSLADGVPSRFSGSGSGTDFT

SEQ ID NO:	Description	Amino Acid Sequence
		LTISLQPEDFATYYCQQVYSIPWTFGGGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTL TLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
64	Anti-hPD-1-D Heavy Chain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYMMS WVRQAPGKGLEWVATISGGGANTYYPD SVKGRFT ISRDN AKNSLYLQMNSLRAEDTAVYYCARQLYYF DYWGQGTTTVTVSSASTKGPSVFPLAPCSRSTSESTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVTVPSSSLGKTYTCNV DHKPSNTKV DKRVESKYGPCCPPCPAPEFLGGPSVFLFPPKPKDT LMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEV HNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLGLK
65	Anti-hPD-1-E CDRL1 (IMGT)	QGISSW
	Anti-hPD-1-E CDRL2 (IMGT)	AAS
66	Anti-hPD-1-E CDRL3 (IMGT)	QQANHLPT
67	Anti-hPD-1-E CDRH1 (IMGT)	GGTFSSYA
68	Anti-hPD-1-E CDRH2 (IMGT)	IIPMFDTA
69	Anti-hPD-1-E CDRH3 (IMGT)	ARAEHSSTGTFDY
70	Anti-hPD-1-E Light Chain Variable	DIQMTQSPSSVSASVGDRVITICRASQGISSWLAW YQQKPGKAPKLLISAASSLQSGVPSRFSGSGSGTDF TLTISLQPEDFATYYCQQANHLPTFGGGTKVEIK
71	Anti-hPD-1-E Heavy Chain Variable	QVQLVQSGAEVKKPGSSVKVSC KASGGTFSSYAIS WVRQAPGQGLEWMGLIIPMFDTAGYAQKFQGRV AITVDESTSTAYMELSSLRSED TAVYYCARAEHSST GTFDYWGQGT LTVSS
72	Anti-hPD-1-E Light Chain	DIQMTQSPSSVSASVGDRVITICRASQGISSWLAW YQQKPGKAPKLLISAASSLQSGVPSRFSGSGSGTDF TLTISLQPEDFATYYCQQANHLPTFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYLSST TLTISKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC

SEQ ID NO:	Description	Amino Acid Sequence
73	Anti-hPD-1-E Heavy Chain	QVQLVQSGAEVKKPGSSVKV SCKASGGTFSSYAIS WVRQAPGQGLEWMGLIIPMFD TAGYAQKFQGRV AITVDESTSTAYMELSSLRSEDTAVYYCARAEHSST GTFDYWGQGTLLVTVSSASTKGPSVFPLAPCSRSTSE STAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLG TKTYTCNV DHKPSN TKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGN VFSCSVMHEALHNHYTQKSLSL SLGK
74	Anti-hPD-1-F CDRL1 (IMGT)	QSLLD SGNQKNF
	Anti-hPD-1-F CDRL2 (IMGT)	WAS
75	Anti-hPD-1-F CDRL3 (IMGT)	QNDYSYPYT
76	Anti-hPD-1-F CDRH1 (IMGT)	GYTFTTYW
77	Anti-hPD-1-F CDRH2 (IMGT)	IYPGTGGS
78	Anti-hPD-1-F CDRH3 (IMGT)	TRWTTGTGAY
79	Anti-hPD-1-F Light Chain Variable	EIVLTQSPATLSLSPGERATL SCKSSQSLLD SGNQK NFLTWYQQKPGQAPRLLIYWASTRESGVPSRFSGS GSGTDFTE TISSLEAEDAATYYCQNDYSYPYTFGQ GTKVEIK
80	Anti-hPD-1-F Heavy Chain Variable	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMH WVRQATGQGLEWMGNIYPGTGGSNFDEKEKNRV TITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGT GAYWGQGT TTVTVSS
81	Anti-hPD-1-F Light Chain	EIVLTQSPATLSLSPGERATL SCKSSQSLLD SGNQK NFLTWYQQKPGQAPRLLIYWASTRESGVPSRFSGS GSGTDFTE TISSLEAEDAATYYCQNDYSYPYTFGQ GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC
82	Anti-hPD-1-F Heavy Chain	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMH WVRQATGQGLEWMGNIYPGTGGSNFDEKEKNRV TITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGT

SEQ ID NO:	Description	Amino Acid Sequence
		GAYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVTVPSSSLGKTKYTCNVDHKPSNTK VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKD TLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVE VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVF SCSVMHEALHNHYTQKSLSLSLQ
83	Anti-hPD-1-G CDRL1 (IMGT)	ESVSND
	Anti-hPD-1-G CDRL2 (IMGT)	YAF
84	Anti-hPD-1-G CDRL3 (IMGT)	HQAYSSPYT
85	Anti-hPD-1-G CDRH1 (IMGT)	GFSLTSYG
86	Anti-hPD-1-G CDRH2 (IMGT)	IYADGST
87	Anti-hPD-1-G CDRH3 (IMGT)	ARAYGNYWYIDV
88	Anti-hPD-1-G Light Chain Variable	DIVMTQSPDSLAVSLGERATINCKSSESVSNDVAW YQQKPGQPPKLLINIAFHRFTGVPDRFSGSGYGTD FTLTISLQAEDVAVYYCHQAYSSPYTFGQGTKLEI K
89	Anti-hPD-1-G Heavy Chain Variable	QVQLQESGPGLVKPSSETLSLTCTVSGFSLTSYGVH WIRQPPGKGLEWIGVIYADGSTNYPNPSLKSRTISK DTSKNQVSLKLSSVTAADTAVYYCARAYGNYWYI DVWGQGTTVTVSS
90	Anti-hPD-1-G Light Chain	DIVMTQSPDSLAVSLGERATINCKSSESVSNDVAW YQQKPGQPPKLLINIAFHRFTGVPDRFSGSGYGTD FTLTISLQAEDVAVYYCHQAYSSPYTFGQGTKLEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC
91	Anti-hPD-1-G Heavy Chain	QVQLQESGPGLVKPSSETLSLTCTVSGFSLTSYGVH WIRQPPGKGLEWIGVIYADGSTNYPNPSLKSRTISK DTSKNQVSLKLSSVTAADTAVYYCARAYGNYWYI DVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVTVPSSSLGKTKYTCNVDHKPSNTKV DKRVESKYGPPCPPCPAPPVAGGPSVFLFPPKPKDT

SEQ ID NO:	Description	Amino Acid Sequence
		LMISRTPEVTCVVVAVSQEDPEVQENWYVDGVEV HNAKTKPREEQENSTYRVVSVLTVVHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLSLGIK
92	Anti-hPD-1-H CDRL1 (IMGT)	LSINTF
	Anti-hPD-1-H CDRL2 (IMGT)	AAS
93	Anti-hPD-1-H CDRL3 (IMGT)	QQSSNTPFT
94	Anti-hPD-1-H CDRH1 (IMGT)	GFTFSNFG
95	Anti-hPD-1-H CDRH2 (IMGT)	ISGGGRDT
96	Anti-hPD-1-H CDRH3 (IMGT)	VKWGNIYFDY
97	Anti-hPD-1-H Light Chain Variable	DIQMTQSPSSLSASVGDSTITCRASLSINTFLNWKYQ QKPGKAPNLLIYAASSLHGGVPSRFSGSGSGTDFTL TIRTLQPEDFATYYCQQSSNTPFTFGPGTVVDVFR
98	Anti-hPD-1-H Heavy Chain Variable	EVQLLESQGVLVQPGGSLRLSAAASGFTFSNFGMT WVRQAPGKGLEWVSGISGGGRDTYFADSVKGRFT ISRDNKNTLYLQMNSLKGEDTAVYYCVKWGNIY FDYWGQGTLLTVSS
99	Anti-hPD-1-H Light Chain	DIQMTQSPSSLSASVGDSTITCRASLSINTFLNWKYQ QKPGKAPNLLIYAASSLHGGVPSRFSGSGSGTDFTL TIRTLQPEDFATYYCQQSSNTPFTFGPGTVVDVFRRT VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKSTYSLSSLT LSKADYEEKHKVYACEVTHQGLSPVTKSFNRGEC
100	Anti-hPD-1-H Heavy Chain	EVQLLESQGVLVQPGGSLRLSAAASGFTFSNFGMT WVRQAPGKGLEWVSGISGGGRDTYFADSVKGRFT ISRDNKNTLYLQMNSLKGEDTAVYYCVKWGNIY FDYWGQGTLLTVSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSVTVPSSSLGKTYTCNVDHKPSNTK VDRKVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVF

