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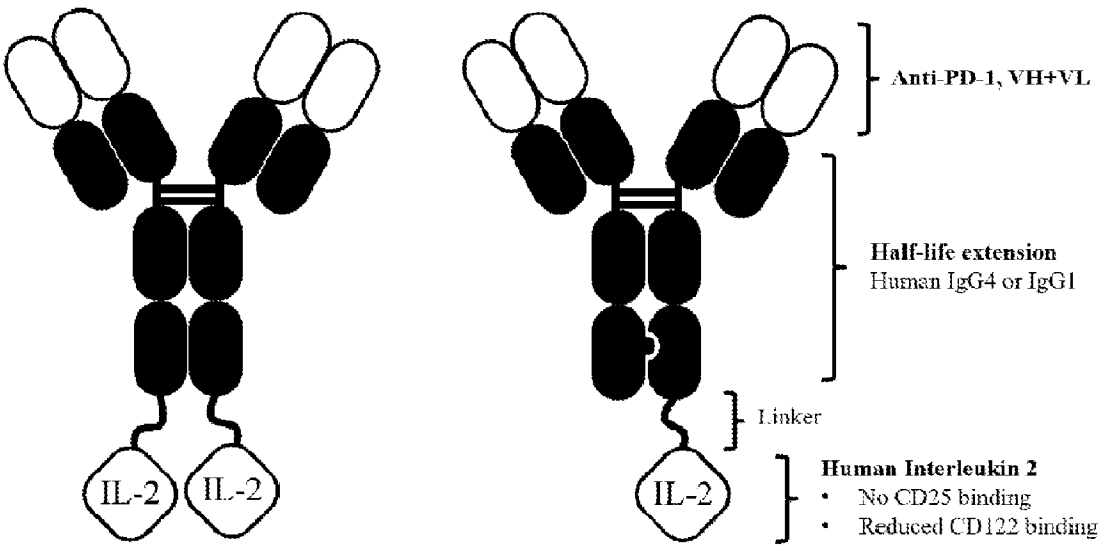
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(54) **Titre : PROTEINES DE FUSION, COMPOSITIONS PHARMACEUTIQUES ET APPLICATIONS THERAPEUTIQUES**
(54) **Title: FUSION PROTEINS, PHARMACEUTICAL COMPOSITIONS, AND THERAPEUTIC APPLICATIONS**

FIG. 1



(57) **Abrégé/Abstract:**
Provided herein are a fusion protein comprising an interleukin-2 domain, a programmed cell death-1 binding domain, and a half-life-extension domain; and a pharmaceutical composition thereof. Also provided herein is a method of their use for treating, preventing, or ameliorating one or more symptoms of a proliferative disease.

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Abstract:

Provided herein are a fusion protein comprising an interleukin-2 domain, a programmed cell death-1 binding domain, and a half-life-extension domain; and a pharmaceutical composition thereof. Also provided herein is a method of their use for treating, preventing, or ameliorating one or more symptoms of a proliferative disease.

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 No. 63/169,132, filed March 31, 2021; the disclosure of which is incorporated herein by reference in its entirety.

FIELD

[0002] Provided herein are a fusion protein comprising an interleukin-2 domain, a programmed cell death-1 binding domain, and a half-life-extension domain; and a pharmaceutical composition thereof. Also provided herein is a method of their 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 in Computer Readable Form (CRF), which is entitled 216A013WO01_SEQ_LIST_ST25.txt of 360,545 bytes in size and created March 30, 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*, 311-17. 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 on T lymphocytes, thereby inhibits further T-cell activation and proliferation. PD-1 expression is induced when T-cell become activated. 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-2Rα or CD25), β chain (IL-2Rβ or CD122), and γ chain (IL-2Rγ, γ_c, or CD132). Wang *et al.*, *Science* **2005**, *310*, 1159-63. The IL-2 binds the IL-2Rα 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-2Rα. Stauber *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 2788-93. The IL-2 binds a heterodimeric complex of the IL-2Rβ and IL-2Rγ, 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-2Rα, IL-2Rβ, and IL-2Rγ, expressed on Treg cells, with a high affinity ($K_d \approx 10$ pM). *Id.* The IL-2 binds the IL-2Rβ alone with a dissociation constant (K_d) of about 100 nM. *Id.* The IL-2Rα by itself has no signal-transducing activity. *Id.* The IL-2 signals through the intermediate-affinity heterodimeric IL-2Rβ/γ complex or the high-affinity heterotrimeric IL-2Rα/β/γ complex. Liao *et al.*, *Curr. Opin.*

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Immunol. **2011**, *23*, 598-604. The binding of the IL-2 to the intermediate-affinity heterodimeric IL-2Rβ/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-2Rα/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 a fusion protein comprising an interleukin-2 (IL-2) domain, a

programmed cell death-1 (PD-1) binding domain, and a half-life-extension domain.

[0010] Also provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and an albumin binding domain.

[0011] Additionally, 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 the amino terminus (*N*-terminus) of the IL-2 domain is connected to the carboxyl terminus (*C*-terminus) of the albumin binding domain directly or *via* the first peptide linker, and the *N*-terminus of the albumin binding domain is connected to the *C*-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

[0012] Furthermore, 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 the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the albumin binding domain directly or *via* the first peptide linker, and the *C*-terminus of the albumin binding domain is connected to the *N*-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

[0013] 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 the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the PD-1 binding domain directly or *via* the first peptide linker, and the *N*-terminus of the PD-1 binding domain is connected to the *C*-terminus of the albumin binding domain directly or *via* the second peptide linker.

[0014] 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 the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the PD-1 binding domain directly or *via* the first peptide linker, and the *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the albumin binding domain directly or *via* the second peptide linker.

[0015] 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 the *N*-terminus of the PD-1 binding domain is connected to the *C*-terminus of the IL-2 domain directly or *via* the first peptide linker, and the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the albumin binding domain directly or *via* the second peptide linker.

[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 the *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 albumin binding domain directly or *via* the second peptide linker.

[0017] Provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and a half-life-extension domain comprising a fragment crystallizable (Fc) domain.

[0018] Provided herein is a fusion protein comprising an IL-2 domain, first and second PD-1 binding domains, a half-life-extension domain that comprises an Fc domain having first and second peptide chains, and optionally a peptide linker; wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first peptide chain of the Fc domain directly or *via* the peptide linker.

[0019] Provided herein is a fusion protein comprising an IL-2 domain, first and second PD-1 binding domains, a half-life-extension domain that comprises an Fc, and optionally a peptide linker; wherein the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the first PD-1 binding domain directly or *via* the peptide linker.

[0020] Provided herein is a fusion protein comprising first and second IL-2 domains, first and second PD-1 binding domains, a half-life-extension domain that comprises an Fc domain having first and second peptide chains, and optionally first and second peptide linkers; wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first peptide chain of the Fc domain directly or *via* the first peptide linker; and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second peptide chain of the Fc domain directly or *via* the second peptide linker.

[0021] Provided herein is a fusion protein comprising an IL-2 domain and an anti-PD-1

antibody.

[0022] Provided herein is a fusion protein comprising an IL-2 domain, an anti-PD-1 antibody comprising light and heavy chains, and optionally a peptide linker, wherein the *N*-terminus of the IL-2 domain is connected to a *C*-terminus of the heavy chains directly or *via* the peptide linker.

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

[0024] Provided herein is a fusion protein comprising an IL-2 domain, an anti-PD-1 antibody comprising light and heavy chains, and optionally a peptide linker, wherein the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the heavy chains directly or *via* the peptide linker.

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

[0026] Provided herein is a pharmaceutical composition comprising a fusion protein provided herein and a pharmaceutically acceptable excipient.

[0027] 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 a fusion protein provided herein.

[0028] 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 a fusion protein provided herein.

[0029] 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 a fusion protein provided herein.

[0030] Provided herein is a method of inhibiting the growth of a cell, comprising contacting the cell with an effective amount of a fusion protein provided herein.

[0031] Provided herein is a method of activating an immune effector cell, comprising contacting the cell with an effective amount of a fusion protein provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] FIG. 1 shows the configuration of an exemplary fusion protein that comprises an anti-PD-1 antibody having two PD-1 binding domains (*e.g.*, two pairs of heavy chain (VH) and light chain variable (VL) regions) and an Fc domain functioning as a half-life-extension domain, and one or two IL-2 domains; wherein the *N*-terminus of an IL-2 domain is connected *via* a peptide linker to the *C*-terminus of a heavy chain of the anti-PD-1 antibody.

[0033] FIG. 2 shows the configuration of an exemplary fusion protein that comprises an anti-PD-1 antibody having two PD-1 binding domains (*e.g.*, two V_HH single domain antibodies) and an Fc domain functioning as a half-life-extension domain, and one or two IL-2 domains; wherein the *N*-terminus of an IL-2 domain is connected *via* a peptide linker to the *C*-terminus of a heavy chain of the anti-PD-1 antibody.

[0034] FIG. 3 shows the configuration of an exemplary fusion protein that comprises an anti-PD-1 domain comprising a single-chain variable fragment (scFv) or V_HH single domain antibody, an IL-2 domain, an anti-HSA V_HH single domain antibody as a half-life extension domain, and first and second peptide linkers; wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the anti-HSA V_HH single domain antibody *via* the first peptide linker, and the *N*-terminus of the anti-HSA V_HH single domain antibody is connected to the *C*-terminus of the anti-PD-1 scFv or V_HH single domain antibody.

[0035] FIG. 4 shows the antitumor effects of anti-PD-1/IL-2 fusion proteins in a xenograft mouse model for colorectal cancer (HT-29 cells).

DETAILED DESCRIPTION

[0036] To facilitate understanding of the disclosure set forth herein, a number of terms are defined below.

[0037] 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.

[0038] 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.

[0039] 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.

[0040] 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.

[0041] 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.

[0042] 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.

[0043] The term "therapeutically effective amount" or "effective amount" is meant to include the amount of a compound (*e.g.*, a 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.

[0044] The term "IC_{50}" or "EC_{50}" refers to an amount, concentration, or dosage of a compound (*e.g.*, a fusion protein) that is required for 50% inhibition of a maximal response in an assay that measures such a response.

[0045] 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, Remington: The Science and Practice of Pharmacy*, 23rd ed.; Adejare Ed.; Academic Press: 2020; *Handbook of Pharmaceutical Excipients*, 9th ed.; Sheskey *et al.*, Eds.; The Pharmaceutical Press: 2020; *Handbook of Pharmaceutical Additives*, 3rd ed.; Ash and

Ash Eds.; Synapse Information Resources, Inc.: 2007; *Pharmaceutical Preformulation and Formulation*, 2nd ed.; Gibson Ed.; CRC Press: 2009.

[0046] 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.

[0047] The terms "substantially pure" and "substantially homogeneous," when referring to a compound (*e.g.* a fusion protein), 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.

Fusion Proteins

[0048] In one embodiment, provided herein is a fusion protein comprising an interleukin-2 (IL-2) domain, a programmed cell death-1 (PD-1) binding domain, and a half-life-extension domain. In certain embodiments, the IL-2 domain causes the fusion protein to signal through a receptor comprising CD122 (IL-2Rβ/IL-15Rβ) and CD132 (IL-2Rγ) subunits.

[0049] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and a half-life-extension domain; wherein the IL-2 domain causes the fusion protein to signal through a receptor consisting of CD122 and CD132 subunits.

[0050] 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 interleukin-2 domain *in vivo* as compared to a wild-type interleukin-2 of SEQ ID NO: 1, 2, 3, 4, or 5.

[0051] In certain embodiments, the half-life-extension domain is an albumin binding domain. In certain embodiments, the half-life-extension domain comprises a fragment crystallizable (Fc) domain. In certain embodiments, the half-life-extension domain is a constant region (C_H1, C_H2, and C_H3) of a heavy chain of an antibody. In certain embodiments, the half-life-extension domain is a Fc domain.

[0052] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and an albumin binding domain. In certain embodiments, the IL-2 domain causes the fusion protein to signal through a receptor comprising CD122 and CD132 subunits.

[0053] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and an albumin binding domain; wherein the IL-2 domain causes the fusion protein to signal through a receptor consisting of CD122 and CD132 subunits.

[0054] In yet another embodiment, 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 the amino terminus (*N*-terminus) of the IL-2 domain is connected to the carboxyl terminus (*C*-terminus) of the albumin binding domain directly or *via* the first peptide linker, and the *N*-terminus of the albumin binding domain is connected to the *C*-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

[0055] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, and an albumin binding domain; wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the albumin binding domain directly, and the *N*-terminus of the albumin binding domain is connected to the *C*-terminus of the PD-1 binding domain directly.

[0056] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and a peptide linker; wherein the

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

[0057] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and first and second peptide linkers; wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the albumin binding domain *via* the first peptide linker, and the *N*-terminus of the albumin binding domain is connected to the *C*-terminus of the PD-1 binding domain *via* the second peptide linker.

[0058] In yet another embodiment, 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 the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the albumin binding domain directly or *via* the first peptide linker, and the *C*-terminus of the albumin binding domain is connected to the *N*-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

[0059] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, and an albumin binding domain; wherein the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the albumin binding domain directly, and the *C*-terminus of the albumin binding domain is connected to the *N*-terminus of the PD-1 binding domain directly.

[0060] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and a peptide linker; wherein the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the albumin binding domain directly, and the *C*-terminus of the albumin binding domain is connected to the *N*-terminus of the PD-1 binding domain *via* the peptide linker; or wherein the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the albumin binding domain *via* the peptide linker; and the *C*-terminus of the albumin binding domain is connected to the *N*-terminus of the PD-1 binding

domain directly.

[0061] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and first and second peptide linkers; wherein the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the albumin binding domain *via* the first peptide linker; and the *C*-terminus of the albumin binding domain is connected to the *N*-terminus of the PD-1 binding domain *via* the second peptide linker.

[0062] In yet another embodiment, 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 the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the PD-1 binding domain directly or *via* the first peptide linker, and the *N*-terminus of the PD-1 binding domain is connected to the *C*-terminus of the albumin binding domain directly or *via* the second peptide linker.

[0063] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, and an albumin binding domain; wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the PD-1 binding domain directly, and the *N*-terminus of the PD-1 binding domain is connected to the *C*-terminus of the albumin binding domain directly.

[0064] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and a peptide linker; wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the PD-1 binding domain directly, and the *N*-terminus of the PD-1 binding domain is connected to the *C*-terminus of the albumin binding domain *via* the peptide linker; or wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the PD-1 binding domain *via* the peptide linker, and the *N*-terminus of the PD-1 binding domain is connected to the *C*-terminus of the albumin binding domain directly.

[0065] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and first and second peptide linkers; wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the PD-1

binding domain *via* the first peptide linker, and the *N*-terminus of the PD-1 binding domain is connected to the *C*-terminus of the albumin binding domain *via* the second peptide linker.

[0066] In yet another embodiment, 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 the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the PD-1 binding domain directly or *via* the first peptide linker, and the *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the albumin binding domain directly or *via* the second peptide linker.

[0067] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, and an albumin binding domain; wherein the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the PD-1 binding domain directly, and the *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the albumin binding domain directly.

[0068] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and a peptide linker; wherein the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the PD-1 binding domain directly, and the *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the albumin binding domain *via* the peptide linker; or wherein the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the PD-1 binding domain *via* the peptide linker, and the *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the albumin binding domain directly.

[0069] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and first and second peptide linkers; wherein the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the PD-1 binding domain *via* the first peptide linker, and the *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the albumin binding domain *via* the second peptide linker.

[0070] In yet another embodiment, 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

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

[0071] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, and an albumin binding domain; wherein the *N*-terminus of the PD-1 binding domain is connected to the *C*-terminus of the IL-2 domain directly, and the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the albumin binding domain directly.

[0072] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and a peptide linker; wherein the *N*-terminus of the PD-1 binding domain is connected to the *C*-terminus of the IL-2 domain directly, and the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the albumin binding domain *via* the peptide linker; or wherein the *N*-terminus of the PD-1 binding domain is connected to the *C*-terminus of the IL-2 domain *via* the peptide linker, and the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the albumin binding domain directly.

[0073] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and first and second peptide linkers; wherein the *N*-terminus of the PD-1 binding domain is connected to the *C*-terminus of the IL-2 domain *via* the first peptide linker, and the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the albumin binding domain *via* the second peptide linker.

[0074] In still another embodiment, 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 the *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 albumin binding domain directly or *via* the second peptide linker.

[0075] In one embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, and an albumin binding domain; wherein the *C*-terminus of the PD-1

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binding domain is connected to the *N*-terminus of the IL-2 domain directly, and the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the albumin binding domain directly.

[0076] In another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and a peptide linker; wherein the *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the IL-2 domain directly, and the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the albumin binding domain *via* the peptide linker; or wherein the *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the IL-2 domain *via* the peptide linker, and the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the albumin binding domain directly.

[0077] In yet another embodiment, the fusion protein provided herein comprises an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and first and second peptide linkers; wherein the *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the IL-2 domain *via* the first peptide linker, and the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the albumin binding domain *via* the second peptide linker.

[0078] 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).

[0079] In certain embodiments, the fusion protein comprising an albumin binding domain 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. In certain embodiments, the fusion protein comprising an albumin binding domain binds to an HSA with a K_d ranging from about 10 pM to about 1,000 nM. In certain embodiments, the fusion protein comprising an albumin binding domain binds to an HSA with a K_d ranging from about 1 to about 500 nM. In certain embodiments, the fusion protein comprising an albumin binding domain binds to an HSA with a K_d ranging from about 1 to about 200 nM. In certain embodiments, the fusion protein comprising an albumin binding domain binds to an HSA with a K_d ranging from about 1 to about 100 nM.