SEQ ID NO:	Description	Amino Acid Sequence
		SCSVMHEALHNHYTQKSLSLGLGK
101	Anti-hPD-1-I CDRL1 (IMGT)	QDVTTA
	Anti-hPD-1-I CDRL2 (IMGT)	WAS
102	Anti-hPD-1-I CDRL3 (IMGT)	QQHYTIPWT
103	Anti-hPD-1-I CDRH1 (IMGT)	GFTFSNYG
104	Anti-hPD-1-I CDRH2 (IMGT)	ISGGGSNI
105	Anti-hPD-1-I CDRH3 (IMGT)	VSYYYGIDF
106	Anti-hPD-1-I Light Chain Variable	DIQMTQSPSSLSASVGDRVTITCKASQDVTAVAW YQQKPGKAPKLLIYWASTRHTGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQHYTIPWTFGGGTKLEI K
107	Anti-hPD-1-I Heavy Chain Variable	QVQLVESGGGLVKPGGSLRLSCAASGFTFSNYGMS WIRQAPGKGLEWVSTISGGGSNIYYADSVKGRFTIS RDNAKNSLYLQMNSLRAEDTAVYYCVSYYYGIDF WGQGTSTVTVSS
108	Anti-hPD-1-I Light Chain	DIQMTQSPSSLSASVGDRVTITCKASQDVTAVAW YQQKPGKAPKLLIYWASTRHTGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQHYTIPWTFGGGTKLEI KRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC
109	Anti-hPD-1-I Heavy Chain	QVQLVESGGGLVKPGGSLRLSCAASGFTFSNYGMS WIRQAPGKGLEWVSTISGGGSNIYYADSVKGRFTIS RDNAKNSLYLQMNSLRAEDTAVYYCVSYYYGIDF WGQGTSTVTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVPSSSLGKTYTCNVDPKPKNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSC SVMHEALHNHYTQKSLSLGLGK

SEQ ID NO:	Description	Amino Acid Sequence
110	Anti-hPD-1-J CDRL1 (IMGT)	ESVDNYGMSF
	Anti-hPD-1-J CDRL2 (IMGT)	AAS
111	Anti-hPD-1-J CDRL3 (IMGT)	QQSKEVPYT
112	Anti-hPD-1-J CDRH1 (IMGT)	GYSFTSYW
113	Anti-hPD-1-J CDRH2 (IMGT)	IHPDSET
114	Anti-hPD-1-J CDRH3 (IMGT)	AREHYGTSPFAY
115	Anti-hPD-1-J Light Chain Variable	EIVLTQSPATLSLSPGERATLSCRASESVDNYGMSF MNWFQQKPGQPPKLLIHAASNQSGVPSRFSGSGS GTDFTLTISSELEPEDFAVYFCQQSKEVPYTFGGGK VEIK
116	Anti-hPD-1-J Heavy Chain Variable	QVQLVQSGAEVKKPGASVKVSCKASGYSTSYWM NWVRQAPGQGLEWIGVIHPSDSETWLDQKFKDRV TITVDKSTSTAYMELSSLRSEDFAVYFCAREHYGT SPFAYWGQGTLLVTVSS
117	Anti-hPD-1-J Light Chain	EIVLTQSPATLSLSPGERATLSCRASESVDNYGMSF MNWFQQKPGQPPKLLIHAASNQSGVPSRFSGSGS GTDFTLTISSELEPEDFAVYFCQQSKEVPYTFGGGK VEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSTLTLSKADYEKHKVYACEVTHQGLSPVTKS FNRGEC
118	Anti-hPD-1-J Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYSTSYWM NWVRQAPGQGLEWIGVIHPSDSETWLDQKFKDRV TITVDKSTSTAYMELSSLRSEDFAVYFCAREHYGT SPFAYWGQGTLLVTVSSASTKGPSVFPLAPCSRSTSE STAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLGKTYTCNVDPKPSN TKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKP KDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYCKVSNKGLPSSIEKISKAKGQPREPQVYTL PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGN VFSCSVMEALHNHYTQKSLSLSLG
119	Anti-hPD-1-V _H H-A CDR1 (IMGT)	GSRIA

SEQ ID NO:	Description	Amino Acid Sequence
120	Anti-hPD-1-V _H H-A CDR2 (IMGT)	ISQNGGST
121	Anti-hPD-1-V _H H-A CDR3 (IMGT)	AAALMTWYGTSYCPHLSTFEGF
122	Anti-hPD-1-V _H H-A	QVKLEESGGGSVQAGGSLRLSCAASGSRIAMGWF RQAPGKEREFGVGAISQNGGSTCYADSVKDRFTISR DNAKNMVYVYLMNSLKPEDTAVYYCAAALMTWY GTSYCPHLSTFEGFWGQGTLLTVSS
123	Anti-hPD-1-V _H H-B CDR1 (IMGT)	GFTLDDYA
124	Anti-hPD-1-V _H H-B CDR2 (IMGT)	ISSSDGST
125	Anti-hPD-1-V _H H-B CDR3 (IMGT)	STDVGCYGSTSPEAY
126	Anti-hPD-1-V _H H-B	QVKLEESGGGLVQPGGSLRLSCAASGFTLDDYALG WFRQAPGKEREVSCISSSDGSTYYADSVKGRFTIS RDNAKNTVYVYLMNSLKPEDTAVYYCSTDVGCYGS TSPEAYWGKGTLLTVSS
127	Anti-hPD-1-V _H H-C CDR1 (IMGT)	GRTFSSYD
128	Anti-hPD-1-V _H H-C CDR2 (IMGT)	VSASGGRS
129	Anti-hPD-1-V _H H-C CDR3 (IMGT)	AARNGAYYITIDPAQYNY
130	Anti-hPD-1-V _H H-C	QVQLVDSGGGLVQAGGSLRLSCAASGRTFSSYDFG WFRQAPGKEREVFAAVSASGGRSGYSDFAKDRFT TSRENAKNTVYVYLMNSLKPEDTAVYYCAARNGAY YITIDPAQYNYWGQGTLLTVSS
131	Anti-hPD-1-V _H H-D CDR1 (IMGT)	GRTFSSYA
132	Anti-hPD-1-V _H H-D CDR2 (IMGT)	ISWSSGIT
133	Anti-hPD-1-V _H H-D CDR3 (IMGT)	AADLNQESSWYDY
134	Anti-hPD-1-V _H H-D	QVQLVESGGGLVQAGDSLTLSCAASGRTFSSYAM GWFRQAPGKEREVFAAISWSSGITYYEDSVKGRFT ISRDNAKNTVYVYLMNSLKPEDTAVYYCAADLNQE SSWYDYWGQGTQTVSS

SEQ ID NO:	Description	Amino Acid Sequence
135	Anti-hPD-1-V _H H-E CDR1 (IMGT)	GSRYA
136	Anti-hPD-1-V _H H-E CDR2 (IMGT)	ISWNGGST
137	Anti-hPD-1-V _H H-E CDR3 (IMGT)	AAALIPKYGTSYCPHISTEFD
138	Anti-hPD-1-V _H H-E	QVKLEESGGGSVQAGGSLRLSCAGSGSRYAMGWF RQAPGKEREVFAAISWNGGSTCYADSVKGRFTISR DNAKNMVYLMNSLKPEDTAVYYCAAALIPKYGT SYCPHISTEFDWVGQGTQVTVSS
	GSG Linker	GSG
139	(GSG) ₂ Linker	GSGGSG
140	(GSG) ₃ Linker	GSGGSGGSG
141	(GSG) ₄ Linker	GSGGSGGSGGSG
142	G3S Linker	GGGS
143	(G3S) ₂ Linker	GGGSGGGS
144	(G3S) ₃ Linker	GGGSGGGSGGGS
145	(G3S) ₄ Linker	GGGSGGGSGGGSGGGS
146	G4S Linker	GGGS
147	(G4S) ₂ Linker	GGGSGGGGS
148	(G4S) ₃ Linker	GGGSGGGSGGGGS
149	(G4S) ₄ Linker	GGGSGGGSGGGSGGGGS
150	SGSG Linker	SGSG
151	S(GSG) ₂ Linker	SGSGGSG
152	S(GSG) ₃ Linker	SGSGGSGGSG
153	S(GSG) ₄ Linker	SGSGGSGGSGGSG

SEQ ID NO:	Description	Amino Acid Sequence
154	SG3S Linker	SGGGS
155	S(G3S) ₂ Linker	SGGGSGGGS
156	S(G3S) ₃ Linker	SGGGSGGGSGGGS
157	S(G3S) ₄ Linker	SGGGSGGGSGGGSGGGS
158	SG4S Linker	SGGGGS
159	S(G4S) ₂ Linker	SGGGGSGGGGGS
160	S(G4S) ₃ Linker	SGGGGSGGGGSGGGGS
161	S(G4S) ₄ Linker	SGGGGSGGGGSGGGSGGGGS
162	EAAAK Linker	EAAAK
163	(EAAAK) ₂ Linker	EAAAKEAAAK
164	(EAAAK) ₃ Linker	EAAAKEAAAKEAAAK
165	(EAAAK) ₄ Linker	EAAAKEAAAKEAAAKEAAAK
166	PAPAP Linker	PAPAP
167	(PAPAP) ₂ Linker	PAPAPPAPAP
168	(PAPAP) ₃ Linker	PAPAPPAPAPPAPAP
169	(PAPAP) ₄ Linker	PAPAPPAPAPPAPAPPAPAP
170	VLVH Linker	IKRTVAAP
171	RAKPS Linker	RAKPS
172	(RAKPS) ₂ Linker	RAKPSRAKPS
173	ASTKG Linker	ASTKG
174	(ASTKG) ₂ Linker	ASTKGASTKG

SEQ ID NO:	Description	Amino Acid Sequence
175	AKTHT Linker	AKTHT
176	(AKTHT) ₂ Linker	AKHTAKTHT
177	ASTKG(G4S) ₂ Linker	ASTKGGGGSGGGGS
178	RAKPS(G4S) ₂ Linker	RAKPSGGGGSGGGGS
179	hIL-2	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFCQSIISTLT
180	hIL-2 (T3A)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLN NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFCQSIISTLT
181	hIL-2 (C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
182	hIL-2 (C125A)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFAQSIISTLT
183	hIL-2 (T3A, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLN NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
184	hIL-2 (T3A, C125A)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLN NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFAQSIISTLT
185	hIL-2 Mutein (R38N, L40T, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TNMTTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
186	hIL-2 Mutein (T3A, R38N, L40T, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTNMTTFKFYMPKKATELKHLQCLEEELKPLEEVLN NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
187	hIL-2 Mutein (E15K, R38N, L40T, C125S)	APTSSSTKKTQLQKHLLLDLQMILNGINNYKNPK LTNMTTFKFYMPKKATELKHLQCLEEELKPLEEVLN

SEQ ID NO:	Description	Amino Acid Sequence
		NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
188	hIL-2 Mutein (T3A, E15K, R38N, L40T, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTNMTTFKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
189	hIL-2 Mutein (E15K, K32E, R38N, L40T, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYENPKL TNMTTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
190	hIL-2 Mutein (T3A, E15K, K32E, R38N, L40T, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYENPK LTNMTTFKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
191	hIL-2 Mutein (E15K, R38N, L40T, K76E, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTNMTTFKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
192	hIL-2 Mutein (T3A, E15K, R38N, L40T, K76E, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTNMTTFKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
193	hIL-2 Mutein (K32E, R38E, F42A, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYENPKL TEMLTAKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
194	hIL-2 Mutein (T3A, K32E, R38E, F42A, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYENPKL TEMLTAKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
195	hIL-2 Mutein (K32E, R38E, F42K, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYENPKL TEMLTKKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
196	hIL-2 Mutein (T3A, K32E, R38E, F42K, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYENPKL TEMLTKKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
197	hIL-2 Mutein (E15K, K32E, R38E, F42K, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYENPKL TEMLTKKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
198	hIL-2 Mutein (T3A, E15K, K32E, R38E,	APASSSTKKTQLQLKHLLLDLQMILNGINNYENPKL TEMLTKKFYMPKKATELKHLQCLEEELKPLEEVL

SEQ ID NO:	Description	Amino Acid Sequence
	F42K, C125S)	NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
199	hIL-2 Mutein (R38E, F42A, K76E, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TEMLTAKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSENFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
200	hIL-2 Mutein (T3A, R38E, F42A, K76E, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTEMLTAKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
201	hIL-2 Mutein (R38E, F42K, K76E, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TEMLTKKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSENFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
202	hIL-2 Mutein (T3A, R38E, F42K, K76E, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTEMLTKKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
203	hIL-2 Mutein (E15K, R38E, F42K, K76E, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTEMLTKKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
204	hIL-2 Mutein (T3A, E15K, R38E, F42K, K76E, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTEMLTKKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
205	hIL-2 Mutein (F42C, V69C, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLTCKFYMPKKATELKHLQCLEEELKPLEECLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
206	hIL-2 Mutein (T3A, F42C, V69C, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTRMLTCKFYMPKKATELKHLQCLEEELKPLEECL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
207	hIL-2 Mutein (K32E, F42C, V69C, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYENPKL TRMLTCKFYMPKKATELKHLQCLEEELKPLEECLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
208	hIL-2 Mutein (T3A, K32E, F42C, V69C, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYENPKL TRMLTCKFYMPKKATELKHLQCLEEELKPLEECLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
209	hIL-2 Mutein (F42C, V69C, K76E, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLTCKFYMPKKATELKHLQCLEEELKPLEECLN LAQSENFHLRPRDLISNINVIVLELKGSETTFMCEY

SEQ ID NO:	Description	Amino Acid Sequence
		ADETATIVEFLNRWITFSQSIISTLT
210	hIL-2 Mutein (T3A, F42C, V69C, K76E, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTRMLTCKFYMPKKATELKHLQCLEEEELKPLEECL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
211	hIL-2 Mutein (E15K, F42C, V69C, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTRMLTCKFYMPKKATELKHLQCLEEEELKPLEECL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
212	hIL-2 Mutein (T3A, E15K, F42C, V69C, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTRMLTCKFYMPKKATELKHLQCLEEEELKPLEECL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
213	hIL-2 Mutein (E15K, K32E, F42C, V69C, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYENPKL TRMLTCKFYMPKKATELKHLQCLEEEELKPLEECLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
214	hIL-2 Mutein (T3A, E15K, K32E, F42C, V69C, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYENPK LTRMLTCKFYMPKKATELKHLQCLEEEELKPLEECL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
215	hIL-2 Mutein (E15K, F42C, V69C, K76E, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTRMLTCKFYMPKKATELKHLQCLEEEELKPLEECL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
216	hIL-2 Mutein (T3A, E15K, F42C, V69C, K76E, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTRMLTCKFYMPKKATELKHLQCLEEEELKPLEECL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
217	hIL-2 Fragment (N29 to L40)	NNYKNPKLTRML
218	IL-15 Hinge Fragment- containing Peptide 1	QSMEIDAT
219	IL-15 Hinge Fragment- containing peptide 2	QSMHIDAT
220	IL-2-IL-15 Chimera 1 (C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
221	IL-2-IL-15 Chimera 2 (T3A, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET

SEQ ID NO:	Description	Amino Acid Sequence
		ATIVEFLNRWITFSQSIISTLT
222	IL-2-IL-15 Chimera 3 (E32H, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
223	IL-2-IL-15 Chimera 4 (T3A, E32H, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
224	IL-2-IL-15 Chimera 5 (E15K, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
225	IL-2-IL-15 Chimera 6 (T3A, E15K, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
226	IL-2-IL-15 Chimera 7 (E15K, E32H, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
227	IL-2-IL-15 Chimera 8 (T3A, E15K, E32H, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
228	IL-2-IL-15 Chimera 9 (E15K, E32H, S87D, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLIDNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
229	IL-2-IL-15 Chimera 10 (T3A, E15K, E32H, S87D, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLIDNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
230	IL-2-IL-15 Chimera 11 (H16E, C125S)	APTSSSTKKTQLQLEELLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
231	IL-2-IL-15 Chimera 12 (T3A, H16E, C125S)	APASSSTKKTQLQLEELLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
232	IL-2-IL-15 Chimera 13 (H16F, C125S)	APTSSSTKKTQLQLEFLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET

SEQ ID NO:	Description	Amino Acid Sequence
		ATIVEFLNRWITFSQSIISTLT
233	IL-2-IL-15 Chimera 14 (T3A, H16F, C125S)	APASSSTKKTQLQLEFLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
234	IL-2-IL-15 Chimera 15 (H16I, C125S)	APTSSSTKKTQLQLEILLLDLQMILNGIQSMEIDATT FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT
235	IL-2-IL-15 Chimera 16 (T3A, H16I, C125S)	APASSSTKKTQLQLEILLLDLQMILNGIQSMEIDATT FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT
236	IL-2-IL-15 Chimera 17 (H16V, C125S)	APTSSSTKKTQLQLEVLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
237	IL-2-IL-15 Chimera 18 (T3A, H16V, C125S)	APASSSTKKTQLQLEVLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
238	IL-2-IL-15 Chimera 19 (D20T, C125S)	APTSSSTKKTQLQLEHLLLTLMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
239	IL-2-IL-15 Chimera 20 (T3A, D20T, C125S)	APASSSTKKTQLQLEHLLLTLMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
240	IL-2-IL-15 Chimera 21 (D20A, C125S)	APTSSSTKKTQLQLEHLLLALQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
241	IL-2-IL-15 Chimera 22 (T3A, D20A, C125S)	APASSSTKKTQLQLEHLLLALQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
242	IL-2-IL-15 Chimera 23 (D20E, C125S)	APTSSSTKKTQLQLEHLLLELQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
243	IL-2-IL-15 Chimera 24 (T3A, D20E, C125S)	APASSSTKKTQLQLEHLLLELQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET

SEQ ID NO:	Description	Amino Acid Sequence
		ATIVEFLNRWITFSQSIISTLT
244	IL-2-IL-15 Chimera 25 (D20K, C125S)	APTSSSTKKTQLQLEHLLLKLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
245	IL-2-IL-15 Chimera 26 (T3A, D20K, C125S)	APASSSTKKTQLQLEHLLLKLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
246	IL-2-IL-15 Chimera 27 (N88A, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISAINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
247	IL-2-IL-15 Chimera 28 (T3A, N88A, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISAINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
248	IL-2-IL-15 Chimera 29 (I92A, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVAVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
249	IL-2-IL-15 Chimera 30 (T3A, I92A, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVAVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
250	IL-2-IL-15 Chimera 31 (I92G, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVGVLLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
251	IL-2-IL-15 Chimera 32 (T3A, I92G, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVGVLLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
252	IL-2-IL-15 Chimera 33 (I92D, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVDVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
253	IL-2-IL-15 Chimera 34 (T3A, I92D, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVDVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
254	IL-2-IL-15 Chimera 35 (I92E, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVEVLELKGSETTFMCEYADET

SEQ ID NO:	Description	Amino Acid Sequence
		ATIVEFLNRWITFSQSIISTLT
255	IL-2-IL-15 Chimera 36 (T3A, I92E, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVEVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
256	IL-2-IL-15 Chimera 37 (H16E, I92A, C125S)	APTSSSTKKTQLQLEELLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVAVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
257	IL-2-IL-15 Chimera 38 (T3A, H16E, I92A, C125S)	APASSSTKKTQLQLEELLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVAVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
258	IL-2-IL-15 Chimera 39 (E15K, I92A, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVAVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
259	IL-2-IL-15 Chimera 40 (T3A, E15K, I92A, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVAVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
260	IL-2-IL-15 Chimera 41 (E15K, H16I, C125S)	APTSSSTKKTQLQLKILLLDLQMILNGIQSMEIDATT FKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQSK NFHRLRPDLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT
261	IL-2-IL-15 Chimera 42 (E15K, N88A, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISAINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
262	IL-2-IL-15 Chimera 43 (L18C)	APTSSSTKKTQLQLEHLCLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLT
263	IL-2-IL-15 Chimera 44 (T3A, L18C)	APASSSTKKTQLQLEHLCLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLT
264	IL-2-IL-15 Chimera 45 (C125S, Q126E)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSESIISTLT
265	IL-2-IL-15 Chimera 46 (T3A, C125S, Q126E)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET

SEQ ID NO:	Description	Amino Acid Sequence
		ATIVEFLNRWITFSESIISTLT
266	IL-2-IL-15 Chimera 47 (C125S, Q126K)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSKSIISTLT
267	IL-2-IL-15 Chimera 48 (T3A, C125S, Q126K)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSKSIISTLT
268	IL-2-IL-15 Chimera 49 (C125S, Q126R)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSRSIISTLT
269	IL-2-IL-15 Chimera 50 (T3A, C125S, Q126R)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSRSIISTLT
270	IL-2-IL-15 Chimera 51 (C125S, Q126S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSSSIISTLT
271	IL-2-IL-15 Chimera 52 (T3A, C125S, Q126S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSSSIISTLT
272	IL-2-IL-15 Chimera 53 (C125S, Q126T)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSTSIISTLT
273	IL-2-IL-15 Chimera 54 (T3A, C125S, Q126T)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSTSIISTLT
274	IL-2-IL-15 Chimera 55 (C125S, S130R)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIIRTLT
275	IL-2-IL-15 Chimera 56 (T3A, C125S, S130R)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIIRTLT
276	IL-2-IL-15 Chimera 57 (L18C, Q126E)	APTSSSTKKTQLQLEHLCCLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET

SEQ ID NO:	Description	Amino Acid Sequence
		ATIVEFLNRWITFCESIISTLT
277	IL-2-IL-15 Chimera 58 (T3A, L18C, Q126E)	APASSSTKKTQLQLEHLCLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCESIISTLT
278	IL-2-IL-15 Chimera 59 (L18C, Q126K)	APTSSSTKKTQLQLEHLCLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCKSIISTLT
279	IL-2-IL-15 Chimera 60 (T3A, L18C, Q126K)	APASSSTKKTQLQLEHLCLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCKSIISTLT
280	IL-2-IL-15 Chimera 61 (L18C, Q126R)	APTSSSTKKTQLQLEHLCLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCRSIISTLT
281	IL-2-IL-15 Chimera 62 (T3A, L18C, Q126R)	APASSSTKKTQLQLEHLCLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCRSIISTLT
282	IL-2-IL-15 Chimera 63 (L18C, Q126S)	APTSSSTKKTQLQLEHLCLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCSSIISTLT
283	IL-2-IL-15 Chimera 64 (T3A, L18C, Q126S)	APASSSTKKTQLQLEHLCLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCSSIISTLT
284	IL-2-IL-15 Chimera 65 (I92A, C125S, Q126E)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVAVLELKGSETTFMCEYADET ATIVEFLNRWITFSESIISTLT
285	IL-2-IL-15 Chimera 66 (T3A, I92A, C125S, Q126E)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVAVLELKGSETTFMCEYADET ATIVEFLNRWITFSESIISTLT
286	IL-2-IL-15 Chimera 67 (C125S, Q126T, S130R)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSTSIIRTLT
287	IL-2-IL-15 Chimera 68 (T3A, C125S, Q126T,	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQS KNFHRLRPDLISNINVIVLELKGSETTFMCEYADET

SEQ ID NO:	Description	Amino Acid Sequence
	S130R)	ATIVEFLNRWITFSTSIIRTLT
288	Mouse IL-2-IL-15 Chimera	APTSSSTSSSTAEAAQQQQQQQQQQQHLEQLLMDL QELLSRMQSMEDATTFKFYLPKQATELKDLQCLE DELGPLRHVLDLTQSKSFQLEDAENFISNIRVTVVK LKGSDNTFECQFDDESATVVDFLRRWIAFSQSIISTS PQ
289	hIL-2R α	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRGFR RIKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTT KQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHC REPPPWENEATERIYHFVVGQMVYYQCVQGYRAL HRGPAESVCKMTHGKTRWTQPQLICTGEMETSQFP GEEKPQASPEGRPESETSCSLVTTTDFQIQTEMAATM ETSIFTTEYQ
290	hIL-2R β	AVNGTSQFTCFYNSRANISCVWSQDQALQDTSQCV HAWPDRRRWNQTCELLPVSQASWACNLILGAPDS QKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENL RLMAPISLQVVHVETHRCNISWEISQASHYFERHLE FEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDT QYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALG KD
291	hIL-2R γ	LNTTILTPNGNEDTTADFFLTMTPTDSLVSSTLPLPE VQCFVFNVEYMNCTWNSSSEPQPTNLTLYWYKN SDNDKVQKCSHYLFSEEITSGCQLQKKEIHLYQTFV VQLQDPREPRRQATQMLKLQNLVIPWAPENLTLH KLSESQLELNWNNRFLNHCLEHLVQYRTDWDHSW TEQSVDYRHKFSLPSVDGQKRYTFRVRSRFPNLCG SAQHWSEWSHPIHWGSNTSKENPFLFALEA
292	Anti-PD-1-A- IL-2-IL-15 Chimera 2 (T3A, C125S)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWDGSKRYYADSVKGRF TISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGKTYTCNVDPKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLSLKGKGGGGSGGGGSG

SEQ ID NO:	Description	Amino Acid Sequence
		GGGSAPASSSTKKTQLQLEHLLLDLQMILNGIQSM EIDATTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
293	Anti-PD-1- A- IL-2-IL-15 Chimera 44 (T3A, L18C)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF TISRDN SKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGKTYTCNV DHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQ EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLGLKGGGGSGGGGGSG GGGSAPASSSTKKTQLQLEHLCLDLQMILNGIQSM EIDATTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFCQSIISTLT
294	Anti-PD-1- A-IL-2 Chimera 54 (T3A, C125S, Q126T)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF TISRDN SKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGKTYTCNV DHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLGLKGGGGSGGGGGSG

SEQ ID NO:	Description	Amino Acid Sequence
		GGGSAPASSSTKKTQLQLEHLLLDLQMILNGIQSM EIDATTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSTSIISTLT
295	Anti-PD-1-A- IL-2-IL-15 Chimera 56 (T3A, C125S, S130R)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF TISRDN SKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGT LTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSS GLYSLSSV VTPSSSLG TKTYTCNV DHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLGLKGGGGSGGGGGSG GGGSAPASSSTKKTQLQLEHLLLDLQMILNGIQSM EIDATTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIIRTLT
296	Anti-PD-1- A- IL-2-IL-15 Chimera 58 (T3A, L18C, Q126E)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF TISRDN SKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGT LTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSS GLYSLSSV VTPSSSLG TKTYTCNV DHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQ EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLGLKGGGGSGGGGGSG

SEQ ID NO:	Description	Amino Acid Sequence
		GGGSAPASSSTKKTQLQLEHLCLDLQMILNGIQSM EIDATTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFCESIISTLT
297	Anti-PD-1-A- IL-2-IL-15 Chimera 64 (T3A, L18C, Q126S)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF TISRDN SKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGKTYTCNV DHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQ EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLGLKGGGGSGGGGGSG GGGSAPASSSTKKTQLQLEHLCLDLQMILNGIQSM EIDATTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFCSSIISTLT
298	Anti-PD-1- A- IL-2-IL-15 Chimera 68 (T3A, C125S, Q126T, S130R)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF TISRDN SKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGKTYTCNV DHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLGLKGGGGSGGGGGSG

SEQ ID NO:	Description	Amino Acid Sequence
		GGGSAPASSSTKKTQLQLEHLLLDLQMILNGIQSM EIDATTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSTSIIRTLT
299	Anti-PD-1- A-IL-2 (T3A, F42A, Y45A, L72G, C125S)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAWIWYDGSKRYYADSVKGRF TISRDN SKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGKTYTCNV DHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLGLKGGGGSGGGGGSG GGGSAPASSSTKKTQLQLEHLLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLEEELKPL EEVLNGAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEFLNRWITFAQSIISTLT
300	Anti-PD-1-A (hole)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAWIWYDGSKRYYADSVKGRF TISRDN SKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGKTYTCNV DHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLVSR LTVDKSRWQEGNVFSC SVMHEALHNHYTQKSLSLGLK

SEQ ID NO:	Description	Amino Acid Sequence
301	IL-2-IL-15 Chimera 69 (T3A, L18S, C125S, Q126E)	APASSSTKKTQLQLEHLSLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEEELKPLEEVNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSESIISTLT

* * * * *

[00480] The examples set forth above are provided to give those of ordinary skill in the art with a complete disclosure and description of how to make and use the claimed embodiments, and are not intended to limit the scope of what is disclosed herein. Modifications that are obvious to persons of skill in the art are intended to be within the scope of the following claims. All publications, patents, and patent applications cited in this specification are incorporated herein by reference as if each such publication, patent or patent application were specifically and individually indicated to be incorporated herein by reference.