[0080] In one embodiment, the albumin binding domain is an antibody or a fragment

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thereof, comprising (i) complementarity determining region 1 (CDR1) of SEQ ID NO: 83, complementarity determining region 2 (CDR2) of SEQ ID NO: 84, and complementarity determining region 3 (CDR3) of SEQ ID NO: 85; or (ii) CDR1 of SEQ ID NO: 91, CDR2 of SEQ ID NO: 92, and CDR3 of SEQ ID NO: 93. In another embodiment, the albumin binding domain comprises CDR1 of SEQ ID NO: 83, CDR2 of SEQ ID NO: 84, and CDR3 of SEQ ID NO: 85. In yet another embodiment, the albumin binding domain comprises CDR1 of SEQ ID NO: 91, CDR2 of SEQ ID NO: 92, and CDR3 of SEQ ID NO: 93. In yet another embodiment, the albumin binding domain comprises an amino acid sequence of SEQ ID NO: 90 or 97. In yet another embodiment, the albumin binding domain comprises an amino acid sequence of SEQ ID NO: 90. In still another embodiment, the albumin binding domain comprises an amino acid sequence of SEQ ID NO: 97.

[0081] In certain embodiments, the antibody is a human antibody. In certain embodiments, the antibody is a humanized antibody.

[0082] In certain embodiments, the antibody is a single domain antibody (sdAb) that binds to an albumin. In certain embodiments, the antibody is an sdAb that binds to an HSA.

[0083] In certain embodiments, the 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. In certain embodiments, the sdAb binds to an HSA with a K_d ranging from about 10 pM to about 1,000 nM. In certain embodiments, the sdAb binds to an HSA with a K_d ranging from about 1 to about 500 nM. In certain embodiments, the sdAb binds to an HSA with a K_d ranging from about 1 to about 200 nM. In certain embodiments, the sdAb binds to an HSA with a K_d ranging from about 1 to about 100 nM.

[0084] In one embodiment, the sdAb comprises (i) CDR1 of SEQ ID NO: 83, CDR2 of SEQ ID NO: 84, and CDR3 of SEQ ID NO: 85; or (ii) CDR1 of SEQ ID NO: 91, CDR2 of SEQ ID NO: 92, and CDR3 of SEQ ID NO: 93. In another embodiment, the sdAb comprises CDR1 of SEQ ID NO: 83, CDR2 of SEQ ID NO: 84, and CDR3 of SEQ ID NO: 85. In yet another embodiment, the sdAb comprises CDR1 of SEQ ID NO: 91, CDR2 of SEQ ID NO: 92, and CDR3 of SEQ ID NO: 93.

[0085] In one embodiment, the sdAb has the structure of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

CDR1, CDR2, and CDR3 are:

(i) CDR1 of SEQ ID NO: 83, CDR2 of SEQ ID NO: 84, and CDR3 of SEQ ID NO: 85; or

(ii) CDR1 of SEQ ID NO: 91, CDR2 of SEQ ID NO: 92, and CDR3 of SEQ ID NO: 93;

FR1 is an amino acid sequence of SEQ ID NO: 86 or 94;

FR2 is an amino acid sequence of SEQ ID NO: 87 or 95;

FR3 is an amino acid sequence of SEQ ID NO: 88; and

FR4 is an amino acid sequence of SEQ ID NO: 89 or 96.

[0086] In another embodiment, the sdAb has the structure of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

CDR1, CDR2, and CDR3 are:

(i) CDR1 of SEQ ID NO: 83, CDR2 of SEQ ID NO: 84, and CDR3 of SEQ ID NO: 85; or

(ii) CDR1 of SEQ ID NO: 91, CDR2 of SEQ ID NO: 92, and CDR3 of SEQ ID NO: 93;

FR1 is an amino acid sequence of SEQ ID NO: 86;

FR2 is an amino acid sequence of SEQ ID NO: 87;

FR3 is an amino acid sequence of SEQ ID NO: 88; and

FR3 is an amino acid sequence of SEQ ID NO: 89.

[0087] In yet another embodiment, the sdAb has the structure of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

CDR1, CDR2, and CDR3 are:

(i) CDR1 of SEQ ID NO: 83, CDR2 of SEQ ID NO: 84, and CDR3 of SEQ ID NO: 85; or

(ii) CDR1 of SEQ ID NO: 91, CDR2 of SEQ ID NO: 92, and CDR3 of SEQ ID NO: 93;

FR1 is an amino acid sequence of SEQ ID NO: 94;

FR2 is an amino acid sequence of SEQ ID NO: 95;
FR3 is an amino acid sequence of SEQ ID NO: 88; and
FR3 is an amino acid sequence of SEQ ID NO: 96.

[0088] In one embodiment, the sdAb has an amino acid sequence of SEQ ID NO: 90 or 97. In another embodiment, the sdAb has an amino acid sequence of SEQ ID NO: 90. In yet another embodiment, the sdAb has an amino acid sequence of SEQ ID NO: 97.

[0089] In certain embodiments, the antibody is a V_HH single domain antibody that binds to an albumin. In certain embodiments, the antibody is a V_HH single domain antibody that binds to an HSA.

[0090] In certain embodiments, the V_HH single domain 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. In certain embodiments, the V_HH single domain antibody binds to an HSA with a K_d ranging from about 10 pM to about 1,000 nM. In certain embodiments, the V_HH single domain antibody binds to an HSA with a K_d ranging from about 1 to about 500 nM. In certain embodiments, the V_HH single domain antibody binds to an HSA with a K_d ranging from about 1 to about 200 nM. In certain embodiments, the V_HH single domain antibody binds to an HSA with a K_d ranging from about 1 to about 100 nM.

[0091] In one embodiment, the V_HH single domain antibody comprises (i) heavy chain CDR1 of SEQ ID NO: 83, heavy chain CDR2 of SEQ ID NO: 84, and heavy chain CDR3 of SEQ ID NO: 85; or (ii) heavy chain CDR1 of SEQ ID NO: 91, heavy chain CDR2 of SEQ ID NO: 92, and heavy chain CDR3 of SEQ ID NO: 93. In another embodiment, the V_HH single domain antibody comprises heavy chain CDR1 of SEQ ID NO: 83, heavy chain CDR2 of SEQ ID NO: 84, and heavy chain CDR3 of SEQ ID NO: 85. In yet another embodiment, the V_HH single domain antibody comprises heavy chain CDR1 of SEQ ID NO: 91, heavy chain CDR2 of SEQ ID NO: 92, and heavy chain CDR3 of SEQ ID NO: 93.

[0092] In one embodiment, the V_HH single domain antibody has the structure of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

CDR1, CDR2, and CDR3 are:

(i) CDR1 of SEQ ID NO: 83, CDR2 of SEQ ID NO: 84, and CDR3 of SEQ ID NO: 85; or

(ii) CDR1 of SEQ ID NO: 91, CDR2 of SEQ ID NO: 92, and CDR3 of SEQ ID NO: 93;

FR1 is an amino acid sequence of SEQ ID NO: 86 or 94;

FR2 is an amino acid sequence of SEQ ID NO: 87 or 95;

FR3 is an amino acid sequence of SEQ ID NO: 88; and

FR4 is an amino acid sequence of SEQ ID NO: 89 or 96.

[0093] In another embodiment, the V_HH single domain antibody has the structure of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

CDR1, CDR2, and CDR3 are:

(i) CDR1 of SEQ ID NO: 83, CDR2 of SEQ ID NO: 84, and CDR3 of SEQ ID NO: 85; or

(ii) CDR1 of SEQ ID NO: 91, CDR2 of SEQ ID NO: 92, and CDR3 of SEQ ID NO: 93;

FR1 is an amino acid sequence of SEQ ID NO: 86;

FR2 is an amino acid sequence of SEQ ID NO: 87;

FR3 is an amino acid sequence of SEQ ID NO: 88; and

FR3 is an amino acid sequence of SEQ ID NO: 89.

[0094] In yet another embodiment, the V_HH single domain antibody has the structure of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

CDR1, CDR2, and CDR3 are:

(i) CDR1 of SEQ ID NO: 85, CDR2 of SEQ ID NO: 86, and CDR3 of SEQ ID NO: 87; or

(ii) CDR1 of SEQ ID NO: 93, CDR2 of SEQ ID NO: 94, and CDR3 of SEQ ID NO: 95;

FR1 is an amino acid sequence of SEQ ID NO: 94;

FR2 is an amino acid sequence of SEQ ID NO: 95;

FR3 is an amino acid sequence of SEQ ID NO: 88; and

FR3 is an amino acid sequence of SEQ ID NO: 96.

[0095] In one embodiment, the V_HH single domain antibody has an amino acid sequence of SEQ ID NO: 90 or 97. In another embodiment, the V_HH single domain antibody has an amino acid sequence of SEQ ID NO: 90. In yet another embodiment, the V_HH single domain antibody has an amino acid sequence of SEQ ID NO: 97.

[0096] In certain embodiments, the V_HH single domain antibody is a human antibody. In certain embodiments, the V_HH single domain antibody is a humanized antibody.

[0097] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and a half-life-extension domain that comprises a fragment crystallizable (Fc) domain. In certain embodiments, the IL-2 domain causes the fusion protein to signal through a receptor comprising CD122 and CD132 subunits.

[0098] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain, a PD-1 binding domain, and a half-life-extension domain that comprises an Fc domain; wherein the IL-2 domain causes the fusion protein to signal through a receptor consisting of CD122 and CD132 subunits.

[0099] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, first and second PD-1 binding domains, a half-life-extension domain that comprises an Fc domain having first and second peptide chains, and optionally a peptide linker; wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first peptide chain of the Fc domain directly or *via* the peptide linker.

[00100] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, first and second PD-1 binding domains, a half-life-extension domain that comprises an Fc domain having first and second peptide chains, and a peptide linker; wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first peptide chain of the Fc domain *via* the peptide linker.

[00101] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, first and second PD-1 binding domains, a half-life-extension domain that comprises an Fc domain having first and second peptide chains, and optionally first and second peptide linkers; wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-

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

[00102] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, first and second PD-1 binding domains, a half-life-extension domain that comprises an Fc domain having first and second peptide chains, and first and second peptide linkers; wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first peptide chain of the Fc domain *via* the first peptide linker; and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second peptide chain of the Fc domain *via* the second peptide linker.

[00103] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain, first and second PD-1 binding domains, a half-life-extension domain that comprises an Fc, and optionally a peptide linker; wherein the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the first PD-1 binding domain directly or *via* the peptide linker.

[00104] In still another embodiment, provided herein is a fusion protein comprising an IL-2 domain, first and second PD-1 binding domains, a half-life-extension domain that comprises an Fc, and a peptide linker; wherein the *C*-terminus of the IL-2 domain is connected to an *N*-terminus of the first PD-1 binding domain *via* the peptide linker.

[00105] 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.

[00106] In one embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, or 109. In another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 98. In yet another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 99. In yet another embodiment, the

FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 100. In yet another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 101. In yet another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 102. In yet another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 103. In yet another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 104. In yet another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 105. In yet another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 106. In yet another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 107. In yet another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 108. In still another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence of SEQ ID NO: 109.

[00107] In one embodiment, the FC domain as a half-life-extension domain comprises a pair of chains in a knobs-in-holes (KIH) configuration. Thus, in one embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence pair of SEQ ID NOs: 101 and 102, 103 and 104, 105 and 106, or108 and 109 in a knobs-in-holes configuration. In another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence pair of SEQ ID NOs: 101 and 102 in a knobs-in-holes configuration. In yet another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence pair of SEQ ID NOs: 103 and 104 in a knobs-in-holes configuration. In yet another embodiment, the Fc domain as a half-life-extension domain comprises an amino acid sequence pair of SEQ ID NOs: 105 and 106 in a knobs-in-holes configuration. In still another embodiment, the FC domain as a half-life-extension domain comprises an amino acid sequence pair of SEQ ID NOs: 108 and 109 in a knobs-in-holes configuration.

[00108] In one embodiment, the PD-1 binding domain and the half-life-extension domain that comprises an Fc are a part of an intact anti-PD-1 antibody comprising two light chains and two heavy chains.

[00109] 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.

[00110] 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 optionally a peptide linker, wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain directly or *via* the peptide linker.

[00111] 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 *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker.

[00112] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an anti-PD-1 antibody comprising two light chains and first and second heavy chains, and optionally first and second peptide linkers, wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain directly or *via* the first peptide linker; and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain directly or *via* the second peptide linker.

[00113] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, an anti-PD-1 antibody comprising light chains and first and second heavy chains, and first and second peptide linkers, wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker; and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker.

[00114] 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 optionally a peptide linker, wherein the *C*-terminus of the IL-2 domain is connected to the *N*-terminus of the first heavy chain directly or *via* the peptide linker.

[00115] In still another embodiment, provided herein is a fusion protein comprising an IL-

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

[00116] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain of any one of SEQ ID NOs: 42 to 81, 255, and 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215, wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00117] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 56, 60, 68, 70, 80, 255, or 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00118] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 42, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00119] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 50, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a

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CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00120] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 52, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00121] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 54, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00122] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 56, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00123] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 60, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of

SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00124] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 68, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00125] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 70, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00126] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 80, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00127] In still another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00128] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain of any one of SEQ ID NOs: 42 to 81, 255, and 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy is of SEQ ID NO: 117.

[00129] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 56, 60, 68, 70, 80, 255, or 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00130] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 42, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00131] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 50, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00132] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 52, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the

peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00133] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 54, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00134] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 56, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00135] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 60, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00136] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 68, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00137] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 70, an intact anti-PD-1 antibody comprising two light chains and first

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and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00138] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 80, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00139] In still another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00140] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each independently of any one of SEQ ID NOs: 42 to 81, 255, and 256; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00141] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each independently of SEQ ID NO: 56, 60, 68, 70, 80, 255, or 256; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the

first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00142] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 42; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00143] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 50; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00144] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 52; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker;

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and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00145] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 54; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00146] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 56; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00147] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 60; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114,

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and a CDRH3 of SEQ ID NO: 115.

[00148] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 68; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00149] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 70; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00150] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 80; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00151] In still another embodiment, provided herein is a fusion protein comprising first

and second IL-2 domains, each of SEQ ID NO: 256; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00152] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each independently of any one of SEQ ID NOs: 42 to 81, 255, and 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00153] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each independently of SEQ ID NO: 56, 60, 68, 70, 80, 255, or 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00154] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 42, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of

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SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00155] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 50, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00156] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 52, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00157] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 54, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

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[00158] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 56, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00159] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 60, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00160] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 68, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00161] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 70, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-

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2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00162] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 80, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00163] In still another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 116 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 117.

[00164] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain of any one of SEQ ID NOs: 42 to 81, 255, and 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00165] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 56, 60, 68, 70, 80, 255, or 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO:

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215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00166] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 42, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00167] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 50, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00168] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 52, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00169] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 54, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the

peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00170] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 56, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00171] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 60, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00172] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 68, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00173] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 70, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a

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CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00174] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 80, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00175] In still another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00176] In one embodiment, provided herein is a fusion protein comprising an IL-2 domain of any one of SEQ ID NOs: 42 to 81, 255, and 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00177] In another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 56, 60, 68, 70, 80, 255, or 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ

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ID NO: 135.

[00178] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 42, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00179] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 50, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00180] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 52, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00181] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 54, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00182] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 56, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-

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terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00183] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 60, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00184] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 68, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00185] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 70, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00186] In yet another embodiment, provided herein is a fusion protein comprising an IL-2 domain of SEQ ID NO: 80, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00187] In still another embodiment, provided herein is a fusion protein comprising an IL-

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2 domain of SEQ ID NO: 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and a peptide linker (*e.g.*, of SEQ ID NO: 215), wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00188] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each independently of any one of SEQ ID NOs: 42 to 81, 255, and 256; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00189] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each independently of SEQ ID NO: 56, 60, 68, 70, 80, 255, or 256; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00190] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 42; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker;

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and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00191] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 50; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00192] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 52; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00193] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 54; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132,

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and a CDRH3 of SEQ ID NO: 133.

[00194] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 56; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00195] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 60; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00196] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 68; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00197] In yet another embodiment, provided herein is a fusion protein comprising first

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and second IL-2 domains, each of SEQ ID NO: 70; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00198] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 80; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00199] In still another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 256; an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein the anti-PD-1 antibody comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL1 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00200] In one embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each independently of any one of SEQ ID NOs: 42 to 81, 255, and 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and

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first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00201] In another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each independently of SEQ ID NO: 56, 60, 68, 70, 80, 255, or 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00202] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 42, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00203] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 50, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker;

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and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00204] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 52, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00205] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 54, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00206] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 56, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00207] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 60, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of

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SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00208] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 68, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00209] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 70, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00210] In yet another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 80, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00211] In still another embodiment, provided herein is a fusion protein comprising first and second IL-2 domains, each of SEQ ID NO: 256, an intact anti-PD-1 antibody comprising two light chains and first and second heavy chains, and first and second peptide linkers (*e.g.*, each of SEQ ID NO: 215), wherein the *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the second peptide linker; and wherein each light chain comprises the amino acid sequence of SEQ ID NO: 134 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 135.

[00212] In one embodiment, the fusion protein provided herein comprising an intact anti-PD-1 antibody is in a knobs-in-holes configuration. In another embodiment, the fusion protein provided herein comprising an intact anti-PD-1 IgG1 antibody is in a knobs-in-holes configuration. In yet another embodiment, the fusion protein provided herein comprising an intact anti-PD-1 IgG4 antibody is in a knobs-in-holes configuration.

[00213] In one embodiment, provided herein is an anti-hPD-1/hIL-2 fusion protein in a knobs-in-holes configuration, comprising a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 248, and an hIL-2 fused heavy chain of SEQ ID NO: 249 that comprises an hIL-2 domain of SEQ ID NO: 42 and a peptide linker of SEQ ID NO: 215, wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker.