What is claimed is:

1. A fusion protein comprising an interleukin-2 (IL-2) domain and an anti-PD-1 antibody; wherein the IL-2 domain comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a replacement of the amino acid residues from positions N29 to L40 with an IL-15 hinge fragment-containing peptide; a disulfide bond formed between two amino acid residues from positions N30 to L80; or an amino acid substitution at position E15, H16, D20, K32, R38, M39, L40, T41, F42, K43, F44, Y45, K76, S87, N88, or I92.
2. An interleukin-2 (IL-2) mutein comprising: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a replacement of the amino acid residues from positions N29 to L40 with an IL-15 hinge fragment-containing peptide; a disulfide bond formed between two amino acid residues from positions N30 to L80; or an amino acid substitution at position E15, H16, D20, K32, R38, M39, L40, T41, F42, K43, F44, Y45, K76, S87, N88, or I92.
3. The fusion protein of claim 1 or the IL-2 mutein of claim 2, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position L18.
4. The fusion protein or IL-2 mutein of any one of claim 1 to 3, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of L18C.
5. The fusion protein or IL-2 mutein of any one of claim 1 to 3, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of L18S.
6. The fusion protein or IL-2 mutein of any one of claim 1 to 5, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position Q126.
7. The fusion protein or IL-2 mutein of any one of claim 1 to 6, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of Q126E, Q126K, Q126R, Q126S, or Q126T.
8. The fusion protein or IL-2 mutein of any one of claim 1 to 7, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of Q126E, Q126K, or Q126R.
9. The fusion protein or IL-2 mutein of any one of claim 1 to 8, wherein the IL-2

domain or IL-2 mutein comprises an amino acid substitution of Q126E.

10. The fusion protein or IL-2 mutein of any one of claim 1 to 9, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position S130.

11. The fusion protein or IL-2 mutein of any one of claim 1 to 10, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of S130A, S130E, S130Q, or S130R.

12. The fusion protein or IL-2 mutein of any one of claim 1 to 10, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitution of (i) Q126E or Q126T; (ii) L18C, and Q126E, Q126K, Q126R, or Q126S; (iii) L18S and Q126E; (iv) Q126T and S130R; or (v) L18C, Q126E, and S130E.

13. The fusion protein or IL-2 mutein of any one of claim 1 to 11, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions of L18S and Q126E.

14. The fusion protein or IL-2 mutein of any one of claim 1 to 13, wherein the IL-2 domain or IL-2 mutein comprises a replacement of the amino acid residues from positions N29 to L40 with an IL-15 hinge fragment-containing peptide, and optionally an amino acid substitution at position E15, H16, D20, S87, N88, or I92.

15. The fusion protein or IL-2 mutein of any one of claim 1 to 14, wherein the IL-2 domain or IL-2 mutein comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219, and optionally an amino acid substitution of E15K, H16E, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

16. The fusion protein or IL-2 mutein of any one of claim 1 to 15, wherein the IL-2 domain or IL-2 mutein comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219.

17. The fusion protein or IL-2 mutein of any one of claim 1 to 16, wherein the IL-2

domain or IL-2 mutein comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219, and optionally an amino acid substitution of E15K.

18. The fusion protein or IL-2 mutein of any one of claim 1 to 17, wherein the IL-2 domain or IL-2 mutein comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219, and optionally an amino acid substitution of H16E, H16F, H16I, or H16V.

19. The fusion protein or IL-2 mutein of any one of claim 1 to 18, wherein the IL-2 domain or IL-2 mutein comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219, and optionally an amino acid substitution of D20A, D20E, D20K, or D20T.

20. The fusion protein or IL-2 mutein of any one of claim 1 to 19, wherein the IL-2 domain or IL-2 mutein comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219, and optionally an amino acid substitution of S87D.

21. The fusion protein or IL-2 mutein of any one of claim 1 to 20, wherein the IL-2 domain or IL-2 mutein comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219, and optionally an amino acid substitution of N88A.

22. The fusion protein or IL-2 mutein of any one of claim 1 to 21, wherein the IL-2 domain or IL-2 mutein comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219, and optionally an

amino acid substitution of I92A, I92D, I92E, or I92G.

23. The fusion protein or IL-2 mutein of any one of claim 1 to 22, wherein the IL-2 domain or IL-2 mutein comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 217 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 218 or 219, and one or two amino acid substitutions of E15K, H16I, D20T, N88A, or I92A.

24. The fusion protein or IL-2 mutein of any one of claim 1 to 23, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position E15, H16, D20, K32, R38, L40, F42, K76, S87, N88, or I92.

25. The fusion protein or IL-2 mutein of any one of claim 1 to 24, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position E15, D20, H16, N88, or I92.

26. The fusion protein or IL-2 mutein of any one of claim 1 to 25, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position E15.

27. The fusion protein or IL-2 mutein of any one of claim 1 to 26, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of E15K.

28. The fusion protein or IL-2 mutein of any one of claim 1 to 27, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position H16.

29. The fusion protein or IL-2 mutein of any one of claim 1 to 28, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of H16E, H16I, or H16V.

30. The fusion protein or IL-2 mutein of any one of claim 1 to 29, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position D20.

31. The fusion protein or IL-2 mutein of any one of claim 1 to 30, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of D20A, D20E, D20K, or D20T.

32. The fusion protein or IL-2 mutein of any one of claim 1 to 31, wherein the IL-2

domain or IL-2 mutein comprises an amino acid substitution at position K32.

33. The fusion protein or IL-2 mutein of any one of claim 1 to 32, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of K32E.

34. The fusion protein or IL-2 mutein of any one of claim 1 to 33, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position R38.

35. The fusion protein or IL-2 mutein of any one of claim 1 to 34, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of R38E or R38N.

36. The fusion protein or IL-2 mutein of any one of claim 1 to 35, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position L40.

37. The fusion protein or IL-2 mutein of any one of claim 1 to 36, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of L40T.

38. The fusion protein or IL-2 mutein of any one of claim 1 to 37, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position F42.

39. The fusion protein or IL-2 mutein of any one of claim 1 to 38, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of F42A or F42K.

40. The fusion protein or IL-2 mutein of any one of claim 1 to 39, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position K76.

41. The fusion protein or IL-2 mutein of any one of claim 1 to 40, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of K76E.

42. The fusion protein or IL-2 mutein of any one of claim 1 to 41, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position S87.

43. The fusion protein or IL-2 mutein of any one of claim 1 to 42, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of S87D.

44. The fusion protein or IL-2 mutein of any one of claim 1 to 43, wherein the IL-2

domain or IL-2 mutein comprises an amino acid substitution at position N88.

45. The fusion protein or IL-2 mutein of any one of claim 1 to 44, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of N88A.

46. The fusion protein or IL-2 mutein of any one of claim 1 to 45, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution at position I92.

47. The fusion protein or IL-2 mutein of any one of claim 1 to 46, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of I92A, I92D, I92E, or I92G.

48. The fusion protein or IL-2 mutein of any one of claim 1 to 47, wherein the IL-2 domain or IL-2 mutein comprises an amino acid substitution of I92A.

49. The fusion protein or IL-2 mutein of any one of claim 1 to 48, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitution of I92A and Q126E.

50. The fusion protein or IL-2 mutein of any one of claim 1 to 49, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions at positions R38 and L40.

51. The fusion protein or IL-2 mutein of any one of claim 1 to 50, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions of R38N and L40T.

52. The fusion protein or IL-2 mutein of any one of claim 1 to 51, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions at positions E15, R38, and L40.

53. The fusion protein or IL-2 mutein of any one of claim 1 to 52, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions of E15K, R38N, and L40T.

54. The fusion protein or IL-2 mutein of any one of claim 1 to 53, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions at positions E15, K32, R38, and L40.

55. The fusion protein or IL-2 mutein of any one of claim 1 to 54, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions of E15K, K32E, R38N, and L40T.

56. The fusion protein or IL-2 mutein of any one of claim 1 to 55, wherein the IL-2

domain or IL-2 mutein comprises amino acid substitutions at positions E15, R38, L40, and K76.

57. The fusion protein or IL-2 mutein of any one of claim 1 to 56, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions of E15K, R38N, L40T, and K76E.

58. The fusion protein or IL-2 mutein of any one of claim 1 to 57, wherein the IL-2 domain or IL-2 mutein is *N*-glycosylated.

59. The fusion protein or IL-2 mutein of any one of claim 1 to 58, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions at positions R38 and F42.

60. The fusion protein or IL-2 mutein of any one of claim 1 to 59, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions of R38E and F42A or F42K.

61. The fusion protein or IL-2 mutein of any one of claim 1 to 60, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions at positions K32, R38, and F42.

62. The fusion protein or IL-2 mutein of any one of claim 1 to 61, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions of K32E, R38E, and F42A or F42K.

63. The fusion protein or IL-2 mutein of any one of claim 1 to 61, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions at positions E15, K32, R38, and F42.

64. The fusion protein or IL-2 mutein of any one of claim 1 to 63, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions of E15K, K32E, R38E, and F42A or F42K.

65. The fusion protein or IL-2 mutein of any one of claim 1 to 59, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions at positions R38, F42, and K76.

66. The fusion protein or IL-2 mutein of any one of claim 1 to 59 and 65, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions of R38E, F42A or F42K, and K76E.