[00214] In another embodiment, provided herein is an anti-hPD-1/hIL-2 fusion protein in a knobs-in-holes configuration, comprising a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 248, and an hIL-2 fused heavy chain of SEQ ID NO: 250 that comprises an hIL-2 domain of SEQ ID NO: 54 and a peptide linker of SEQ ID NO: 215, wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker.

[00215] In yet another embodiment, provided herein is an anti-hPD-1/hIL-2 fusion protein in a knobs-in-holes configuration has a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 248, and an hIL-2 fused heavy chain of SEQ ID NO: 251 that comprises an hIL-2 domain of SEQ ID NO: 68 and a peptide linker of SEQ ID NO: 215; and wherein the *N*-terminus of the

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hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker.

[00216] In yet another embodiment, provided herein is an anti-hPD-1/hIL-2 fusion protein in a knobs-in-holes configuration comprising a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 248, and an hIL-2 fused heavy chain of SEQ ID NO: 252 that comprises an hIL-2 domain of SEQ ID NO: 70 and a peptide linker of SEQ ID NO: 215, wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker.

[00217] In yet another embodiment, provided herein is an anti-hPD-1/hIL-2 fusion protein in a knobs-in-holes configuration, comprising a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 248, and an hIL-2 fused heavy chain of SEQ ID NO: 253 that comprises an hIL-2 domain of SEQ ID NO: 80 and a peptide linker of SEQ ID NO: 215, wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker.

[00218] In still another embodiment, provided herein is an anti-hPD-1/hIL-2 fusion protein in a knobs-in-holes configuration, comprising a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 248, and an hIL-2 fused heavy chain of SEQ ID NO: 254 that comprising an hIL-2 domain of SEQ ID NO: 50 and a peptide linker of SEQ ID NO: 215, wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker.

[00219] In one embodiment, provided herein is an anti-hPD-1/hIL-2 fusion protein in a knobs-in-holes configuration, comprising a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 248, and an hIL-2 fused heavy chain of SEQ ID NO: 249, 250, 251, 252, 253, or 254.

[00220] In another embodiment, provided herein is an anti-hPD-1/hIL-2 fusion protein in a knobs-in-holes configuration, comprising a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 257, and an hIL-2 fused heavy chain of SEQ ID NO: 258, 259, 260, 261, 262, or 263.

[00221] In yet another embodiment, provided herein is an anti-hPD-1/hIL-2 fusion protein

in a knobs-in-holes configuration, comprising a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 264, and an hIL-2 fused heavy chain of SEQ ID NO: 202, 265, 266, 267, 268, or 269.

[00222] In yet another embodiment, provided herein is an anti-hPD-1/hIL-2 fusion protein in a knobs-in-holes configuration, comprising a light chain of SEQ ID NO: 118, a heavy chain of SEQ ID NO: 201, and an hIL-2 fused heavy chain of SEQ ID NO: 203 or 204.

[00223] In still another embodiment, provided herein is an anti-hPD-1/hIL-2 fusion protein in a knobs-in-holes configuration, comprising a light chain of SEQ ID NO: 118, and an hIL-2 fused heavy chain of SEQ ID NO: 205.

Interleukin-2 Domain

[00224] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) 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, S127, or S130; (ii) a disulfide bond formed between two amino acid residues from positions N30 to L80; or (iii) a replacement of the amino acid residues from positions N29 to A50 with a peptide comprising an amino acid sequence of an IL-15 hinge or a fragment thereof ("an IL-15 hinge fragment-containing peptide").

[00225] In another embodiment, the IL-2 domain in a fusion protein provided herein comprises: (i) a replacement of the amino acid residues from positions N29 to A50 with an IL-15 hinge fragment-containing peptide; (ii) an amino acid substitution at position E15, H16, D20, K32, K76, S87, N88, or I92; (iii) an amino acid substitution at a position from R38 to Y45; and/or (iv) a disulfide bond formed between two amino acid residues from positions N30 to L80.

[00226] In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution at position E15, H16, D20, K32, R38, L40, F42, K76, S87, N88, or I92; (ii) a disulfide bond formed between two amino acid residues from positions N30 to L80; or (iii) a replacement of the amino

acid residues from positions N29 to A50 with an IL-15 hinge fragment-containing peptide.

[00227] In certain embodiments, the amino acid at position E15 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 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 at position E15 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is K.

[00228] In certain embodiments, the amino acid at position H16 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is one of the twenty natural amino acids other than H. In certain embodiments, the amino acid at position H16 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is E, F, I, or V. In certain embodiments, the amino acid at position H16 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is E, I, or V. In certain embodiments, the amino acid at position H16 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is E. In certain embodiments, the amino acid at position H16 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is F. In certain embodiments, the amino acid at position H16 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is I. In certain embodiments, the amino acid at position H16 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is V.

[00229] In certain embodiments, the amino acid at position D20 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is one of the twenty natural amino acids other than D. In certain embodiments, the amino acid at position D20 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is A, E, K, or T. In certain embodiments, the amino acid at position D20 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is A. In certain embodiments, the amino acid at position D20 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is E. In certain embodiments, the amino acid at position D20 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is K. In certain embodiments, the amino acid at position D20 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is T.

[00230] In certain embodiments, the amino acid at position K32 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is one of the twenty natural amino acids other than K. In certain embodiments, the amino acid at position K32 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is D, E, or Q. In certain embodiments, the amino acid at position K32 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is D. In certain embodiments, the amino acid at position K32 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is E. In certain embodiments, the amino acid at position K32 as set forth in SEQ

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ID NO: 1, 2, 3, 4, or 5 is Q.

[00231] In certain embodiments, the amino acid at position R38 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is one of the twenty natural amino acids other than R. In certain embodiments, the amino acid at position R38 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is E or N. In certain embodiments, the amino acid at position R38 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is E. In certain embodiments, the amino acid at position R38 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is N.

[00232] In certain embodiments, the amino acid at position L40 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is one of the twenty natural amino acids other than L. In certain embodiments, the amino acid at position L40 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is S or T. In certain embodiments, the amino acid at position L40 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is S. In certain embodiments, the amino acid at position L40 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is T.

[00233] In certain embodiments, the amino acid at position F42 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is one of the twenty natural amino acids other than F. In certain embodiments, the amino acid at position F42 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is A, C, K, or N. In certain embodiments, the amino acid at position F42 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is A or K. In certain embodiments, the amino acid at position F42 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is A. In certain embodiments, the amino acid at position F42 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is C. In certain embodiments, the amino acid at position F42 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is K. In certain embodiments, the amino acid at position F42 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is N.

[00234] In certain embodiments, the amino acid at position K76 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is one of the twenty natural amino acids other than K. In certain embodiments, the amino acid at position K76 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is D, E, or Q. In certain embodiments, the amino acid at position K76 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is D. In certain embodiments, the amino acid at position K76 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is E. In certain embodiments, the amino acid at position K76 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is Q.

[00235] In certain embodiments, the amino acid at position S87 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is one of the twenty natural amino acids other than S. In certain embodiments, the amino acid at position S87 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is D or E. In certain embodiments, the amino acid at position S87 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is D. In certain embodiments, the amino acid at position S87 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is E.

[00236] In certain embodiments, the amino acid at position N88 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is one of the twenty natural amino acids other than N. In certain embodiments, the amino acid at position N88 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is A.

[00237] In certain embodiments, the amino acid at position I92 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is one of the twenty natural amino acids other than I. In certain embodiments, the amino acid at position I92 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is A, D, E, or G. In certain embodiments, the amino acid at position I92 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is A. In certain embodiments, the amino acid at position I92 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is D. In certain embodiments, the amino acid at position I92 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is E. In certain embodiments, the amino acid at position I92 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 is G.

[00238] In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) 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, S127W, S130A, S130E, S130F, or S130W; (ii) a disulfide bond formed between two different amino acid residues, each independently at position K35, R38, F42, Y45, E62, V69, or L72; or (iii) a replacement of the amino acid residues from positions N29 to A50 having an

amino acid sequence of SEQ ID NO: 38 with an IL-15 hinge fragment-containing peptide.

[00239] In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) 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; (ii) a disulfide bond formed between F42C and V69C; or (iii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide.

[00240] In one embodiment, the IL-15 hinge fragment-containing peptide comprises an amino acid sequence of SEQ ID NO: 40 or 41. In another embodiment, the IL-15 hinge fragment-containing peptide comprises an amino acid sequence of SEQ ID NO: 40. In yet another embodiment, the IL-15 hinge fragment-containing peptide comprises an amino acid sequence of SEQ ID NO: 41.

[00241] In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) 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; (ii) a disulfide bond formed between F42C and V69C; or (iii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41.

[00242] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises 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, S127, or S130 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00243] In another embodiment, the IL-2 domain in a fusion protein provided herein comprises 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, S127W, S130A, S130E, S130F, or S130W as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00244] In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution at position E15, H16, D20, K32, R38, L40, F42, K76, S87, N88, or I92 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00245] In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises 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 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00246] In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises 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 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00247] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution at position E15 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of E15K as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00248] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution at position H16 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of H16E, H16F, H16I, or H16V as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of H16E, H16I, or H16V as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In

yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of H16E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of H16F as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of H16I as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of H16V as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00249] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution at position D20 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of D20A, D20E, D20K, or D20T as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of D20A as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of D20E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of D20K as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of D20T as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00250] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution at position K32 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of K32E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00251] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution at position R38 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of R38E or R38N as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid

substitution of R38E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of R38N as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00252] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution at position L40 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of L40S or L40T as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of L40S as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of L40T as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00253] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution at position F42 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of F42A, F42C, or F42K as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of F42A or F42K as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of F42A as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of F42C as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of F42K as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00254] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution at position K76 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of K76E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00255] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution at position S87 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

In another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of S87D as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00256] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution at position N88 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of N88A as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00257] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution at position I92 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of I92A, I92D, I92E, or I92G as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of I92A as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of I92D as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of I92E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid substitution of I92G as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00258] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions R38 and L40, and optionally an amino acid substitution at position E15, H16, D20, K32, K76, S87, N88, or I92. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions R38 and L40, and optionally an amino acid substitution at position E15, H16, D20, K76, S87, N88, or I92. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions E15, R38, and L40, and optionally an amino acid substitution at position H16, D20, K76, S87, N88, or I92. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions R38, L40, and K76,

and optionally an amino acid substitution at position E15, H16, D20, S87, N88, or I92. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions E15, R38, L40, and K76, and optionally an amino acid substitution at position H16, D20, S87, N88, or I92.

[00259] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N and L40S or L40T, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N and L40S or L40T, and optionally an amino acid substitution of E15K, H16E, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, R38N and L40S or L40T, and optionally an amino acid substitution of H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N, L40S or L40T, and K76E, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, R38N, L40S or L40T, and K76E, and optionally an amino acid substitution of H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00260] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N and L40S, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, R38N and L40S, and optionally an amino acid substitution of H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A,

I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N, L40S, and K76E, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, R38N, L40S, and K76E, and optionally an amino acid substitution of H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00261] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N and L40T, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, R38N and L40T, and optionally an amino acid substitution of H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N, L40T, and K76E, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, R38N, L40T, and K76E, and optionally an amino acid substitution of H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00262] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises amino acid substitutions at positions R38 and L40 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises amino acid substitutions of R38N and L40S or L40T as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises amino acid substitutions of R38N and L40S as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises amino acid

substitutions of R38N and L40T as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00263] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises amino acid substitutions at positions E15, R38, and L40 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises amino acid substitutions of E15K, R38N, and L40S or L40T as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises amino acid substitutions of E15K, R38N, and L40S as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises amino acid substitutions of E15K, R38N, and L40T as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00264] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises amino acid substitutions at positions E15, R38, L40, and K76 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises amino acid substitutions of E15K, R38N, L40S or L40T, and K76E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises amino acid substitutions of E15K, R38N, L40S, and K76E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises amino acid substitutions of E15K, R38N, L40T, and K76E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00265] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 6 to 13.

[00266] In one embodiment, the IL-2 domain in a fusion protein provided is glycosylated. In another embodiment, the IL-2 domain in a fusion protein provided is *N*-glycosylated. In yet another embodiment, the IL-2 domain in the fusion protein provided herein is glycosylate at the nitrogen in the side chain of an asparagine residue. In still another embodiment, the IL-2 domain in a fusion protein provided comprises an amino acid substitution of R38N that is *N*-glycosylated.

[00267] In one embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-

glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions R38 and L40, and optionally an amino acid substitution at position E15, H16, D20, E32, K76, S87, N88, or I92. In another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions R38 and L40, and optionally an amino acid substitution at position E15, H16, D20, K76, S87, N88, or I92. In yet another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions E15, R38, and L40, and optionally an amino acid substitution at position H16, D20, K76, S87, N88, or I92. In yet another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions R38, L40, and K76, and optionally an amino acid substitution at position E15, H16, D20, S87, N88, or I92. In still another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions E15, R38, L40, and K76, and optionally an amino acid substitution at position H16, D20, S87, N88, or I92.

[00268] In one embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N and L40S or L40T, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, E32K, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N and L40S or L40T, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, R38N and L40S or L40T, and optionally an amino acid substitution of H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N, L40S or L40T, and K76E, and optionally an amino acid substitution of

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E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G. In still another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, R38N, L40S or L40T, and K76E, and optionally an amino acid substitution of H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00269] In one embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N and L40S, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, R38N and L40S, and optionally an amino acid substitution of H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N, L40S, and K76E, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G. In still another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, R38N, L40S, and K76E, and optionally an amino acid substitution of H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00270] In one embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N and L40T, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, R38N and L40T, and optionally an amino acid substitution of H16E,

H16F, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38N, L40T, and K76E, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G. In still another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, R38N, L40T, and K76E, and optionally an amino acid substitution of H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

[00271] In one embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, amino acid substitutions at positions R38 and L40 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, amino acid substitutions of R38N and L40S or L40T as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, amino acid substitutions of R38N and L40S as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, amino acid substitutions of R38N and L40T as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00272] In one embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, amino acid substitutions at positions E15, R38, and L40 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, amino acid substitutions of E15K, R38N, and L40S or L40T as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, amino acid substitutions of E5K, R38N, and L40S as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, amino acid substitutions of E15K, R38N, and L40T as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00273] In one embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, amino acid substitutions at positions E15, R38, L40, and K76 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, amino acid substitutions of E15K, R38N, L40S or L40T, and K76E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, amino acid substitutions of E15K, R38N, L40S, and K76E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the IL-2 domain in a fusion protein provided herein is an *N*-glycosylated IL-2 domain comprising, amino acid substitutions of E15K, R38N, L40T, and K76E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00274] In one embodiment, the *N*-glycosylated IL-2 domain in a fusion protein provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 6 to 13.

[00275] In one embodiment, the *N*-glycosylated IL-2 domain in the fusion protein provided herein has one glycan. In another embodiment, the *N*-glycosylated IL-2 domain in the fusion protein provided herein has one glycan attached to the nitrogen in the side chain of an asparagine residue. In yet another embodiment, the *N*-glycosylated IL-2 domain in the fusion protein provided herein has one glycan attached to the nitrogen in the side chain of an asparagine residue at position R38N.

[00276] In one embodiment, the *N*-glycosylated IL-2 domain in the fusion protein provided herein has two glycans. In another embodiment, the *N*-glycosylated IL-2 domain in the fusion protein provided herein has 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 domain in the fusion protein provided herein has two glycans, each of which is attached to the nitrogen in the side chain of an asparagine residue.

[00277] In one embodiment, the *N*-glycosylated IL-2 domain in a fusion protein provided herein has three glycans. In one embodiment, the glycan is an *N*-glycan.

[00278] In one embodiment, the *N*-glycan on the *N*-glycosylated IL-2 domain in a fusion protein provided herein is oligomannose-type. In another embodiment, the *N*-glycan on the *N*-

glycosylated IL-2 domain in a fusion protein provided herein is complex-type. In another embodiment, the *N*-glycan on the *N*-glycosylated IL-2 domain in a fusion protein provided herein is hydride-type.

[00279] In one embodiment, the *N*-glycan on the *N*-glycosylated IL-2 domain in a fusion protein provided herein is biantennary complex-type. In another embodiment, the *N*-glycan on the *N*-glycosylated IL-2 domain in a fusion protein provided herein is triantennary complex-type. In yet another embodiment, the *N*-glycan on the *N*-glycosylated IL-2 domain in a fusion protein provided herein is tetraantennary complex-type.

[00280] In one embodiment, the *N*-glycan on the *N*-glycosylated IL-2 domain in a fusion protein 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.

[00281] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions R38 and F42, and optionally an amino acid substitution at position E15, H16, D20, K32, K76, S87, N88, or I92. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions K32, R38, and F42, and optionally an amino acid substitution at position E15, H16, D20, K76, S87, N88, or I92. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions R38, F42, and K76, and optionally an amino acid substitution at position E15, H16, D20, K32, S87, N88, or I92. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions E15, K32, R38, and F42, and optionally an amino acid substitution at position H16, D20, K76, S87, N88, or I92. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions at positions E15, R38, F42, and K76, and optionally an amino acid substitution at position H16, D20, K32, S87, N88, or I92.

[00282] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38E and

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F42A or F42K, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of K32E, R38E, and F42A or F42K, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of R38E, F42A or F42K, and K76E, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, S87D, N88A, I92A, I92D, I92E, or I92G. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, K32E, R38E, and F42A or F42K, and optionally an amino acid substitution of H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, amino acid substitutions of E15K, R38E, F42A or F42K, and K76E, and optionally an amino acid substitution of H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, S87D, N88A, I92A, I92D, I92E, or I92G.