67. The fusion protein or IL-2 mutein of any one of claim 1 to 59, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions at positions E15, R38, F42, and K76.

68. The fusion protein or IL-2 mutein of any one of claim 1 to 59 and 67, wherein the IL-2 domain or IL-2 mutein comprises amino acid substitutions of E15K, R38E, F42A or F42K, and K76E.

69. The fusion protein or IL-2 mutein of any one of claim 1 to 68, wherein the IL-2 domain or IL-2 mutein comprises a disulfide bond formed between two different amino acid residues from positions N30 to L80.

70. The fusion protein or IL-2 mutein of any one of claim 1 to 69, wherein the IL-2 domain or IL-2 mutein comprises a disulfide bond formed between two different amino acid residues, each independently at K35, R38, F42, Y45, E62, V69, or L72.

71. The fusion protein or IL-2 mutein of any one of claim 1 to 70, wherein the IL-2 domain or IL-2 mutein comprises a disulfide bond formed between K35C and L72C, R38C and L72C, F42C and V69C, Y42C and L72C, or Y45C and E62C.

72. The fusion protein or IL-2 mutein of any one of claim 1 to 71, wherein the IL-2 domain or IL-2 mutein comprises a disulfide bond formed between F42C and V69C.

73. The fusion protein or IL-2 mutein of any one of claim 1 to 72, wherein the IL-2 domain or IL-2 mutein comprises a disulfide bond formed between F42C and V69C and an amino acid substitution of E15K, K32E, or K76E.

74. The fusion protein or IL-2 mutein of claim 73, wherein the IL-2 domain or IL-2 mutein comprises a disulfide bond formed between F42C and V69C and an amino acid substitution of E15K.

75. The fusion protein or IL-2 mutein of claim 73, wherein the IL-2 domain or IL-2 mutein comprises a disulfide bond formed between F42C and V69C and an amino acid substitution of K32E.

76. The fusion protein or IL-2 mutein of claim 73, wherein the IL-2 domain or IL-2 mutein comprises a disulfide bond formed between F42C and V69C and an amino acid substitution of K76E.

77. The fusion protein or IL-2 mutein of claim 73, wherein the IL-2 domain or IL-2 mutein comprises a disulfide bond formed between F42C and V69C and amino acid substitutions of E15K and K32E.

78. The fusion protein or IL-2 mutein of claim 73, wherein the IL-2 domain or IL-2 mutein comprises a disulfide bond formed between F42C and V69C and amino acid substitutions of E15K and K76E.

79. The fusion protein of claim 1 or the IL-2 mutein of claim 2, wherein the IL-2 domain or IL-2 mutein comprises an amino acid sequence of any one of SEQ ID NOs: 185 to 261, wherein the IL-2 mutein comprises an amino acid substitution of L18C, L18S, Q126E, Q126K, Q126R, Q126S, Q126T, S130A, S130E, S130Q, or S130R; wherein the amino acid residue at position T3 is T or A, and the amino acid residue at position C125 is C or S; and wherein, when the IL-2 mutein comprises an amino acid substitution of L18C, the amino acid residue at position C125 is C.

80. The fusion protein of claim 1 or the IL-2 mutein of claim 2, wherein the IL-2 domain or IL-2 mutein comprises an amino acid sequence of any one of SEQ ID NO: 262 to 287 and 301.

81. A fusion protein comprising an IL-2 domain and a half-life-extension domain, wherein the IL-2 domain comprises the amino acid sequence of the IL-2 mutein of any one of claims 2 to 80.

82. The fusion protein of claim 81, wherein the half-life-extension domain comprises an albumin binding domain, a fragment crystallizable (Fc) domain, a serum albumin, a polyethylene glycol group, or a fatty acyl group.

83. The fusion protein of claim 81 or 82, wherein the half-life-extension domain is an albumin binding domain.

84. The fusion protein of claim 82 or 83, wherein the albumin binding domain is an antibody or a fragment thereof that binds to a human serum albumin (HSA).

85. The fusion protein of claim 84, wherein the albumin binding domain is an anti-

HSA single domain antibody.

86. The fusion protein of claim 84 or 85, wherein the albumin binding domain is an anti-HSA V_HH single domain antibody.

87. The fusion protein of any one of claims 81 to 86, comprising an IL-2 domain, an anti-HSA V_HH single domain antibody, and optionally a peptide linker; wherein the C-terminus of the anti-HSA V_HH single domain antibody is connected to the N-terminus of the IL-2 domain directly or *via* the peptide linker.

88. The fusion protein of any one of claims 85 to 87, wherein the single domain antibody comprises (i) a CDR1 of SEQ ID NO: 1, a CDR2 of SEQ ID NO: 2, and a CDR3 of SEQ ID NO: 3; or (ii) a CDR1 of SEQ ID NO: 9, a CDR2 of SEQ ID NO: 10, and a CDR3 of SEQ ID NO: 11.

89. The fusion protein of any one of claims 85 to 88, wherein the single domain antibody has an amino acid sequence of SEQ ID NO: 8 or 15.

90. The fusion protein of claim 81 or 82, wherein the half-life-extension domain is a Fc domain.

91. The fusion protein of claim 90, comprising an IL-2 domain, an Fc domain comprising first and second peptide chains, and optionally a peptide linker; wherein the C-terminus of the first peptide chain of the Fc domain is connected to the N-terminus of the IL-2 domain directly or *via* the peptide linker.

92. The fusion protein of any one of claims 81 to 91, further comprising a programmed cell death-1 (PD-1) binding domain.

93. The fusion protein of claim 92, wherein the PD-1 binding domain is an anti-PD-1 single domain antibody.

94. The fusion protein of claim 92 or 93, wherein the PD-1 binding domain is an anti-PD-1 V_HH single domain antibody.

95. The fusion protein of any one of claims 92 to 94, comprising an IL-2 domain, an anti-PD-1 V_HH single domain antibody, and an Fc domain.

96. The fusion protein of any one of claims 92 to 95, comprising an IL-2 domain, first and second PD-1 binding domains, an Fc domain comprising first and second peptide chains, and optionally a peptide linker; wherein a C-terminus of the first PD-1 binding domain is connected to the N-terminus of the first peptide chain of the Fc domain directly or *via* a peptide linker, and the C-terminus of the first peptide chain of the Fc domain is connected to the N-terminus of the IL-2 domain directly or *via* the peptide linker; and wherein a C-terminus of the second PD-1 binding domain is connected to the N-terminus of the second peptide chain of the Fc domain directly or *via* a peptide linker.

97. The fusion protein of any one of claims 94 to 96, wherein each anti-PD-1 V_HH single domain antibody independently comprises: (i) a CDR1 of SEQ ID NO: 119, a CDR2 of SEQ ID NO: 120, and a CDR3 of SEQ ID NO: 121; (ii) a CDR1 of SEQ ID NO: 123, a CDR2 of SEQ ID NO: 124, and a CDR3 of SEQ ID NO: 125; (iii) a CDR1 of SEQ ID NO: 127, a CDR2 of SEQ ID NO: 128, and a CDR3 of SEQ ID NO: 129; (iv) a CDR1 of SEQ ID NO: 131, a CDR2 of SEQ ID NO: 132, and a CDR3 of SEQ ID NO: 133; or (v) a CDR1 of SEQ ID NO: 135, a CDR2 of SEQ ID NO: 136, and a CDR3 of SEQ ID NO: 137.

98. The fusion protein of any one of claims 90 to 97, wherein the Fc domain is a hIgG1 Fc domain, a hIgG2 Fc domain, a hIgG4 Fc domain, or a mutein thereof.

99. The fusion protein of any one of claims 90 to 98, wherein the Fc domain is in a knobs-in-holes configuration.

100. The fusion protein of claim 92, wherein the anti-PD-1 binding domain and the FC domain form parts of an intact anti-PD-1 antibody comprising two light chains and two heavy chains.

101. The fusion protein of any one of claims 1 to 80 and 100, comprising an IL-2 domain, and an intact anti-PD-1 antibody comprising two light chains and two heavy chains.

102. The fusion protein of any one of claims 1 to 80, 100, and 101, comprising an IL-2

domain, an intact anti-PD-1 antibody comprising first and second light chains and first and second heavy chains, and optionally a peptide linker; wherein the *C*-terminus of the first heavy chain of the intact anti-PD-1 antibody is connected to the *N*-terminus of the IL-2 domain directly or *via* the peptide linker.

103. The fusion protein of any one of claims 1 to 80 and 100, comprising two IL-2 domains, and an intact anti-PD-1 antibody comprising two light chains and two heavy chains.

104. The fusion protein of any one of claims 1 to 80, 100, and 103, comprising first and second IL-2 domains, an intact anti-PD-1 antibody comprising first and second light chains and first and second heavy chains, and optionally first and second peptide linkers; wherein the *C*-terminus of the first heavy chain of the intact anti-PD-1 antibody is connected to the *N*-terminus of the first IL-2 domain directly or *via* the first peptide linker, and the *C*-terminus of the second heavy chain of the intact anti-PD-1 antibody is connected to the *N*-terminus of the second IL-2 domain directly or *via* the second peptide linker.