[00283] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions at positions K32, R38, and F42 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions of K32E, R38E, and F42A or F42K as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions of K32E, R38E, and F42A as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions of K32E, R38E, and F42K as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00284] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions at positions R38, F42, and K76 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the interleukin-2 domain in a fusion protein provided

herein comprises amino acid substitutions of R38E, F42A or F42K, and K76E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions of R38E, F42A, and K76E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions of R38E, F42K, and K76E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00285] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions at positions E15, K32, R38, and F42 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions of E15K, K32E, R38E, and F42A or F42K as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions of E15K, K32E, R38E, and F42A as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions of E15K, K32E, R38E, and F42K as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00286] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions at positions E15, R38, F42, and K76 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions of E15K, R38E, F42A or F42K, and K76E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions of E15K, R38E, F42A, and K76E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises amino acid substitutions of E15K, R38E, F42K, and K76E as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00287] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 14 to 25.

[00288] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a disulfide bond formed between two different amino acid residues from positions N30 to L80; and optionally an amino acid

substitution at position E15, H16, D20, K32, K76, S87, N88, or I92. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a disulfide bond formed between two different amino acid residues from positions K35 to L72; and optionally an amino acid substitution at position E15, H16, D20, K32, K76, S87, N88, or I92. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a disulfide bond formed between two different amino acid residues from positions K35 to L69; and optionally an amino acid substitution at position E15, H16, D20, K32, K76, S87, N88, or I92.

[00289] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a disulfide bond formed between two different amino acid residues from positions N30 to L80; and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a disulfide bond formed between two different amino acid residues from positions K35 to L72; and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a disulfide bond formed between two different amino acid residues from positions K35 to L69; and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[00290] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises a disulfide bond formed between two different amino acid residues from positions N30 to L80 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises a disulfide bond formed between two different amino acid residues from positions K35 to L72 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises a disulfide bond formed between two different amino acid residues from positions K35 to L69 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

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[00291] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises a disulfide bond formed between two different amino acid residues, each independently at K35, R38, F42, Y45, E62, V69, or L72 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises 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 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises a disulfide bond formed between an amino acid residue at position F42 and an amino acid residue at position E62, V69, or L72 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In still another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises a disulfide bond formed between an amino acid residue at position K35, R38, F42, or Y45, and an amino acid residue at position V69 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

[00292] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises 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 interleukin-2 domain in the fusion protein comprises a disulfide bond formed between K35C and L72C. In yet another embodiment, the interleukin-2 domain in the fusion protein comprises a disulfide bond formed between R38C and L72C. In yet another embodiment, the interleukin-2 domain in the fusion protein comprises a disulfide bond formed between F42C and V69C. In yet another embodiment, the interleukin-2 domain in the fusion protein comprises a disulfide bond formed between Y42C and L72C. In still another embodiment, the interleukin-2 domain in the fusion protein comprises a disulfide bond formed between Y45C and E62C.

[00293] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) 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, S127, or S130; and (ii) a disulfide bond formed between two different amino acid residues from positions N30 to L80.

[00294] In another embodiment, the interleukin-2 domain in a fusion protein provided

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herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) 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, S127W, S130A, S130E, S130F, or; and (ii) a disulfide bond formed between two different amino acid residues, each independently at position K35, R38, F42, Y45, E62, V69, or L72.

[00295] In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G; and (ii) a disulfide bond formed between F42C and V69C.

[00296] In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of E15K, H16E, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G; and (ii) a disulfide bond formed between F42C and V69C.

[00297] In still another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of E15K, K32E, or K76E; and (ii) a disulfide bond formed between F42C and V69C.

[00298] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises a disulfide bond formed between F42C and V69C as set forth in SEQ ID NO: 1, 2, 3, 4, or 5. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a disulfide bond formed between F42C and V69C, an amino acid substitution at position K32 or K76, and optionally an amino acid substitution at position E15. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a disulfide bond formed between F42C and V69C, and an amino acid substitution of K32E. In yet another

embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a disulfide bond formed between F42C and V69C, and an amino acid substitution of K76E. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a disulfide bond formed between F42C and V69C, and amino acid substitutions of E15K and K32E. In still another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a disulfide bond formed between F42C and V69C, and amino acid substitutions of E15K and K76E.

[00299] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 26 to 37.

[00300] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a replacement of the amino acid residues from positions N29 to A50 with an IL-15 hinge fragment-containing peptide, and optionally an amino acid substitution at position E15, H16, D20, K32, K76, S87, N88, or I92. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, 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, K32, K76, S87, N88, or I92. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41, and optionally an amino acid substitution at position E15, H16, D20, K32, K76, S87, N88, or I92.

[00301] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a replacement of the amino acid residues from positions N29 to A50 with an IL-15 hinge fragment-containing peptide, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5,

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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 of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41, and optionally an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, K32E, K76E, S87D, N88A, I92A, I92D, I92E, or I92G.

[00302] In one embodiment, the IL-15 hinge fragment-containing peptide has an amino acid sequence of SEQ ID NO: 40 or 41. In another embodiment, the IL-15 hinge fragment-containing peptide has an amino acid sequence of SEQ ID NO: 40. In yet another embodiment, the IL-15 hinge fragment-containing peptide has an amino acid sequence of SEQ ID NO: 41.

[00303] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises a replacement of the amino acid residues from positions N29 to A50 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 with an IL-15 hinge fragment-containing peptide. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises a replacement of the amino acid residues from positions N29 to L40 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 with an IL-15 hinge fragment-containing peptide. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41.

[00304] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) 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, S127, or S130; and (ii) a replacement of the amino acid residues from positions N29 to A50 with an IL-15 hinge fragment-containing peptide.

[00305] In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) 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, S127W, S130A, S130E, S130F, or S130W; and (ii) a replacement of the amino acid residues from positions N29 to A50 having an amino acid sequence of SEQ ID NO: 38 with an IL-15 hinge fragment-containing peptide.

[00306] In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) 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, S127W, S130A, S130E, S130F, or S130W; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide.

[00307] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution at position E15, H16, D20, S87, N88, or I92; and (ii) a replacement of the amino acid residues from positions N29 to A50 with an IL-15 hinge fragment-containing peptide.

[00308] In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G; and (ii) a replacement of the amino acid residues from positions N29 to A50 having an amino acid sequence of SEQ ID NO: 38 with an IL-15 hinge fragment-containing peptide.

[00309] In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide.

[00310] In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide.

[00311] In still another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of E15K, H16E, H16F, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41.

[00312] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution at E15; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of E15K; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41.

[00313] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of H16; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino

acid sequence of SEQ ID NO: 40 or 41. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of H16E, H16F, H16I, or H16V; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of H16E, H16I, or H16V; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of H16E; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of H16F; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of H16I; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In still another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of H16V; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41.

[00314] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of D20; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid

sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of D20A, D20E, D20K, or D20T; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of D20A; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of D20E; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of D20K; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In still another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of D20T; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41.

[00315] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution at S87; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of S87D; and (ii) a replacement of the amino acid residues from positions N29

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to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41.

[00316] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution at N88; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of N88A; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41.

[00317] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution at I92; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of I92A, I92D, I92E, or I92G; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of I92A; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of I92D; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth

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in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of I92E; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In still another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of I92G; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41.

[00318] In one embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) one or two amino acid substitutions of E15K, H16I, D20T, N88A, and I92A; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of E15K, H16I, D20T, N88A, or I92A; and (ii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41. In yet another embodiment, the interleukin-2 domain in a fusion protein provided herein comprises, as set forth in SEQ ID NO: 1, 2, 3, 4, or 5, (i) an amino acid substitution of E15K; (ii) an amino acid substitution of H16I, D20T, N88A, or I92A; and (iii) a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41.

[00319] In one embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid sequence of any one of SEQ ID NOs: 42 to 81, 255, and 256. In another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid sequence of SEQ ID NO: 56, 60, 68, 70, 80, 255, or 256. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid sequence of SEQ ID NO: 56. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid sequence of SEQ ID NO: 60. In yet another embodiment, the IL-2

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domain in a fusion protein provided herein comprises an amino acid sequence of SEQ ID NO: 68. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid sequence of SEQ ID NO: 70. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid sequence of SEQ ID NO: 80. In yet another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid sequence of SEQ ID NO: 255. In still another embodiment, the IL-2 domain in a fusion protein provided herein comprises an amino acid sequence of SEQ ID NO: 256.

[00320] In certain embodiments, the interleukin-2 domain in a fusion protein 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: 1, 2, 3, 4, or 5. In certain embodiments, the interleukin-2 domain in a fusion protein provided herein comprises an amino acid sequence that is no less than about 80% identical to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, or 5. In certain embodiments, the interleukin-2 domain in a fusion protein provided herein comprises an amino acid sequence that is no less than about 85% identical to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, or 5. In certain embodiments, the interleukin-2 domain in a fusion protein provided herein comprises an amino acid sequence that is no less than about 90% identical to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, or 5. In certain embodiments, the interleukin-2 domain in a fusion protein provided herein comprises an amino acid sequence that is no less than about 91% identical to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, or 5. In certain embodiments, the interleukin-2 domain in a fusion protein provided herein comprises an amino acid sequence that is no less than about 92% identical to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, or 5. In certain embodiments, the interleukin-2 domain in a fusion protein provided herein comprises an amino acid sequence that is no less than about 93% identical to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, or 5. In certain embodiments, the interleukin-2 domain in a fusion protein provided herein comprises an amino acid sequence that is no less than about 94% identical to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, or 5. In certain embodiments, the interleukin-2 domain in a fusion protein provided herein comprises an amino acid sequence that is no less than about 95% identical to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, or 5. In certain

embodiments, the interleukin-2 domain in a fusion protein provided herein comprises an amino acid sequence that is no less than about 96% identical to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, or 5. In certain embodiments, the interleukin-2 domain in a fusion protein provided herein comprises an amino acid sequence that is no less than about 97% identical to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, or 5. In certain embodiments, the interleukin-2 domain in a fusion protein provided herein comprises an amino acid sequence that is no less than about 98% identical to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, or 5. In certain embodiments, the interleukin-2 domain in a fusion protein provided herein comprises an amino acid sequence that is no less than about 99% identical to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, or 5.

[00321] In certain embodiments, the interleukin-2 domain in a fusion protein 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.

[00322] In certain embodiments, the interleukin-2 domain in a fusion protein provided herein further includes one or more additional substitutions, deletions, and/or insertions; and/or one or more additional post-translational modifications.

PD-1 Binding Domain

[00323] In one embodiment, the PD-1 binding domain comprises:

(i) a light chain complementarity determining region 1 (CDRL1) of SEQ ID NO: 111,
a light chain complementarity determining region 2 (CDRL2) of DAS,
a light chain complementarity determining region 3 (CDRL3) of SEQ ID NO: 112,
a heavy chain complementarity determining region 1 (CDRH1) of SEQ ID NO: 113,
a heavy chain complementarity determining region 2 (CDRH2) of SEQ ID NO: 114, and
a heavy chain complementarity determining region 3 (CDRH3) of SEQ ID NO: 115;

(ii) a CDRL1 of SEQ ID NO: 120, a CDRL2 of LAS, a CDRL3 of SEQ ID NO: 121, a CDRH1 of SEQ ID NO: 122, a CDRH2 of SEQ ID NO: 123, and a CDRH3 of SEQ ID NO: 124;

(iii) a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL3 of SEQ ID NO: 130, a

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CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133;

(iv) a CDRL1 of SEQ ID NO: 138, a CDRL2 of TAT, a CDRL3 of SEQ ID NO: 139, a CDRH1 of SEQ ID NO: 140, a CDRH2 of SEQ ID NO: 141, and a CDRH3 of SEQ ID NO: 142;

(v) a CDRL1 of SEQ ID NO: 147, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 148, a CDRH1 of SEQ ID NO: 149, a CDRH2 of SEQ ID NO: 150, and a CDRH3 of SEQ ID NO: 151;

(vi) a CDRL1 of SEQ ID NO: 156, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 157, a CDRH1 of SEQ ID NO: 158, a CDRH2 of SEQ ID NO: 159, and a CDRH3 of SEQ ID NO: 160;

(vii) a CDRL1 of SEQ ID NO: 165, a CDRL2 of YAF, a CDRL3 of SEQ ID NO: 166, a CDRH1 of SEQ ID NO: 167, a CDRH2 of SEQ ID NO: 168, and a CDRH3 of SEQ ID NO: 169;

(viii) a CDRL1 of SEQ ID NO: 174, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 175, a CDRH1 of SEQ ID NO: 176, a CDRH2 of SEQ ID NO: 177, and a CDRH3 of SEQ ID NO: 178;

(ix) a CDRL1 of SEQ ID NO: 183, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 184, a CDRH1 of SEQ ID NO: 185, a CDRH2 of SEQ ID NO: 186, and a CDRH3 of SEQ ID NO: 187; or

(x) a CDRL1 of SEQ ID NO: 192, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 193, a CDRH1 of SEQ ID NO: 194, a CDRH2 of SEQ ID NO: 195, and a CDRH3 of SEQ ID NO: 196.

[00324] In one embodiment, the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

[00325] In another embodiment, the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 120, a CDRL2 of LAS, a CDRL3 of SEQ ID NO: 121, a CDRH1 of SEQ ID NO: 122, a CDRH2 of SEQ ID NO: 123, and a CDRH3 of SEQ ID NO: 124.

[00326] In yet another embodiment, the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL3 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

[00327] In yet another embodiment, the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 138, a CDRL2 of TAT, a CDRL3 of SEQ ID NO: 139, a CDRH1 of SEQ ID NO: 140, a CDRH2 of SEQ ID NO: 141, and a CDRH3 of SEQ ID NO: 142.

[00328] In yet another embodiment, the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 147, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 148, a CDRH1 of SEQ ID NO: 149, a CDRH2 of SEQ ID NO: 150, and a CDRH3 of SEQ ID NO: 151.

[00329] In yet another embodiment, the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 156, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 157, a CDRH1 of SEQ ID NO: 158, a CDRH2 of SEQ ID NO: 159, and a CDRH3 of SEQ ID NO: 160.

[00330] In yet another embodiment, the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 165, a CDRL2 of YAF, a CDRL3 of SEQ ID NO: 166, a CDRH1 of SEQ ID NO: 167, a CDRH2 of SEQ ID NO: 168, and a CDRH3 of SEQ ID NO: 169.

[00331] In yet another embodiment, the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 174, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 175, a CDRH1 of SEQ ID NO: 176, a CDRH2 of SEQ ID NO: 177, and a CDRH3 of SEQ ID NO: 178.

[00332] In yet another embodiment, the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 183, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 184, a CDRH1 of SEQ ID NO: 185, a CDRH2 of SEQ ID NO: 186, and a CDRH3 of SEQ ID NO: 187.

[00333] In still another embodiment, the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 192, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 193, a CDRH1 of SEQ ID NO: 194, a CDRH2 of SEQ ID NO: 195, and a CDRH3 of SEQ ID NO: 196.

[00334] In certain embodiments, the CDRs provided herein are defined according to the IMGT or Kabat numbering system. In certain embodiments, the CDRs provided herein are defined according to the IMGT numbering system. In certain embodiments, the CDRs provided

herein are defined according to the Kabat numbering system.

[00335] In another embodiment, the PD-1 binding domain comprises:

(i) a light chain variable region of SEQ ID NO: 116 and a heavy chain variable region of SEQ ID NO: 117;

(ii) a light chain variable region of SEQ ID NO: 125 and a heavy chain variable region of SEQ ID NO: 126;

(iii) a light chain variable region of SEQ ID NO: 134 and a heavy chain variable region of SEQ ID NO: 135;

(iv) a light chain variable region of SEQ ID NO: 143 and a heavy chain variable region of SEQ ID NO: 144;

(v) a light chain variable region of SEQ ID NO: 152 and a heavy chain variable region of SEQ ID NO: 153;

(vi) a light chain variable region of SEQ ID NO: 161 and a heavy chain variable region of SEQ ID NO: 162;

(vii) a light chain variable region of SEQ ID NO: 170 and a heavy chain variable region of SEQ ID NO: 171;

(viii) a light chain variable region of SEQ ID NO: 179 and a heavy chain variable region of SEQ ID NO: 180;

(ix) a light chain variable region of SEQ ID NO: 188 and a heavy chain variable region of SEQ ID NO: 189;

(x) a light chain variable region of SEQ ID NO: 197 and a heavy chain variable region of SEQ ID NO: 198.

[00336] In one embodiment, the PD-1 binding domain comprises a light chain variable region of SEQ ID NO: 116 and a heavy chain variable region of SEQ ID NO: 117. In another embodiment, the PD-1 binding domain comprises a light chain variable region of SEQ ID NO: 125 and a heavy chain variable region of SEQ ID NO: 126. In yet another embodiment, the PD-1 binding domain comprises a light chain variable region of SEQ ID NO: 134 and a heavy chain variable region of SEQ ID NO: 135. In yet another embodiment, the PD-1 binding domain comprises a light chain variable region of SEQ ID NO: 143 and a heavy chain variable region of SEQ ID NO: 144. In yet another embodiment, the PD-1 binding domain comprises a light chain

variable region of SEQ ID NO: 152 and a heavy chain variable region of SEQ ID NO: 153. In yet another embodiment, the PD-1 binding domain comprises a light chain variable region of SEQ ID NO: 161 and a heavy chain variable region of SEQ ID NO: 162. In yet another embodiment, the PD-1 binding domain comprises a light chain variable region of SEQ ID NO: 170 and a heavy chain variable region of SEQ ID NO: 171. In yet another embodiment, the PD-1 binding domain comprises a light chain variable region of SEQ ID NO: 179 and a heavy chain variable region of SEQ ID NO: 180. In yet another embodiment, the PD-1 binding domain comprises a light chain variable region of SEQ ID NO: 188 and a heavy chain variable region of SEQ ID NO: 189. In still another embodiment, the PD-1 binding domain comprises a light chain variable region of SEQ ID NO: 197 and a heavy chain variable region of SEQ ID NO: 198.

[00337] 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, or minibody.

[00338] In another embodiment, the PD-1 binding domain is an scFv. In yet another embodiment, the PD-1 binding domain is an 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 light chain directly or *via* the peptide linker, or wherein the *N*-terminal of the light chain is connected to the *C*-terminal of the light chain directly or *via* the peptide linker. In yet another embodiment, the PD-1 binding domain is an scFv comprising a light chain (V_L), a heavy chain (V_H), and a peptide linker, wherein the *C*-terminal of the light chain is connected to the *N*-terminal of the light chain *via* the peptide linker, or wherein the *N*-terminal of the light chain is connected to the *C*-terminal of the light chain *via* the peptide linker. In yet another embodiment, the PD-1 binding domain is an scFv comprising a light chain (V_L), a heavy chain (V_H), and a peptide linker, wherein the *C*-terminal of the light chain is connected to the *N*-terminal of the light chain *via* the peptide linker. In yet another embodiment, the PD-1 binding domain is an scFv comprising a light chain (V_L), a heavy chain (V_H), and a peptide linker, wherein the *N*-terminal of the light chain is connected to the *C*-terminal of the light chain *via* the peptide linker. In certain embodiments, the peptide linker has the amino acid sequence of SEQ ID NO: 215 or 216.

[00339] In yet another embodiment, the PD-1 binding domain is a Fab. In yet another

embodiment, the PD-1 binding domain is a Fab'. In yet another embodiment, the PD-1 binding domain is a $F(ab)_2$. In yet another embodiment, the PD-1 binding domain is a $F(ab')_2$. In yet another embodiment, the PD-1 binding domain is a Fv. In yet another embodiment, the PD-1 binding domain is a diabody. In yet another embodiment, the PD-1 binding domain is a triabody. In yet another embodiment, the PD-1 binding domain is a tetrabody. In yet another embodiment, the PD-1 binding domain is a minibody. In still another embodiment, the PD-1 binding domain is a V_HH single domain antibody.

[00340] In one embodiment, the PD-1 binding domain and the half-life extension domain are parts of an intact antibody comprising two light chains and two heavy chains.

[00341] In one embodiment, the intact antibody is an IgA, IgD, IgE, IgG, or IgM antibody. In another embodiment, the intact antibody is an IgA antibody. In yet another embodiment, the intact antibody is an IgD antibody. In yet another embodiment, the intact antibody is an IgE antibody. In yet another embodiment, the intact antibody is an IgG antibody. In still another embodiment, the intact antibody is an IgM antibody.

[00342] In one embodiment, the intact antibody is an IgA1, IgA2, IgG1, IgG2, IgG3, or IgG4 antibody. In another embodiment, the intact antibody is an IgA1 or IgA2. In yet another embodiment, the intact antibody is an IgA1. In yet another embodiment, the intact antibody is an IgA2. In yet another embodiment, the intact antibody is an IgG1, IgG2, IgG3, or IgG4 antibody. In yet another embodiment, the intact antibody is an IgG1 antibody. In yet another embodiment, the intact antibody is an IgG2 antibody. In yet another embodiment, the intact antibody is an IgG3 antibody. In still another embodiment, the intact antibody is an IgG4 antibody.

[00343] In one embodiment, the light chain is a kappa or lambda chain. In another embodiment, the light chain is a kappa chain. In yet another embodiment, the light chain is a lambda chain.

[00344] In one embodiment, the antibody comprises:

(i) a light chain of SEQ ID NO: 118 and a heavy chain of SEQ ID NO: 119;

(ii) a light chain of SEQ ID NO: 127 and a heavy chain of SEQ ID NO: 128;

(iii) a light chain of SEQ ID NO: 136 and a heavy chain of SEQ ID NO: 137;

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(iv) a light chain of SEQ ID NO: 145 and a heavy chain of SEQ ID NO: 146;
(v) a light chain of SEQ ID NO: 154 and a heavy chain of SEQ ID NO: 155;
(vi) a light chain of SEQ ID NO: 163 and a heavy chain of SEQ ID NO: 164;
(vii) a light chain of SEQ ID NO: 172 and a heavy chain of SEQ ID NO: 173;
(viii) a light chain of SEQ ID NO: 181 and a heavy chain of SEQ ID NO: 182;
(ix) a light chain of SEQ ID NO: 190 and a heavy chain of SEQ ID NO: 191;
(x) a light chain of SEQ ID NO: 199 and a heavy chain of SEQ ID NO: 200.

[00345] In one embodiment, the antibody comprises a light chain of SEQ ID NO: 118 and a heavy chain of SEQ ID NO: 119. In another embodiment, the antibody comprises a light chain of SEQ ID NO: 127 and a heavy chain of SEQ ID NO: 128. In yet another embodiment, the antibody comprises a light chain of SEQ ID NO: 136 and a heavy chain of SEQ ID NO: 137. In yet another embodiment, the antibody comprises a light chain of SEQ ID NO: 145 and a heavy chain of SEQ ID NO: 146. In yet another embodiment, the antibody comprises a light chain of SEQ ID NO: 154 and a heavy chain of SEQ ID NO: 156. In yet another embodiment, the antibody comprises a light chain of SEQ ID NO: 163 and a heavy chain of SEQ ID NO: 164. In yet another embodiment, the antibody comprises a light chain of SEQ ID NO: 172 and a heavy chain of SEQ ID NO: 173. In yet another embodiment, the antibody comprises a light chain of SEQ ID NO: 181 and a heavy chain of SEQ ID NO: 182. In yet another embodiment, the antibody comprises a light chain of SEQ ID NO: 190 and a heavy chain of SEQ ID NO: 191. In still another embodiment, the antibody comprises a light chain of SEQ ID NO: 199 and a heavy chain of SEQ ID NO: 200.

Peptide Linkers

[00346] 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: 206 to 245. In another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of GSG or SEQ ID NO: 206, 207, or 208. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 209, 210, 211, or 212. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid

sequence of SEQ ID NO: 213, 214, 215, or 216. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 217, 218, 219, or 220. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 221, 222, 223, or 224. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 225, 226, 227, or 228. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 229, 230, 231, or 232. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 233, 234, 235, or 236. In yet another embodiment, each peptide linker in a fusion protein provided herein independently comprises an amino acid sequence of SEQ ID NO: 237. In yet another embodiment, each peptide linker in a fusion protein provided herein is independently comprises an amino acid sequence of SEQ ID NO: 238 or 239. In yet another embodiment, each peptide linker in a fusion protein provided herein is independently comprises an amino acid sequence of SEQ ID NO: 240 or 241. In yet another embodiment, each peptide linker in a fusion protein provided herein is independently comprises an amino acid sequence of SEQ ID NO: 242 or 243. In still another embodiment, each peptide linker in a fusion protein provided herein is independently comprises an amino acid sequence of SEQ ID NO: 244 or 245.

Pharmaceutical Compositions

[00347] In one embodiment, provided herein is a pharmaceutical composition comprising a fusion protein provided herein and a pharmaceutically acceptable excipient.

[00348] In one embodiment, the pharmaceutical composition is formulated as single dosage form.

[00349] 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.

[00350] 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

[00351] 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 a fusion protein provided herein.

[00352] In certain embodiments, the disorder, disease, or condition mediated by a PD-1 is a proliferative disease.

[00353] 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 a fusion protein provided herein.

[00354] In certain embodiments, the disorder, disease, or condition mediated by an IL-2 is a proliferative disease.

[00355] 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 a fusion protein provided herein.

[00356] In one embodiment, the proliferative disease is cancer. In another embodiment, the proliferative disease is colorectal cancer.

[00357] 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.

[00358] In certain embodiments, the cancer is drug-resistant. In certain embodiment, 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.

[00359] In certain embodiments, the subject is a mammal. In certain embodiments, the subject is a human.

[00360] In another embodiment, provided herein is a method of inhibiting the growth of a cell, comprising contacting the cell with an effective amount of a 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.

[00361] In yet another embodiment, provided herein is a method of activating an immune effector cell, comprising contacting the effector cell with an effective amount of a fusion protein provided herein. In certain embodiments, the effector cell is a human effector cell.

[00362] 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.

[00363] The disclosure will be further understood by the following non-limiting examples.

EXAMPLES

Example 1

Cloning, Expression, and Purification of Fusion Proteins

[00364] The amino acid sequence of human IL-2 (hIL-2) was obtained from UNIPROT (hIL-2: P60568, 21-153 aa). A mutant human IL-2 was generated by introducing a mutation to abolish the CD25 binding as well as reducing the CD122 binding. The amino acid sequence of a human anti-PD-1 antibody (VH & VL) was obtained from the Therapeutic Antibody Database (TABS). The deoxyoligonucleotide (DNA) sequences encoding the mutant human IL2 and human anti-PD1 antibody were codon optimized for CHO cell expression.

[00365] Certain configurations of fusion proteins containing (i) an hIL-2 mutein and (ii) a human anti-PD-1 antibody or a fragment thereof are illustrated in FIGS. 1 and 2. The deoxyoligonucleotide sequences encoding (i) the hIL-2 mutein, (ii) the human anti-PD-1 antibody or a fragment thereof, and (iii) other sequences such as 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.

[00366] The oligonucleotide sequence encoding a fusion protein was transiently expressed in EXPICHO™ cells. Briefly, on Day -1, EXPICHO-S™ cells were seeded at 3-4 x 10^6 cells/mL with the EXPICHO™ expression medium in a vented Erlenmeyer shake flask. The flask was placed on a 125-rpm orbital shaker in a 37 °C incubator with 8% CO_2. On Day 0, plasmid DNA was mixed with the EXPIFECTAMINE™ CHO reagent. The mixture was then slowly added to the cells. After 16 hours, the cells were transferred to a 32 °C incubator with 5% CO_2. The cells were fed twice on Day 1 and Day 5 with the EXPICHO ™ feed. The CHO cells were harvested on Day 8-12.

[00367] 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) resin and ion exchange chromatography (*e.g.*, CAPTO™ Q ImpRes).

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[00368] For the protein A affinity chromatography, a protein A affinity column was loaded with a clarified CHO medium and then washed once with 20 mM sodium phosphate at pH 7.5, once with 20 mM sodium phosphate with 0.5 M NaCl at pH 7.5, and once again with 20 mM sodium phosphate at pH 7.5. The fusion protein was eluted with 50 mM sodium acetate at pH 3.0 supplied with 1% isopropanol by volume.

[00369] The purified fusion protein was then buffer exchanged into 20 mM Tris-HCl at pH 8.5 in preparation of AKTA™ purification. The fusion protein was loaded onto 1 mL HiTrap CAPTO™ Q ImpRes column. The column was then washed with 20 mM Tris-HCl at pH 8.5 for 5 column volumes (CV) and eluted with 20 mM Tris-HCl at pH 8.5 plus 1 M NaCl by a gradient of 0-20% in 20 CV. The fusion protein was eluted off between 9~12 mS/cm. Eluted fractions were pooled and buffer exchanged into a solution containing 20 mM sodium phosphate at pH 6.5 for storage.

Example 2

Stability Determination

[00370] The thermostability of purified fusion proteins was measured *via* dynamic light scattering using a DYNAPRO® NANOSTAR® dynamic light scattering detector. The fusion proteins were each buffer exchanged into 20 mM sodium phosphate at pH 6.5 and then incubated in a continuous temperature ramp ranging from 30 to 80 °C while measuring the radius of each fusion protein every 1 °C. The results are summarized in Table 1. The fusion proteins were observed to have two T_{onsets}. T_{onset} 1 was calculated to be between 55-59 °C depending on mutations in the IL-2 muteins. T_{onset} 2 was calculated to be 70 °C.

[00371] Anti-hPD-1/hIL-2 fusion protein **A1** in a knobs-in-holes configuration has a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 248, and an hIL-2 fused heavy chain of SEQ ID NO: 249, comprising an hIL-2 domain of SEQ ID NO: 42 and a peptide linker of SEQ ID NO: 215, wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker. Anti-hPD-1/hIL-2 fusion protein **A2** in a knobs-in-holes configuration has a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 248, and an hIL-2 fused heavy chain of SEQ ID NO: 250, comprising an hIL-2 domain of SEQ ID NO: 54 and a peptide linker of SEQ ID NO: 215, wherein the *N*-terminus of the hIL-2 domain is

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connected to the *C*-terminus of the second heavy chain *via* the peptide linker. Anti-hPD-1/hIL-2 fusion protein **A3** in a knobs-in-holes configuration has a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 248, and an hIL-2 fused heavy chain of SEQ ID NO: 251, comprising an hIL-2 domain of SEQ ID NO: 68 and a peptide linker of SEQ ID NO: 215; and wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker. Anti-hPD-1/hIL-2 fusion protein **A4** in a knobs-in-holes configuration has a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 248, and an hIL-2 fused heavy chain of SEQ ID NO: 252, comprising an hIL-2 domain of SEQ ID NO: 70 and a peptide linker of SEQ ID NO: 215, wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker. Anti-hPD-1/hIL-2 fusion protein **A5** in a knobs-in-holes configuration has a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 248, and an hIL-2 fused heavy chain of SEQ ID NO: 253, comprising an hIL-2 domain of SEQ ID NO: 80 and a peptide linker of SEQ ID NO: 215, wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker. Anti-hPD-1/hIL-2 fusion protein **A6** in a knobs-in-holes configuration has a light chain of SEQ ID NO: 136, a heavy chain of SEQ ID NO: 248, and an hIL-2 fused heavy chain of SEQ ID NO: 254, comprising an hIL-2 domain of SEQ ID NO: 50 and a peptide linker of SEQ ID NO: 215, wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker. Anti-hPD-1/hIL-2 fusion protein **A19** in a knobs-in-holes configuration has a light chain of SEQ ID NO: 118, a heavy chain of SEQ ID NO: 201, and an hIL-2 fused heavy chain of SEQ ID NO: 203, comprising an hIL-2 domain of SEQ ID NO: 70 and a peptide linker of SEQ ID NO: 215, wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker. Anti-hPD-1/hIL-2 fusion protein **A20** in a knobs-in-holes configuration has a light chain of SEQ ID NO: 118, a heavy chain of SEQ ID NO: 201, and an hIL-2 fused heavy chain of SEQ ID NO: 204, comprising an hIL-2 domain of SEQ ID NO: 256 and a peptide linker of SEQ ID NO: 215, wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker. Anti-hPD-1/hIL-2 fusion protein **A21** has a light chain of SEQ ID NO: 118, and an hIL-2 fused heavy chain of SEQ ID NO: 205, comprising an hIL-2 domain of SEQ ID NO: 256 and a peptide linker of SEQ ID NO: 215, wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the

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second heavy chain *via* the peptide linker.

Table 1. Thermostability Determination of Fusion Proteins

Protein	T_{onset} 1 (°C)	T_{onset} 2 (°C)
Anti-hPD-1/hIL-2 fusion protein **A1**	57.7	69.5
Anti-hPD-1/hIL-2 fusion protein **A2**	58.2	70
Anti-hPD-1/hIL-2 fusion protein **A3**	57.5	70.3
Anti-hPD-1/hIL-2 fusion protein **A4**	56.8	70.6
Anti-hPD-1/hIL-2 fusion protein **A5**	55.3	68.8
Anti-hPD-1/hIL-2 fusion protein **A6**	57.9	69.8
Anti-hPD-1/hIL-2 fusion protein **A19**	62.5	N.A.
Anti-hPD-1/hIL-2 fusion protein **A20**	62.3	N.A.
Anti-hPD-1/hIL-2 fusion protein **A21**	62	N.A.

Example 3

Binding Studies of IL-2 Muteins to CD25 and CD122

[00372] OCTET® RED96 was used to characterize the interactions of wildtype IL-2 and IL-2 muteins with CD25 and CD122. Briefly, an CD25- or CD122-Fc fusion protein was loaded onto an anti-human IgG Fc capture (AHC) biosensor. The biosensor was then dipped into a solution containing wild-type human IL-2 or an IL-2 mutein at 400 nM. The results are summarized in Table 2. All IL-2 muteins tested were found to have abolished or significantly reduced CD25 binding.

Table 2. Interactions of Wildtype IL-2 and IL-2 Muteins with CD25 and CD122

Fusion Protein	SEQ ID NO:	Binding Response	
		CD25	CD122
Wildtype hIL-2	2	1	1
hIL-2 Mutein (R38N, L40T, C125S)	6	<0.05	>0.5

Fusion Protein	SEQ ID NO:	Binding Response	
		CD25	CD122
hIL-2 Mutein (K32E, R38E, F42K, C125S)	16	<0.05	>0.5
hIL-2 Mutein (K32E, F42C, V69C, C125S)	28	<0.05	>0.5
IL-2-IL-15 Chimera 1 (C125S)	42	<0.05	>0.5
IL-2-IL-15 Chimera 5 (E15K, C125S)	46	<0.05	>0.5
IL-2-IL-15 Chimera 11 (H16E, C125S)	52	<0.05	<0.2
IL-2-IL-15 Chimera 15 (H16I, C125S)	56	<0.05	<0.1
IL-2-IL-15 Chimera 17 (H16V, C125S)	58	<0.05	<0.1
IL-2-IL-15 Chimera 19 (D20T, C125S)	60	<0.05	<0.1
IL-2-IL-15 Chimera 21 (D20A, C125S)	62	<0.05	<0.1
IL-2-IL-15 Chimera 23 (D20E, C125S)	64	<0.05	<0.1
IL-2-IL-15 Chimera 25 (D20K, C125S)	66	<0.05	<0.1
IL-2-IL-15 Chimera 27 (N88A, C125S)	68	<0.05	<0.1
IL-2-IL-15 Chimera 29 (I92A, C125S)	70	<0.05	<0.1
IL-2-IL-15 Chimera 29 (I92G, C125S)	72	<0.05	<0.2
IL-2-IL-15 Chimera 29 (I92D, C125S)	74	<0.05	<0.1
IL-2-IL-15 Chimera 29 (I92E, C125S)	76	<0.05	<0.1

Example 4

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

[00373] 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 10^5 cells in PBS (50 μL) are injected subcutaneously into anesthetized C57BL/6 mice using an 18-gauge needle. A stock solution of a fusion protein is diluted in PBS on the day of dosing and the mice are dosed intraperitoneally with PBS (control), a mouse anti-PD-1 (anti-mPD-1) (1 to 20 μg), a corresponding hIL-2 fusion protein (1 to 100 μg), a combination of the anti-mPD-1 (1 to 100 μg) and corresponding hIL-2 fusion protein (1 to 100 μg), or an anti-mPD-1/hIL-2 fusion protein (20 μg) in PBS (100 μL) 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. The hIL-2 fusion protein comprises an hIL-2 domain described herein and an anti-HSA domain, wherein the *C*-terminus of the hIL-2 domain is connected to the *N*-terminus of the anti-HSA domain *via* the peptide linker. The anti-mPD-1/hIL-2 fusion protein is in a knobs-in-holes configuration, comprising the same hIL-2 domain and a peptide linker, wherein the *N*-terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker.