105. The fusion protein of any one of claims 1 to 80, and 100 to 104, wherein the PD-1 antibody comprises:

- (i) a CDRL1 of SEQ ID NO: 29, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 30, a CDRH1 of SEQ ID NO: 31, a CDRH2 of SEQ ID NO: 32, and a CDRH3 of SEQ ID NO: 33;
- (ii) a CDRL1 of SEQ ID NO: 38, a CDRL2 of LAS, a CDRL3 of SEQ ID NO: 39, a CDRH1 of SEQ ID NO: 40, a CDRH2 of SEQ ID NO: 41, and a CDRH3 of SEQ ID NO: 42;
- (iii) a CDRL1 of SEQ ID NO: 47, a CDRL2 of KVS, a CDRL3 of SEQ ID NO: 48, a CDRH1 of SEQ ID NO: 49, a CDRH2 of SEQ ID NO: 50, and a CDRH3 of SEQ ID NO: 51;
- (iv) a CDRL1 of SEQ ID NO: 56, a CDRL2 of TAT, a CDRL3 of SEQ ID NO: 57, a CDRH1 of SEQ ID NO: 58, a CDRH2 of SEQ ID NO: 59, and a CDRH3 of SEQ ID NO: 60;
- (v) a CDRL1 of SEQ ID NO: 65, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 66, a CDRH1 of SEQ ID NO: 67, a CDRH2 of SEQ ID NO: 68, and a CDRH3 of SEQ ID NO: 69;
- (vi) a CDRL1 of SEQ ID NO: 74, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 75, a CDRH1 of SEQ ID NO: 76, a CDRH2 of SEQ ID NO: 77, and a CDRH3 of SEQ ID NO: 78;
- (vii) a CDRL1 of SEQ ID NO: 83, a CDRL2 of YAF, a CDRL3 of SEQ ID NO: 84, a CDRH1

- of SEQ ID NO: 85, a CDRH2 of SEQ ID NO: 86, and a CDRH3 of SEQ ID NO: 87;
- (viii) a CDRL1 of SEQ ID NO: 92, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 93, a CDRH1 of SEQ ID NO: 94, a CDRH2 of SEQ ID NO: 95, and a CDRH3 of SEQ ID NO: 96;
 - (ix) a CDRL1 of SEQ ID NO: 101, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 102, a CDRH1 of SEQ ID NO: 103, a CDRH2 of SEQ ID NO: 104, and a CDRH3 of SEQ ID NO: 105; or
 - (x) a CDRL1 of SEQ ID NO: 110, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 111, a CDRH1 of SEQ ID NO: 112, a CDRH2 of SEQ ID NO: 113, and a CDRH3 of SEQ ID NO: 114.

106. The fusion protein of any one of claims 1 to 80, and 100 to 105, wherein the PD-1 antibody comprises, comprising:

- (i) a light chain variable region of SEQ ID NO: 34 and a heavy chain variable region of SEQ ID NO: 35;
- (ii) a light chain variable region of SEQ ID NO: 43 and a heavy chain variable region of SEQ ID NO: 44;
- (iii) a light chain variable region of SEQ ID NO: 52 and a heavy chain variable region of SEQ ID NO: 53;
- (iv) a light chain variable region of SEQ ID NO: 61 and a heavy chain variable region of SEQ ID NO: 62;
- (v) a light chain variable region of SEQ ID NO: 70 and a heavy chain variable region of SEQ ID NO: 71;
- (vi) a light chain variable region of SEQ ID NO: 79 and a heavy chain variable region of SEQ ID NO: 80;
- (vii) a light chain variable region of SEQ ID NO: 88 and a heavy chain variable region of SEQ ID NO: 89;
- (viii) a light chain variable region of SEQ ID NO: 97 and a heavy chain variable region of SEQ ID NO: 98;
- (ix) a light chain variable region of SEQ ID NO: 106 and a heavy chain variable region of SEQ ID NO: 107; or
- (x) a light chain variable region of SEQ ID NO: 115 and a heavy chain variable region of SEQ ID NO: 116.

107. The fusion protein of any one of claims 1 to 80, and 100 to 106, wherein the PD-1 antibody comprises:

- (i) a light chain of SEQ ID NO: 36 and a heavy chain of SEQ ID NO: 37;
- (ii) a light chain of SEQ ID NO: 45 and a heavy chain of SEQ ID NO: 46;
- (iii) a light chain of SEQ ID NO: 54 and a heavy chain of SEQ ID NO: 55;
- (iv) a light chain of SEQ ID NO: 63 and a heavy chain of SEQ ID NO: 64;
- (v) a light chain of SEQ ID NO: 72 and a heavy chain of SEQ ID NO: 73;
- (vi) a light chain of SEQ ID NO: 81 and a heavy chain of SEQ ID NO: 82;
- (vii) a light chain of SEQ ID NO: 90 and a heavy chain of SEQ ID NO: 91;
- (viii) a light chain of SEQ ID NO: 99 and a heavy chain of SEQ ID NO: 100;
- (ix) a light chain of SEQ ID NO: 108 and a heavy chain of SEQ ID NO: 109; or
- (x) a light chain of SEQ ID NO: 117 and a heavy chain of SEQ ID NO: 118.

108. The fusion protein of any one of claims 1 to 80, and 100 to 107, wherein the anti-PD-1 antibody is a human or humanized antibody.

109. The fusion protein of any one of claims 1 to 80, and 100 to 108, wherein the anti-PD-1 antibody, wherein the intact anti-PD-1 antibody is an IgA, IgD, IgE, IgG, or IgM antibody.

110. The fusion protein of any one of claims 1 to 80, and 100 to 109, wherein the anti-PD-1 antibody is an IgG antibody.

111. The fusion protein of any one of claims 1 to 80, and 100 to 110, wherein the anti-PD-1 antibody is an IgG1 or IgG4 antibody.

112. The fusion protein of any one of claims 1 to 80, and 100 to 111, wherein the anti-PD-1 antibody is in a knobs-in-holes configuration.

113. The fusion protein of any one of claims 1 to 80, and 100 to 112, wherein the anti-PD-1 antibody is a non-blocking antibody.

114. The fusion protein of any one of claims 1 to 80, and 100 to 112, wherein the anti-PD-1 antibody is a blocking antibody.

115. The fusion protein of claim 1, where the fusion protein is:

fusion protein **A1** comprising amino acid sequences of SEQ ID NOs: 36, 292, and 300;
fusion protein **A2** comprising amino acid sequences of SEQ ID NOs: 36, 293, and 300;
fusion protein **A3** comprising amino acid sequences of SEQ ID NOs: 36, 294, and 300;
fusion protein **A4** comprising amino acid sequences of SEQ ID NOs: 36, 295, and 300;
fusion protein **A5** comprising amino acid sequences of SEQ ID NOs: 36, 296, and 300;
fusion protein **A6** comprising amino acid sequences of SEQ ID NOs: 36, 297, and 300; or
fusion protein **A7** comprising amino acid sequences of SEQ ID NOs: 36, 298, and 300.

116. A pharmaceutical composition comprising the fusion protein of any one of claims 1 to 115 or the IL-2 mutein of any one of claims 2 to 80, and a pharmaceutically acceptable excipient.

117. The pharmaceutical composition of claim 116, wherein the pharmaceutical composition is in single dosage form.

118. The pharmaceutical composition of claim 116 or 117, wherein the pharmaceutical composition is a solid.

119. The pharmaceutical composition of claim 116 or 117, wherein the pharmaceutical composition is in a parenteral dosage form.

120. The pharmaceutical composition of claim 119, the pharmaceutical composition is in an intravenous dosage form.

121. The pharmaceutical composition of any one of claims 116, 117, 119, or 120, wherein the pharmaceutical composition is a solution.

122. A method of treating one or more symptoms of treating, preventing, or ameliorating one or more symptoms of a proliferative disease in a subject, comprising administering to the subject in need thereof a therapeutically effective amount of the fusion protein of any one of claims 1 to 115, the IL-2 mutein of any one of claims 2 to 80, or the pharmaceutical composition of any one of claims 116 to 121.

123. The method of claim 122, wherein the proliferative disease is cancer.

124. The method of claim 122 or 123, wherein the proliferative disease is metastatic cancer.

125. A method of inhibiting the growth of a cell, comprising contacting the cell with an effective amount of the fusion protein of any one of claims 1 to 115, the IL-2 mutein of any one of claims 2 to 80, or the pharmaceutical composition of any one of claims 116 to 121.