Example 5. Antitumor Activity of Anti-PD-1/IL-2 Fusion Protein in a Xenograft Model using PD-1 Knock-in Mice

[00374] 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 10^5 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 a fusion protein is diluted in PBS on the day of dosing and the mice are dosed intraperitoneally with PBS (control), a human anti-PD-1 (anti-hPD-1) (1 to 20 μg), a corresponding hIL-2 fusion protein (1 to 100 μg), a combination of the anti-hPD-1 (1 to 100 μg) and corresponding hIL-2 fusion protein (1 to 100 μg), or an anti-hPD-1/hIL-2 fusion protein (20 μg) in PBS (100 μL) 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. The hIL-2 fusion protein comprises an hIL-2 domain described herein and an anti-HSA domain, wherein the *C*-terminus of the hIL-2 domain is connected to the *N*-terminus of the anti-HSA domain *via* the peptide linker. The anti-hPD-1/hIL-2 fusion protein is in a knobs-in-holes configuration, comprising the same hIL-2 domain and a peptide linker, wherein the *N*-

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terminus of the hIL-2 domain is connected to the *C*-terminus of the second heavy chain *via* the peptide linker.

Example 6

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

[00375] 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 x 10^6 cells in PBS (100 µL) are injected subcutaneously into anesthetized BALB/c mice using an 18-gauge needle. A stock solution of a fusion protein is diluted in PBS on the day of dosing and the mice are dosed intraperitoneally twice per week for two weeks with (i) PBS (control), an anti-mPD-1 (20 µg), an anti-mPD-1/hIL-2 fusion protein (5 or 20 µg), or an anti-mPD-1/mIL-2 fusion protein (5 or 20 µg). 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 7

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

[00376] HT-29 cells were cultured and maintained in McCoys 5a media supplemented with 10% fetal bovine serum and penicillin/streptomycin. The cells were trypsinized, washed with media, counted, and washed with PBS. The cell suspension (1 x 10^6 cells in PBS (100 µL)) was injected subcutaneously into anesthetized NCG mice using a 27-gauge needle. After 6 days, human PBMCs (1 x 10^7 cells in PBS (100 µL)) were injected into the tail vein of each mouse. A stock solution of a fusion protein was diluted in PBS on the day of dosing and the mice were dosed intraperitoneally twice per week for two weeks. Tumor sizes (length (L) and width (W)) were measured twice per week using a digital caliper, and the tumor volume was calculated (L x W x W)/2. The results are shown in FIG. 4.

Example 8

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

[00377] Anti-PD-1/IL-2 fusion proteins were evaluated for their effect on pSTAT5

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signaling *via* its IL-2 domain in CD3 T-cells. Briefly, CD3 T-cells (100,000) were treated with an anti-PD-1/IL-2 fusion protein for 30 min at 37 °C and 5% CO_2 in Hanks balanced salt solution containing 10 mM HEPES. Phospho-STAT5 was measured using a phosphor-STAT5 (Tyr694) HTRF assay. The signal ratio at 665 nm/620 nm was multiplied by 1,000 and the data was analyzed with global fitting to determine EC_{50} values. The results are summarized in Table 3, where "A" represents an EC_{50} value of no greater than 10 nM, "B" represents an EC_{50} value of greater than 10 nM and no greater than 100 nM, "C" represents an EC_{50} value of greater than 100 nM and no greater than 1,000 nM, and "D" represents an EC_{50} value of greater than 1,000 nM.

Table 3. STAT5 Signaling in $CD3^+$ T-Cells

Fusion Protein	EC_{50} (nM)		
	Mouse T-cell (CTLL2)	Human $CD3^+$ T-cells	Human $CD3^+$ $PD1^+$ T-cells
Anti-hPD-1/hIL-2 **A1**	ND	A	A
Anti-hPD-1/hIL-2 **A2**	ND	C	B
Anti-hPD-1/hIL-2 **A3**	B	C	B
Anti-hPD-1/hIL-2 **A4**	A	B	A
Anti-hPD-1/hIL-2 **A5**	ND	B	ND
Anti-hPD-1/hIL-2 **A6**	ND	A	A
Anti-hPD-1/hIL-2 **A19**	B	B	A
Anti-hPD-1/hIL-2 **A20**	D	D	C
Anti-hPD-1/hIL-2 **A21**	ND	C	N.D.

[00378] Sequences described herein are provided in the sequence table below.

SEQUENCE TABLE

SEQ ID NO:	Description	Amino Acid Sequence
1	hIL-2	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFCQSIISTLT
2	hIL 2 (C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT

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SEQ ID NO:	Description	Amino Acid Sequence
3	hIL-2 (C125A)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFAQSIISTLT
4	hIL-2 (T3A, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
5	hIL-2 (T3A, C125A)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFAQSIISTLT
6	hIL-2 Mutein (R38N, L40T, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TNMTTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
7	hIL-2 Mutein (T3A, R38N, L40T, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTNMTTFKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
8	hIL-2 Mutein (E15K, R38N, L40T, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTNMTTFKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
9	hIL-2 Mutein (T3A, E15K, R38N, L40T, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTNMTTFKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
10	hIL-2 Mutein (E15K, K32E, R38N, L40T, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYENPKL TNMTTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
11	hIL-2 Mutein (T3A, E15K, K32E, R38N, L40T, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYENPK LTNMTTFKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
12	hIL-2 Mutein (E15K, R38N, L40T, K76E, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTNMTTFKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
13	hIL-2 Mutein (T3A, E15K, R38N, L40T, K76E, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTNMTTFKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT

SEQ ID NO:	Description	Amino Acid Sequence
14	hIL-2 Mutein (K32E, R38E, F42A, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYENPKL TEMLTAKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
15	hIL-2 Mutein (T3A, K32E, R38E, F42A, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYENPKL TEMLTAKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
16	hIL-2 Mutein (K32E, R38E, F42K, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYENPKL TEMLTKKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
17	hIL-2 Mutein (T3A, K32E, R38E, F42K, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYENPKL TEMLTKKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
18	hIL-2 Mutein (E15K, K32E, R38E, F42K, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYENPKL TEMLTKKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
19	hIL-2 Mutein (T3A, E15K, K32E, R38E, F42K, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYENPK LTEMLTKKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
20	hIL-2 Mutein (R38E, F42A, K76E, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TEMLTAKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSENFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
21	hIL-2 Mutein (T3A, R38E, F42A, K76E, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTEMLTAKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
22	hIL-2 Mutein (R38E, F42K, K76E, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TEMLTKKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSENFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
23	hIL-2 Mutein (T3A, R38E, F42K, K76E, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTEMLTKKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
24	hIL-2 Mutein (E15K, R38E, F42K, K76E, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTEMLTKKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT

SEQ ID NO:	Description	Amino Acid Sequence
25	hIL-2 Mutein (T3A, E15K, R38E, F42K, K76E, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTEMLTKKFYMPKKATELKHLQCLEEELKPLEEVL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
26	hIL-2 Mutein (F42C, V69C, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLTCKFYMPKKATELKHLQCLEEELKPLEECLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
27	hIL-2 Mutein (T3A, F42C, V69C, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTRMLTCKFYMPKKATELKHLQCLEEELKPLEECL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
28	hIL-2 Mutein (K32E, F42C, V69C, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYENPKL TRMLTCKFYMPKKATELKHLQCLEEELKPLEECLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
29	hIL-2 Mutein (T3A, K32E, F42C, V69C, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYENPKL TRMLTCKFYMPKKATELKHLQCLEEELKPLEECLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
30	hIL-2 Mutein (F42C, V69C, K76E, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKL TRMLTCKFYMPKKATELKHLQCLEEELKPLEECLN LAQSENFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
31	hIL-2 Mutein (T3A, F42C, V69C, K76E, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTRMLTCKFYMPKKATELKHLQCLEEELKPLEECL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
32	hIL-2 Mutein (E15K, F42C, V69C, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTRMLTCKFYMPKKATELKHLQCLEEELKPLEECL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
33	hIL-2 Mutein (T3A, E15K, F42C, V69C, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTRMLTCKFYMPKKATELKHLQCLEEELKPLEECL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
34	hIL-2 Mutein (E15K, K32E, F42C, V69C, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYENPKL TRMLTCKFYMPKKATELKHLQCLEEELKPLEECLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
35	hIL-2 Mutein (T3A, E15K, K32E, F42C, V69C, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYENPK LTRMLTCKFYMPKKATELKHLQCLEEELKPLEECL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT

SEQ ID NO:	Description	Amino Acid Sequence
36	hIL-2 Mutein (E15K, F42C, V69C, K76E, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTRMLTCKFYMPKKATELKHLQCLEEELKPLEECL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
37	hIL-2 Mutein (T3A, E15K, F42C, V69C, K76E, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGINNYKNPK LTRMLTCKFYMPKKATELKHLQCLEEELKPLEECL NLAQSENFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFSQSIISTLT
38	hIL-2 Fragment (N29 to A50)	NNYKNPKLTRMLTFKFYMPKKA
39	hIL-2 Fragment (N29 to L40)	NNYKNPKLTRML
40	IL-15 Hinge Fragment-containing Peptide 1	QSMEIDAT
41	IL-15 Hinge Fragment-containing peptide 2	QSMHIDAT
42	IL-2-IL-15 Chimera 1 (C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
43	IL-2-IL-15 Chimera 2 (T3A, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
44	IL-2-IL-15 Chimera 3 (E32H, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
45	IL-2-IL-15 Chimera 4 (T3A, E32H, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
46	IL-2-IL-15 Chimera 5 (E15K, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
47	IL-2-IL-15 Chimera 6 (T3A, E15K, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
48	IL-2-IL-15 Chimera 7 (E15K, E32H, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET

SEQ ID NO:	Description	Amino Acid Sequence
		ATIVEFLNRWITFSQSIISTLT
49	IL-2-IL-15 Chimera 8 (T3A, E15K, E32H, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
50	IL-2-IL-15 Chimera 9 (E15K, E32H, S87D, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLIDNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
51	IL-2-IL-15 Chimera 10 (T3A, E15K, E32H, S87D, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLIDNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
52	IL-2-IL-15 Chimera 11 (H16E, C125S)	APTSSSTKKTQLQLEELLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
53	IL-2-IL-15 Chimera 12 (T3A, H16E, C125S)	APASSSTKKTQLQLEELLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
54	IL-2-IL-15 Chimera 13 (H16F, C125S)	APTSSSTKKTQLQLEFLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
55	IL-2-IL-15 Chimera 14 (T3A, H16F, C125S)	APASSSTKKTQLQLEFLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
56	IL-2-IL-15 Chimera 15 (H16I, C125S)	APTSSSTKKTQLQLEILLLDLQMILNGIQSMEIDATT FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT
57	IL-2-IL-15 Chimera 16 (T3A, H16I, C125S)	APASSSTKKTQLQLEILLLDLQMILNGIQSMEIDATT FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT
58	IL-2-IL-15 Chimera 17 (H16V, C125S)	APTSSSTKKTQLQLEVLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
59	IL-2-IL-15 Chimera 18 (T3A, H16V, C125S)	APASSSTKKTQLQLEVLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET

SEQ ID NO:	Description	Amino Acid Sequence
		ATIVEFLNRWITFSQSIISTLT
60	IL-2-IL-15 Chimera 19 (D20T, C125S)	APTSSSTKKTQLQLEHLLLTLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
61	IL-2-IL-15 Chimera 20 (T3A, D20T, C125S)	APASSSTKKTQLQLEHLLLTLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
62	IL-2-IL-15 Chimera 21 (D20A, C125S)	APTSSSTKKTQLQLEHLLLALQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
63	IL-2-IL-15 Chimera 22 (T3A, D20A, C125S)	APASSSTKKTQLQLEHLLLALQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
64	IL-2-IL-15 Chimera 23 (D20E, C125S)	APTSSSTKKTQLQLEHLLLELQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
65	IL-2-IL-15 Chimera 24 (T3A, D20E, C125S)	APASSSTKKTQLQLEHLLLELQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
66	IL-2-IL-15 Chimera 25 (D20K, C125S)	APTSSSTKKTQLQLEHLLLKLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
67	IL-2-IL-15 Chimera 26 (T3A, D20K, C125S)	APASSSTKKTQLQLEHLLLKLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
68	IL-2-IL-15 Chimera 27 (N88A, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISAINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
69	IL-2-IL-15 Chimera 28 (T3A, N88A, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISAINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
70	IL-2-IL-15 Chimera 29 (I92A, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVAVLELKGSETTFMCEYADET

SEQ ID NO:	Description	Amino Acid Sequence
		ATIVEFLNRWITFSQSIISTLT
71	IL-2-IL-15 Chimera 30 (T3A, I92A, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVAVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
72	IL-2-IL-15 Chimera 31 (I92G, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVGVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
73	IL-2-IL-15 Chimera 32 (T3A, I92G, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVGVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
74	IL-2-IL-15 Chimera 33 (I92D, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVDVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
75	IL-2-IL-15 Chimera 34 (T3A, I92D, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVDVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
76	IL-2-IL-15 Chimera 35 (I92E, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVEVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
77	IL-2-IL-15 Chimera 36 (T3A, I92E, C125S)	APASSSTKKTQLQLEHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVEVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
78	IL-2-IL-15 Chimera 37 (H16E, I92A, C125S)	APTSSSTKKTQLQLEELLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVAVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
79	IL-2-IL-15 Chimera 38 (T3A, H16E, I92A, C125S)	APASSSTKKTQLQLEELLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVAVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
80	IL-2-IL-15 Chimera 39 (E15K, I92A, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVAVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
81	IL-2-IL-15 Chimera 40 (T3A, E15K, I92A, C125S)	APASSSTKKTQLQLKHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISNINVAVLELKGSETTFMCEYADET

SEQ ID NO:	Description	Amino Acid Sequence
		ATIVEFLNRWITFSQSIISTLT
82	Mouse IL-2-IL-15 Chimera	APTSSSTSSSTAEAQQQQQQQQQQQQHLEQLLMDL QELLSRMQSMEIDATTFKFYLPKQATELKDLQCLE DELGPLRHVLDLTQSKSFQLEDAENFISNIRVTVVK LKGSDNTFECQFDDESATVVDFLRRWIAFSQSIISTS PQ
83	Anti-HSA-1 CDR1	GSTWSINT
84	Anti-HSA-1 CDR2	ISSGGST
85	Anti-HSA-1 CDR3	YAQSTWYPPS
86	Anti-HSA-1 FR1	EVQLVESGGGLVQPGGSLRLSCAAS
87	Anti-HSA-1 FR2	LAWYRQAPGKQRDLVAR
88	Anti-HSA-1 FR3	YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV YYC
89	Anti-HSA-1 FR4	WGQGTLVTVSS
90	Anti-HSA-1	EVQLVESGGGLVQPGGSLRLSCAASGSTWSINTLA WYRQAPGKQRDLVARISSGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCYAQSTWYPP SWGQGTLVTVSS
91	Anti-HSA-2 CDR1	GFAFRGFG
92	Anti-HSA-2 CDR2	INNGGSDT
93	Anti-HSA-2 CDR3	AIGGPGASP
94	Anti-HSA-2 FR1	QVQLVESGGGVVQPGGSLRLSCAAS
95	Anti-HSA-2 FR2	MSWVRQAPGKGLEWVSS
96	Anti-HSA-2 FR4	SGQGTQVTVSS
97	Anti-HSA-2	QVQLVESGGGVVQPGGSLRLSCAASGFAFRGFGM SWVRQAPGKGLEWVSSINNGGSDTYYADSVKGRF TISRDNSKNTLYLQMNSLRAEDTAVYYCAIGGPGA SPSGQGTQVTVSS
98	hIgG1 C_{H1}	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT

SEQ ID NO:	Description	Amino Acid Sequence
		HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
99	hIgG1 C_H Mutein (N297A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
100	hIgG1 C_H Mutein (LALA, N297A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
101	hIgG1 C_H Mutein (S354C, T366W)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQ VSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
102	hIgG1 C_H Mutein (Y349C, T366S, L368A, Y407V)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK

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SEQ ID NO:	Description	Amino Acid Sequence
103	hIgG1 C_H Mutein (S354C, T366W, N297A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQ VSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
104	hIgG1 C_H Mutein (Y349C, T366S, L368A, Y407V, N297A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
105	hIgG1 C_H Mutein (S354C, T366W, LALA, N297A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQ VSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
106	hIgG1 C_H Mutein (Y349C, T366S, L368A, Y407V, LALA, N297A)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVCTLPPSREEMTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
107	hIgG4 C_H	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS

SEQ ID NO:	Description	Amino Acid Sequence
		FFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT QKSLSLSLGK
108	hIgG4 C_H Mutein (T366W)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY TQKSLSLSLGK
109	hIgG4 C_H Mutein (T366S, T368A)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLSC AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLVSRLTVDKSRWQEGNVFSCSVMHEALHNHY TQKSLSLSLGK
110	hKappa C_L	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC
111	Anti-hPD-1-A CDRL1 (IMGT)	QSVSSY
	Anti-hPD-1-A CDRL2 (IMGT)	DAS
112	Anti-hPD-1-A CDRL3 (IMGT)	QQSSNWPRT
113	Anti-hPD-1-A CDRH1 (IMGT)	GITFSNSG
114	Anti-hPD-1-A CDRH2 (IMGT)	IWYDGSKR
115	Anti-hPD-1-A CDRH3 (IMGT)	ATNDDY
116	Anti-hPD-1-A Light Chain Variable	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWY QQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFT LTISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIK
117	Anti-hPD-1-A Heavy Chain	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF

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SEQ ID NO:	Description	Amino Acid Sequence
	Variable	TISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLVTVSS
118	Anti-hPD-1-A Light Chain	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWY QQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFT LTISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
119	Anti-hPD-1-A Heavy Chain	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF TISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSC SVMHEALHNHYTQKSLSLSLGK
120	Anti-hPD-1-B CDRL1 (IMGT)	KGVSTSGYSY
	Anti-hPD-1-B CDRL2 (IMGT)	LAS
121	Anti-hPD-1-B CDRL3 (IMGT)	QHSRDLPLT
122	Anti-hPD-1-B CDRH1 (IMGT)	GYTFTNYY
123	Anti-hPD-1-B CDRH2 (IMGT)	INPSNGGT
124	Anti-hPD-1-B CDRH3 (IMGT)	ARRDYRFDMGFDY
125	Anti-hPD-1-B Light Chain Variable	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYL HWYQQKPGQAPRLLIYLASYLESGVPARFSGSGSG TDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKV EIK
126	Anti-hPD-1-B Heavy Chain Variable	QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYM YWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNR VTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDY RFDMGFDYWGQGTTVTVSS
127	Anti-hPD-1-B Light Chain	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYL HWYQQKPGQAPRLLIYLASYLESGVPARFSGSGSG

SEQ ID NO:	Description	Amino Acid Sequence
		TDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKV EIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC
128	Anti-hPD-1-B Heavy Chain	QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYM YWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNR VTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDY RFDMGFDYWGQGTTVTVSSASTKGPSVFPLAPCSR STSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH KPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMHEALHNHYTQKSLSLSLGK
129	Anti-hPD-1-C CDRL1 (IMGT)	QSIVHSNGNTY
	Anti-hPD-1-C CDRL2 (IMGT)	KVS
130	Anti-hPD-1-C CDRL3 (IMGT)	FQGSHVPLT
131	Anti-hPD-1-C CDRH1 (IMGT)	GYTFTDYE
132	Anti-hPD-1-C CDRH2 (IMGT)	IESETGGT
133	Anti-hPD-1-C CDRH3 (IMGT)	AREGITTVATTYYWYFDV
134	Anti-hPD-1-C Light Chain Variable	DVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNT YLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSG SGTDFTLKISRVEAEDVGVYYCFQGSHVPLTFGQG TKLEIK
135	Anti-hPD-1-C Heavy Chain Variable	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSS
136	Anti-hPD-1-C Light Chain (Kappa)	DVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNT YLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSG SGTDFTLKISRVEAEDVGVYYCFQGSHVPLTFGQG TKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT

SEQ ID NO:	Description	Amino Acid Sequence
		KSFNRGEC
137	Anti-hPD-1-C Heavy Chain	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
138	Anti-hPD-1-D CDRL1 (IMGT)	QTIGTW
	Anti-hPD-1-D CDRL2 (IMGT)	TAT
139	Anti-hPD-1-D CDRL3 (IMGT)	QQVYSIPWT
140	Anti-hPD-1-D CDRH1 (IMGT)	GFTFSSYM
141	Anti-hPD-1-D CDRH2 (IMGT)	ISGGGANT
142	Anti-hPD-1-D CDRH3 (IMGT)	ARQLYYFDY
143	Anti-hPD-1-D Light Chain Variable	DIQMTQSPSSLSASVGDRVTITCLASQTIGTWLTWY QQKPGKAPKLLIYTATSLADGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQVYSIPWTFGGGTKVEIK
144	Anti-hPD-1-D Heavy Chain Variable	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYMMS WVRQAPGKGLEWVATISGGGANTYYPDSVKGRFT ISRDNAKNSLYLQMNSLRAEDTAVYYCARQLYYF DYWGQGTTVTVSS
145	Anti-hPD-1-D Light Chain	DIQMTQSPSSLSASVGDRVTITCLASQTIGTWLTWY QQKPGKAPKLLIYTATSLADGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQVYSIPWTFGGGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
146	Anti-hPD-1-D Heavy Chain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYMMS WVRQAPGKGLEWVATISGGGANTYYPDSVKGRFT ISRDNAKNSLYLQMNSLRAEDTAVYYCARQLYYF

SEQ ID NO:	Description	Amino Acid Sequence
		DYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV DKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEV HNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLSLGK
147	Anti-hPD-1-E CDRL1 (IMGT)	QGISSW
	Anti-hPD-1-E CDRL2 (IMGT)	AAS
148	Anti-hPD-1-E CDRL3 (IMGT)	QQANHLPFT
149	Anti-hPD-1-E CDRH1 (IMGT)	GGTFSSYA
150	Anti-hPD-1-E CDRH2 (IMGT)	IIPMFDTA
151	Anti-hPD-1-E CDRH3 (IMGT)	ARAEHSSTGTFDY
152	Anti-hPD-1-E Light Chain Variable	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAW YQQKPGKAPKLLISAASSLQSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQANHLPFTFGGGTKVEIK
153	Anti-hPD-1-E Heavy Chain Variable	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIS WVRQAPGQGLEWMGLIIPMFDTAGYAQKFQGRV AITVDESTSTAYMELSSLRSEDTAVYYCARAEHSST GTFDYWGQGTLVTVSS
154	Anti-hPD-1-E Light Chain	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAW YQQKPGKAPKLLISAASSLQSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQANHLPFTFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC
155	Anti-hPD-1-E Heavy Chain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIS WVRQAPGQGLEWMGLIIPMFDTAGYAQKFQGRV AITVDESTSTAYMELSSLRSEDTAVYYCARAEHSST GTFDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSE STAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSN TKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG

SEQ ID NO:	Description	Amino Acid Sequence
		VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN VFSCSVMHEALHNHYTQKSLSLSLGK
156	Anti-hPD-1-F CDRL1 (IMGT)	QSLLDSGNQKNF
	Anti-hPD-1-F CDRL2 (IMGT)	WAS
157	Anti-hPD-1-F CDRL3 (IMGT)	QNDYSYPYT
158	Anti-hPD-1-F CDRH1 (IMGT)	GYTFTTYW
159	Anti-hPD-1-F CDRH2 (IMGT)	IYPGTGGS
160	Anti-hPD-1-F CDRH3 (IMGT)	TRWTTGTGAY
161	Anti-hPD-1-F Light Chain Variable	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQK NFLTWYQQKPGQAPRLLIYWASTRESGVPSRFSGS GSGTDFTETISSLEAEDAATYYCQNDYSYPYTFGQ GTKVEIK
162	Anti-hPD-1-F Heavy Chain Variable	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMH WVRQATGQGLEWMGNIYPGTGGSNFDEKEKNRV TITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGT GAYWGQGTTVTVSS
163	Anti-hPD-1-F Light Chain	EIVLTQSPATLSLSPGERATLSCKSSQSLLDSGNQK NFLTWYQQKPGQAPRLLIYWASTRESGVPSRFSGS GSGTDFTETISSLEAEDAATYYCQNDYSYPYTFGQ GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC
164	Anti-hPD-1-F Heavy Chain	EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWMH WVRQATGQGLEWMGNIYPGTGGSNFDEKEKNRV TITADKSTSTAYMELSSLRSEDTAVYYCTRWTTGT GAYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF

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SEQ ID NO:	Description	Amino Acid Sequence
		SCSVMHEALHNHYTQKSLSLSLQG
165	Anti-hPD-1-G CDRL1 (IMGT)	ESVSND
	Anti-hPD-1-G CDRL2 (IMGT)	YAF
166	Anti-hPD-1-G CDRL3 (IMGT)	HQAYSSPYT
167	Anti-hPD-1-G CDRH1 (IMGT)	GFSLTSYG
168	Anti-hPD-1-G CDRH2 (IMGT)	IYADGST
169	Anti-hPD-1-G CDRH3 (IMGT)	ARAYGNYWYIDV
170	Anti-hPD-1-G Light Chain Variable	DIVMTQSPDSLAVSLGERATINCKSSESVSNDVAW YQQKPGQPPKLLINYAFHRFTGVPDRFSGSGYGTD FTLTISSLQAEDVAVYYCHQAYSSPYTFGQGTKLEI K
171	Anti-hPD-1-G Heavy Chain Variable	QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVH WIRQPPGKGLEWIGVIYADGSTNYNPSLKSRVTISK DTSKNQVSLKLSSVTAADTAVYYCARAYGNYWYI DVWGQGTTVTVSS
172	Anti-hPD-1-G Light Chain	DIVMTQSPDSLAVSLGERATINCKSSESVSNDVAW YQQKPGQPPKLLINYAFHRFTGVPDRFSGSGYGTD FTLTISSLQAEDVAVYYCHQAYSSPYTFGQGTKLEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC
173	Anti-hPD-1-G Heavy Chain	QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVH WIRQPPGKGLEWIGVIYADGSTNYNPSLKSRVTISK DTSKNQVSLKLSSVTAADTAVYYCARAYGNYWYI DVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV DKRVESKYGPPCPPCPAPPVAGGPSVFLFPPKPKDT LMISRTPEVTCVVVAVSQEDPEVQENWYVDGVEV HNAKTKPREEQENSTYRVVSVLTVVHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLSLGIK

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SEQ ID NO:	Description	Amino Acid Sequence
174	Anti-hPD-1-H CDRL1 (IMGT)	LSINTF
	Anti-hPD-1-H CDRL2 (IMGT)	AAS
175	Anti-hPD-1-H CDRL3 (IMGT)	QQSSNTPFT
176	Anti-hPD-1-H CDRH1 (IMGT)	GFTFSNFG
177	Anti-hPD-1-H CDRH2 (IMGT)	ISGGGRDT
178	Anti-hPD-1-H CDRH3 (IMGT)	VKWGNIYFDY
179	Anti-hPD-1-H Light Chain Variable	DIQMTQSPSSLSASVGDSITITCRASLSINTFLNWYQ QKPGKAPNLLIYAASSLHGGVPSRFSGSGSGTDFTL TIRTLQPEDFATYYCQQSSNTPFTFGPGTVVDFR
180	Anti-hPD-1-H Heavy Chain Variable	EVQLLESGGVLVQPGGSLRLSCAASGFTFSNFGMT WVRQAPGKGLEWVSGISGGGRDTYFADSVKGRFT ISRDNSKNTLYLQMNSLKGEDTAVYYCVKWGNIY FDYWGQGTLVTVSS
181	Anti-hPD-1-H Light Chain	DIQMTQSPSSLSASVGDSITITCRASLSINTFLNWYQ QKPGKAPNLLIYAASSLHGGVPSRFSGSGSGTDFTL TIRTLQPEDFATYYCQQSSNTPFTFGPGTVVDFRRT VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
182	Anti-hPD-1-H Heavy Chain	EVQLLESGGVLVQPGGSLRLSCAASGFTFSNFGMT WVRQAPGKGLEWVSGISGGGRDTYFADSVKGRFT ISRDNSKNTLYLQMNSLKGEDTAVYYCVKWGNIY FDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF SCSVMHEALHNHYTQKSLSLSLGK
183	Anti-hPD-1-I CDRL1 (IMGT)	QDVTTA
	Anti-hPD-1-I CDRL2 (IMGT)	WAS

SEQ ID NO:	Description	Amino Acid Sequence
184	Anti-hPD-1-I CDRL3 (IMGT)	QQHYTIPWT
185	Anti-hPD-1-I CDRH1 (IMGT)	GFTFSNYG
186	Anti-hPD-1-I CDRH2 (IMGT)	ISGGGSNI
187	Anti-hPD-1-I CDRH3 (IMGT)	VSYYYGIDF
188	Anti-hPD-1-I Light Chain Variable	DIQMTQSPSSLSASVGDRVTITCKASQDVTTAVAW YQQKPGKAPKLLIYWASTRHTGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQHYTIPWTFGGGTKLEI K
189	Anti-hPD-1-I Heavy Chain Variable	QVQLVESGGGLVKPGGSLRLSCAASGFTFSNYGMS WIRQAPGKGLEWVSTISGGGSNIYYADSVKGRFTIS RDNAKNSLYLQMNSLRAEDTAVYYCVSYYYGIDF WGQGTSVTVSS
190	Anti-hPD-1-I Light Chain	DIQMTQSPSSLSASVGDRVTITCKASQDVTTAVAW YQQKPGKAPKLLIYWASTRHTGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQHYTIPWTFGGGTKLEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC
191	Anti-hPD-1-I Heavy Chain	QVQLVESGGGLVKPGGSLRLSCAASGFTFSNYGMS WIRQAPGKGLEWVSTISGGGSNIYYADSVKGRFTIS RDNAKNSLYLQMNSLRAEDTAVYYCVSYYYGIDF WGQGTSVTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSC SVMHEALHNHYTQKSLSLSLGK
192	Anti-hPD-1-J CDRL1 (IMGT)	ESVDNYGMSF
	Anti-hPD-1-J CDRL2 (IMGT)	AAS
193	Anti-hPD-1-J CDRL3 (IMGT)	QQSKEVPYT

SEQ ID NO:	Description	Amino Acid Sequence
194	Anti-hPD-1-J CDRH1 (IMGT)	GYSFTSYW
195	Anti-hPD-1-J CDRH2 (IMGT)	IHPSDSET
196	Anti-hPD-1-J CDRH3 (IMGT)	AREHYGTSPFAY
197	Anti-hPD-1-J Light Chain Variable	EIVLTQSPATLSLSPGERATLSCRASESVDNYGMSF MNWFQQKPGQPPKLLIHAASNQGSGVPSRFSGSGS GTDFTLTISSLEPEDFAVYFCQQSKEVPYTFGGGTK VEIK
198	Anti-hPD-1-J Heavy Chain Variable	QVQLVQSGAEVKKPGASVKVSCKASGYSFTSYWM NWVRQAPGQGLEWIGVIHPSDSETWLDQKFKDRV TITVDKSTSTAYMELSSLRSEDTAVYYCAREHYGT SPFAYWGQGTLVTVSS
199	Anti-hPD-1-J Light Chain	EIVLTQSPATLSLSPGERATLSCRASESVDNYGMSF MNWFQQKPGQPPKLLIHAASNQGSGVPSRFSGSGS GTDFTLTISSLEPEDFAVYFCQQSKEVPYTFGGGTK VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS FNRGEC
200	Anti-hPD-1-J Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYSFTSYWM NWVRQAPGQGLEWIGVIHPSDSETWLDQKFKDRV TITVDKSTSTAYMELSSLRSEDTAVYYCAREHYGT SPFAYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSE STAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSN TKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN VFSCSVMHEALHNHYTQKSLSLSLG
201	Anti-hPD-1-A-1 Heavy Chain	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF TISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLVSRLTVDKSRWQEGNVFSC

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SEQ ID NO:	Description	Amino Acid Sequence
		SVMHEALHNHYTQKSLSLSLGK
202	Anti-hPD-1-hIL-2 (SEQ ID NO: 80) Heavy Chain **A18**	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVCTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGAGGG GSGGGGSGGGGSAPTSSSTKKTQLQLKHLLLDLQM ILNGIQSMEIDATTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISNINVAVLELKGS ETTFMCEYADETATIVEFLNRWITFSQSIISTLT
203	Anti-hPD-1-hIL-2 (SEQ ID NO: 70) Heavy Chain **A19**	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF TISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLSLGKGGGGSGGGGSG GGGSAPTSSSTKKTQLQLEHLLLDLQMILNGIQSME IDATTFKFYMPKKATELKHLQCLEEELKPLEEVLNL AQSKNFHLRPRDLISNINVAVLELKGSETTFMCEYA DETATIVEFLNRWITFSQSIISTLT
204	Anti-hPD-1-hIL-2 (SEQ ID NO: 256) Heavy Chain **A20**	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF TISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLSLGKGGGGSGGGGSG

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SEQ ID NO:	Description	Amino Acid Sequence
		GGGSAPTSSSTKKTQLQLKHLLLDLQMILNGIQSM EIDATTFKFYMPKKATELKHLQCLEEELKPLEEVLN LAQSKNFHLRPRDLISAINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFSQSIISTLT
205	Anti-hPD-1-hIL-2 (SEQ ID NO: 256) Heavy Chain **A21**	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMH WVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRF TISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDY WGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSC SVMHEALHNHYTQKSLSLSLGKGGGGSGGGGSGG GGSAPTSSSTKKTQLQLKHLLLDLQMILNGIQSMEI DATTFKFYMPKKATELKHLQCLEEELKPLEEVLNL AQSKNFHLRPRDLISAINVIVLELKGSETTFMCEYA DETATIVEFLNRWITFSQSIISTLT
	GSG Linker	GSG
206	$(GSG)_2$ Linker	GSGGSG
207	$(GSG)_3$ Linker	GSGGSGGSG
208	$(GSG)_4$ Linker	GSGGSGGSGGSG
209	G3S Linker	GGGS
210	$(G3S)_2$ Linker	GGGSGGGS
211	$(G3S)_3$ Linker	GGGSGGGSGGGS
212	$(G3S)_4$ Linker	GGGSGGGSGGGSGGGS
213	G4S Linker	GGGGS
214	$(G4S)_2$ Linker	GGGGSGGGGS
215	$(G4S)_3$ Linker	GGGGSGGGGSGGGGS
216	$(G4S)_4$ Linker	GGGGSGGGGSGGGGSGGGGS