126. The method of claim 125, wherein the cell is a cancerous cell.

FIG. 1

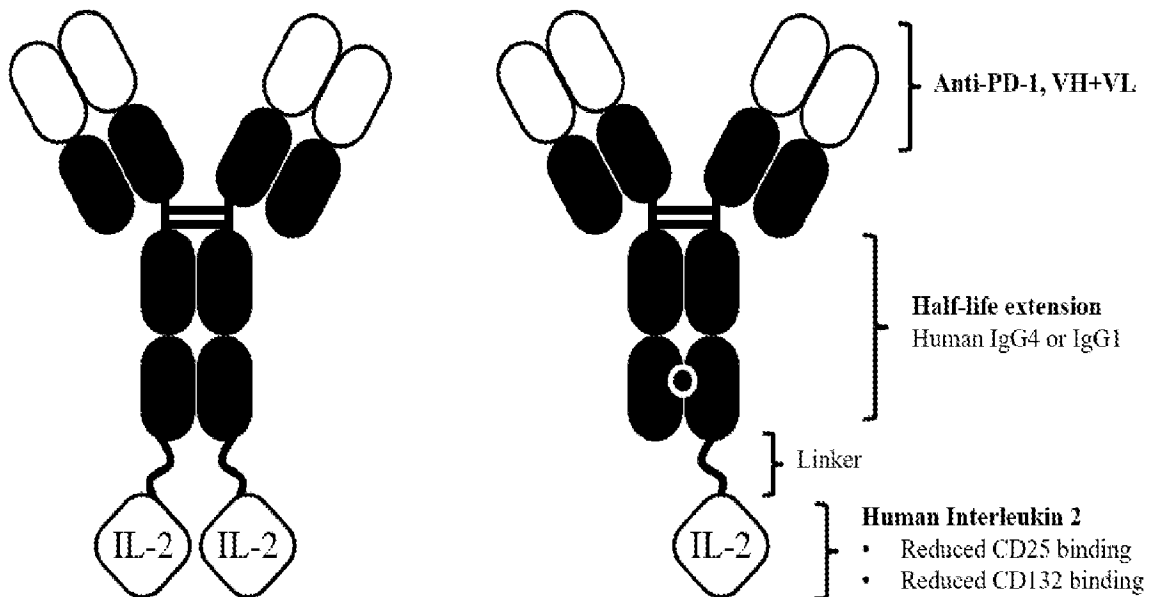


FIG. 2

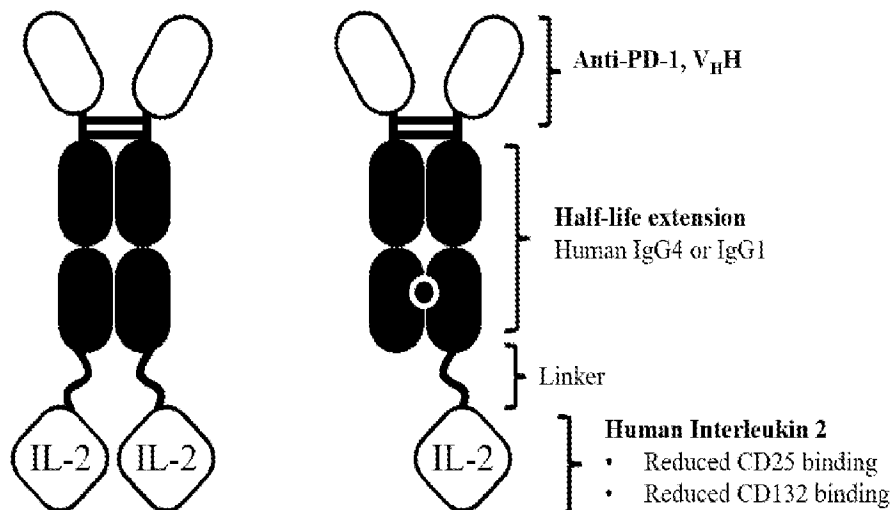
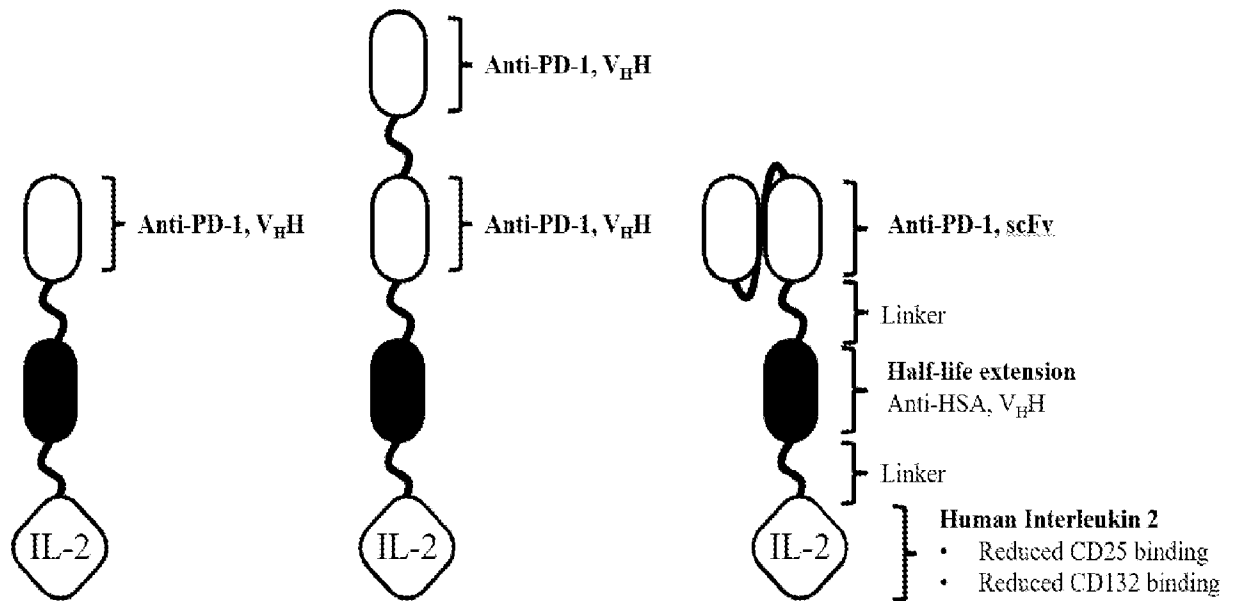


FIG. 3



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/074181

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - INV. - A61K 38/20; C07K 14/55; C07K 16/28 (2022.01)

ADD.

CPC - INV. - A61K 38/2013; C07K 14/55; C07K 16/2803 (2022.08)

ADD. - C07K 2319/74 (2022.08)

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 database consulted during the international search (name of database 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	US 2020/0140513 A1 (ALTOR BIOSCIENCE CORPORATION) 07 May 2020 (07.05.2020) entire document	1, 79, 115
A	WO 2020/247843 A2 (ASHER BIOTHERAPEUTICS INC.) 10 December 2020 (10.12.2020) entire document	1, 79, 115
A	US 2020/0392235 A1 (ASKGENE PHARMA INC.) 17 December 2020 (17.12.2020) entire document	1, 79, 115
A	US 2014/0341902 A1 (MAECKER et al) 20 November 2014 (20.11.2014) entire document	1, 79, 115
A	US 2020/0040080 A1 (SHANGHAI BIOMED-UNION BIOTECHNOLOGY CO LTD) 06 February 2020 (06.02.2020) entire document	1, 79, 115

☐ 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

"D" document cited by the applicant in the international application

"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

31 October 2022

Date of mailing of the international search report

NOV 18 2022

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/074181

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13~~ter~~. 1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:
SEQ ID NOs: 36, 292, and 300 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/074181

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.: 4-78, 81-114, 116-126
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:

See extra sheet(s).

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.:
1, 79, 115

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/US2022/074181

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 need to be paid.

Group I+: claims 1-3, 79, 80, and 115 are drawn to interleukin-2 muteins and fusion proteins comprising an interleukin-2 mutein and a PD-1 antibody.

The first invention of Group I+ is restricted to a fusion protein comprising an interleukin-2 (IL-2) domain and an anti-PD-1 antibody; wherein the fusion protein is selected to be A1 (SEQ ID NO: 36, SEQ ID NO: 292, and SEQ ID NO: 300) and muteins comprising the same. It is believed that claims 1, 79, and 115 read on this first named invention and thus these claims will be searched without fee to the extent that they read on SEQ ID NOs: 36, 292, and 300.

Applicant is invited to elect additional fusion proteins or muteins, and their respective, corresponding, SEQ ID NOs to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be a fusion protein comprising an interleukin-2 (IL-2) domain and an anti-PD-1 antibody; wherein the fusion protein is selected to be A2 (SEQ ID NO: 36, SEQ ID NO: 293, and SEQ ID NO: 300) and muteins comprising the same. Additional fusion proteins or muteins, and their respective, corresponding, SEQ ID NOs will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. 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.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for treating proliferative diseases, requiring the selection of alternative fusion proteins or muteins where "A fusion protein comprising an interleukin-2 (IL-2) domain and an anti-PD-1 antibody; wherein the IL-2 domain comprises: (i) an amino acid substitution at position L18, Q126, or S130; and (ii) a replacement of the amino acid residues from positions N29 to L40 with an IL-15 hinge fragment-containing peptide; a disulfide bond formed between two amino acid residues from positions N30 to L80; or an amino acid substitution at position E15, H16, D20, K32, R38, M39, L40, T41, F42, K43, F44, Y45, K76, S87, N88, or 192."

Additionally, even if Groups I+ were considered to share the technical features of a fusion protein comprising an interleukin-2 (IL-2) domain and an anti-PD-1 Antibody; an IL-15 hinge fragment-containing peptide, and an interleukin-2 (IL-2) mutein. However, these shared technical features do not represent a contribution over the prior art.

Specifically, WO 2020/247843 to Asher Biotherapeutics Inc. discloses a fusion protein (we designed fusion proteins, Para. [0014]) comprising an interleukin-2 (IL-2) domain and an anti-PD-1 antibody ([s]uch fusion proteins comprising mutant IL-2 polypeptides and antibodies binding specific antigens, Para. [0014]; CD8-IL-2 and TAg-IL-2v in combination with anti-PD 1, Para. [0131]); a hinge fragment-containing peptide (the first moiety is a polypeptide comprising an antibody hinge-CH2-CH3 monomer, Para. [0017]), and an interleukin-2 (IL-2) mutein (IL-2 muteins, Para. [0125]).

Further, US 2020/0140513 to Altor Bioscience Corporation teaches an IL-15 hinge (IgG1 hinge-human IL-15, Para. [0195]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.