SEQ ID NO:	Description	Amino Acid Sequence
217	SGSG Linker	SGSG
218	$S(GSG)_2$ Linker	SGSGGSG
219	$S(GSG)_3$ Linker	SGSGGSGGSG
220	$S(GSG)_4$ Linker	SGSGGSGGSGGSG
221	SG3S Linker	SGGGS
222	$S(G3S)_2$ Linker	SGGGSGGGS
223	$S(G3S)_3$ Linker	SGGGSGGGSGGGS
224	$S(G3S)_4$ Linker	SGGGSGGGSGGGSGGGS
225	SG4S Linker	SGGGGS
226	$S(G4S)_2$ Linker	SGGGGSGGGGS
227	$S(G4S)_3$ Linker	SGGGGSGGGGSGGGGS
228	$S(G4S)_4$ Linker	SGGGGSGGGGSGGGGSGGGGS
229	EAAAK Linker	EAAAK
230	$(EAAAK)_2$ Linker	EAAAKEAAAK
231	$(EAAAK)_3$ Linker	EAAAKEAAAKEAAAK
232	$(EAAAK)_4$ Linker	EAAAKEAAAKEAAAKEAAAK
233	PAPAP Linker	PAPAP
234	$(PAPAP)_2$ Linker	PAPAPPAPAP
235	$(PAPAP)_3$ Linker	PAPAPPAPAPPAPAP
236	$(PAPAP)_4$ Linker	PAPAPPAPAPPAPAPPAPAP
237	VLVH Linker	IKRTVAAP

SEQ ID NO:	Description	Amino Acid Sequence
238	RAKPS Linker	RAKPS
239	$(RAKPS)_2$ Linker	RAKPSRAKPS
240	ASTKG Linker	ASTKG
241	$(ASTKG)_2$ Linker	ASTKGASTKG
242	AKTHT Linker	AKTHT
243	$(AKTHT)_2$ Linker	AKTHTAKTHT
244	$ASTKG(G4S)_2$ Linker	ASTKGGGGGSGGGGS
245	$RAKPS(G4S)_2$ Linker	RAKPSGGGGSGGGGS
246	hIL-2-anti-HSA **C1**	APTSSSTKKTQLQLKHLLLDLQMILNGIQSMHIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLIDNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLTGSGGSGGSGGSGEVQ LVESGGGLVQPGGSLRLSCAASGSTWSINTLAWYR QAPGKQRDLVARISSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCYAQSTWYPPSWG QGTLVTVSS
247	Anti-hPD-1 Heavy Chain (IgG1, N297A)	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
248	Anti-hPD-1 Heavy Chain (lgG1, N297A, Last K to A)	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV

SEQ ID NO:	Description	Amino Acid Sequence
		LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGA
249	Anti-hPD-1-hIL-2 Heavy Chain **A1** (IgG1, N297A, Last K to A)	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGAG GGGSGGGGSGGGGSAPTSSSTKKTQLQLEHLLLDL QMILNGIQSMEIDATTFKFYMPKKATELKHLQCLE EELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELK GSETTFMCEYADETATIVEFLNRWITFSQSIISTLT
250	Anti-hPD-1-hIL-2 Heavy Chain **A2** (IgG1, N297A, Last K to A)	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGAG GGGSGGGGSGGGGSAPTSSSTKKTQLQLEELLLDL QMILNGIQSMEIDATTFKFYMPKKATELKHLQCLE EELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELK GSETTFMCEYADETATIVEFLNRWITFSQSIISTLT
251	Anti-hPD-1-hIL-2 (SEQ ID NO: 68) Heavy Chain **A3** (IgG1, N297A, Last K to A)	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV

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SEQ ID NO:	Description	Amino Acid Sequence
		LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGAG GGGSGGGGSGGGGSAPTSSSTKKTQLQLEHLLLDL QMILNGIQSMEIDATTFKFYMPKKATELKHLQCLE EELKPLEEVLNLAQSKNFHLRPRDLISAINVIVLELK GSETTFMCEYADETATIVEFLNRWITFSQSIISTLT
252	Anti-hPD-1-hIL-2 (SEQ ID NO: 80) Heavy Chain **A4** (IgG1, N297A, Last K to A)	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGAG GGGSGGGGSGGGGSAPTSSSTKKTQLQLKHLLLDL QMILNGIQSMEIDATTFKFYMPKKATELKHLQCLE EELKPLEEVLNLAQSKNFHLRPRDLISNINVAVLEL KGSETTFMCEYADETATIVEFLNRWITFSQSIISTLT
253	Anti-hPD-1-hIL-2 (SEQ ID NO: 70) Heavy Chain **A5** (IgG1, N297A, Last K to A)	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGAG GGGSGGGGSGGGGSAPTSSSTKKTQLQLEHLLLDL QMILNGIQSMEIDATTFKFYMPKKATELKHLQCLE EELKPLEEVLNLAQSKNFHLRPRDLISNINVAVLEL KGSETTFMCEYADETATIVEFLNRWITFSQSIISTLT
254	Anti-hPD-1-hIL-2 Heavy Chain **A6** (IgG1, N297A, Last K to A)	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV

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SEQ ID NO:	Description	Amino Acid Sequence
		HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGAG GGGSGGGGSGGGGSAPTSSSTKKTQLQLKHLLLDL QMILNGIQSMHIDATTFKFYMPKKATELKHLQCLE EELKPLEEVLNLAQSKNFHLRPRDLIDNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFSQSIISTLT
255	IL-2-IL-15 Chimera 41 (E15K, H16I, C125S)	APTSSSTKKTQLQLKILLLDLQMILNGIQSMEIDATT FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT
256	IL-2-IL-15 Chimera 42 (E15K, N88A, C125S)	APTSSSTKKTQLQLKHLLLDLQMILNGIQSMEIDAT TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQS KNFHLRPRDLISAINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFSQSIISTLT
257	Anti-hPD-1 Heavy Chain 1 (hIgG4)	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVYTLPPSQEEMTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS RWQEGNVFSCSVMHEALHNHYTQKSLSLSLGA
258	Anti-hPD-1-hIL-2 (SEQ ID NO: 56) Heavy Chain **A7**	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVYTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGAGGG

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SEQ ID NO:	Description	Amino Acid Sequence
		GSGGGGSGGGGSAPTSSSTKKTQLQLEILLLDLQMI LNGIQSMEIDATTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
259	Anti-hPD-1-hIL-2 (SEQ ID NO: 255) Heavy Chain **A8**	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVYTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGAGGG GSGGGGSGGGGSAPTSSSTKKTQLQLKILLLDLQMI LNGIQSMEIDATTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
260	Anti-hPD-1-hIL-2 (SEQ ID NO: 68) Heavy Chain **A9**	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVYTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGAGGG GSGGGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQM ILNGIQSMEIDATTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISAINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
261	Anti-hPD-1-hIL-2 (SEQ ID NO: 256) Heavy Chain **A10**	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ

SEQ ID NO:	Description	Amino Acid Sequence
		DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVYTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGAGGG GSGGGGSGGGGSAPTSSSTKKTQLQLKHLLLDLQM ILNGIQSMEIDATTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISAINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
262	Anti-hPD-1-hIL-2 (SEQ ID NO: 70) Heavy Chain **A11**	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVYTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGAGGG GSGGGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQM ILNGIQSMEIDATTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISNINVAVLELKGS ETTFMCEYADETATIVEFLNRWITFSQSIISTLT
263	Anti-hPD-1-hIL-2 (SEQ ID NO: 80) Heavy Chain **A12**	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVYTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGAGGG GSGGGGSGGGGSAPTSSSTKKTQLQLKHLLLDLQM ILNGIQSMEIDATTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISNINVAVLELKGS ETTFMCEYADETATIVEFLNRWITFSQSIISTLT
264	Anti-hPD-1 Heavy Chain 2 (hIgG4)	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV

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SEQ ID NO:	Description	Amino Acid Sequence
		HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVYTLPPCQEEMTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS RWQEGNVFSCSVMHEALHNHYTQKSLSLSLGA
265	Anti-hPD-1-hIL-2 (SEQ ID NO: 56) Heavy Chain **A13**	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVCTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGAGGG GSGGGGSGGGGSAPTSSSTKKTQLQLEILLLDLQMI LNGIQSMEIDATTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
266	Anti-hPD-1-hIL-2 (SEQ ID NO: 255) Heavy Chain **A14**	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVCTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGAGGG GSGGGGSGGGGSAPTSSSTKKTQLQLKILLLDLQMI LNGIQSMEIDATTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
267	Anti-hPD-1-hIL-2 (SEQ ID NO: 68) Heavy Chain **A15**	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV

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SEQ ID NO:	Description	Amino Acid Sequence
		HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVCTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGAGGG GSGGGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQM ILNGIQSMEIDATTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISAINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
268	Anti-hPD-1-hIL-2 (SEQ ID NO: 256) Heavy Chain **A16**	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVCTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGAGGG GSGGGGSGGGGSAPTSSSTKKTQLQLKHLLLDLQM ILNGIQSMEIDATTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISAINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFSQSIISTLT
269	Anti-hPD-1-hIL-2 (SEQ ID NO: 70) Heavy Chain **A17**	QGQLVQSGAEVKKPGASVKVSCKASGYTFTDYEM HWVRQAPIHGLEWIGVIESETGGTAYNQKFKGRVT ITADKSTSTAYMELSSLRSEDTAVYYCAREGITTVA TTYYWYFDVWGQGTTVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVCTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGAGGG GSGGGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQM ILNGIQSMEIDATTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISNINVAVLELKGS ETTFMCEYADETATIVEFLNRWITFSQSIISTLT

[00379] 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, a programmed cell death-1 (PD-1) binding domain, and a half-life-extension domain; wherein the IL-2 domain comprises: (i) a replacement of the amino acid residues from positions N29 to A50 with an IL-15 hinge fragment-containing peptide; (ii) an amino acid substitution at position E15, H16, D20, K32, K76, S87, N88, or I92; (iii) an amino acid substitution at a position from R38 to Y45; and/or (iv) a disulfide bond formed between two amino acid residues from positions N30 to L80.

2. The fusion protein of claim 1, wherein the IL-2 domain comprises a replacement of the amino acid residues from positions N29 to A50 with an IL-15 hinge fragment-containing peptide, and optionally an amino acid substitution at position E15, H16, D20, S87, N88, or I92.

3. The fusion protein of claim 1 or 2, wherein the IL-2 domain comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41, and optionally an amino acid substitution of E15K, H16E, H16I, H16V, D20A, D20E, D20K, D20T, S87D, N88A, I92A, I92D, I92E, or I92G.

4. The fusion protein of any one of claims 1 to 3, wherein the IL-2 domain comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41, and optionally an amino acid substitution of E15K.

5. The fusion protein of any one of claims 1 to 4, wherein the IL-2 domain comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41, and optionally an amino acid substitution of H16E, H16F, H16I, or H16V.

6. The fusion protein of any one of claims 1 to 5, wherein the IL-2 domain comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an

amino acid sequence of SEQ ID NO: 40 or 41, and optionally an amino acid substitution of D20A, D20E, D20K, or D20T.

7. The fusion protein of any one of claims 1 to 6, wherein the IL-2 domain comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41, and optionally an amino acid substitution of S87D.

8. The fusion protein of any one of claims 1 to 7, wherein the IL-2 domain comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41, and optionally an amino acid substitution of N88A.

9. The fusion protein of any one of claims 1 to 8, wherein the IL-2 domain comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41, and optionally an amino acid substitution of I92A, I92D, I92E, or I92G.

10. The fusion protein of any one of claims 1 to 9, wherein the IL-2 domain comprises a replacement of the amino acid residues from positions N29 to L40 having an amino acid sequence of SEQ ID NO: 39 with an IL-15 hinge fragment-containing peptide having an amino acid sequence of SEQ ID NO: 40 or 41, and one or two amino acid substitutions of E15K, H16I, D20T, N88A, or I92A.

11. The fusion protein of any one of claims 1 to 10, wherein the IL-2 domain comprises an amino acid substitution at position E15, H16, D20, K32, R38, L40, F42, K76, S87, N88, or I92.

12. The fusion protein of any one of claims 1 to 11, wherein the IL-2 domain comprises an amino acid substitution at position E15, D20, H16, N88, or I92.

13. The fusion protein of any one of claims 1 to 12, wherein the IL-2 domain comprises an amino acid substitution at position E15.

14. The fusion protein of any one of claims 1 to 12, wherein the IL-2 domain comprises an amino acid substitution of E15K.

15. The fusion protein of any one of claims 1 to 14, wherein the IL-2 domain comprises an amino acid substitution at position H16.

16. The fusion protein of any one of claims 1 to 15, wherein the IL-2 domain comprises an amino acid substitution of H16E, H16I, or H16V.

17. The fusion protein of any one of claims 1 to 16, wherein the IL-2 domain comprises an amino acid substitution at position D20.

18. The fusion protein of any one of claims 1 to 17, wherein the IL-2 domain comprises an amino acid substitution of D20A, D20E, D20K, or D20T.

19. The fusion protein of any one of claims 1 to 18, wherein the IL-2 domain comprises an amino acid substitution at position K32.

20. The fusion protein of any one of claims 1 to 19, wherein the IL-2 domain comprises an amino acid substitution of K32E.

21. The fusion protein of any one of claims 1 to 20, wherein the IL-2 domain comprises an amino acid substitution at position R38.

22. The fusion protein of any one of claims 1 to 21, wherein the IL-2 domain comprises an amino acid substitution of R38E or R38N.

23. The fusion protein of any one of claims 1 to 22, wherein the IL-2 domain comprises an amino acid substitution at position L40.

24. The fusion protein of any one of claims 1 to 23, wherein the IL-2 domain comprises an amino acid substitution of L40T.

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25. The fusion protein of any one of claims 1 to 24, wherein the IL-2 domain comprises an amino acid substitution at position F42.

26. The fusion protein of any one of claims 1 to 25, wherein the IL-2 domain comprises an amino acid substitution of F42A or F42K.

27. The fusion protein of any one of claims 1 to 26, wherein the IL-2 domain comprises an amino acid substitution at position K76.

28. The fusion protein of any one of claims 1 to 27, wherein the IL-2 domain comprises an amino acid substitution of K76E.

29. The fusion protein of any one of claims 1 to 28, wherein the IL-2 domain comprises an amino acid substitution at position S87.

30. The fusion protein of any one of claims 1 to 29, wherein the IL-2 domain comprises an amino acid substitution of S87D.

31. The fusion protein of any one of claims 1 to 30, wherein the IL-2 domain comprises an amino acid substitution at position N88.

32. The fusion protein of any one of claims 1 to 31, wherein the IL-2 domain comprises an amino acid substitution of N88A.

33. The fusion protein of any one of claims 1 to 32, wherein the IL-2 domain comprises an amino acid substitution at position I92.

34. The fusion protein of any one of claims 1 to 33, wherein the IL-2 domain comprises an amino acid substitution of I92A, I92D, I92E, or I92G.

35. The fusion protein of any one of claims 1 to 34, wherein the IL-2 domain comprises amino acid substitutions at positions R38 and L40 as set forth in SEQ ID NO: 1, 2, 3, 4, or 5.

36. The fusion protein of any one of claims 1 to 35, wherein the IL-2 domain comprises amino acid substitutions of R38N and L40T.

37. The fusion protein of any one of claims 1 to 36, wherein the IL-2 domain comprises amino acid substitutions at positions E15, R38, and L40.

38. The fusion protein of any one of claims 1 to 37, wherein the IL-2 domain comprises amino acid substitutions of E15K, R38N, and L40T.

39. The fusion protein of any one of claims 1 to 38, wherein the IL-2 domain comprises amino acid substitutions at positions E15, K32, R38, and L40.

40. The fusion protein of any one of claims 1 to 39, wherein the IL-2 domain comprises amino acid substitutions of E15K, K32E, R38N, and L40T.

41. The fusion protein of any one of claims 1 to 38, wherein the IL-2 domain comprises amino acid substitutions at positions E15, R38, L40, and K76.

42. The fusion protein of any one of claims 1 to 39 and 41, wherein the IL-2 domain comprises amino acid substitutions of E15K, R38N, L40T, and K76E.

43. The fusion protein of any one of claims 36 to 42, wherein the IL-2 domain is *N*-glycosylated.

44. The fusion protein of any one of claims 1 to 34, wherein the IL-2 domain comprises amino acid substitutions at positions R38 and F42.

45. The fusion protein of any one of claims 1 to 34, wherein the IL-2 domain comprises amino acid substitutions of R38E and F42A or F42K.

46. The fusion protein of any one of claims 1 to 34, wherein the IL-2 domain comprises amino acid substitutions at positions K32, R38, and F42.

47. The fusion protein of any one of claims 1 to 34, wherein the IL-2 domain comprises amino acid substitutions of K32E, R38E, and F42A or F42K.

48. The fusion protein of any one of claims 1 to 34, wherein the IL-2 domain comprises amino acid substitutions at positions E15, K32, R38, and F42.

49. The fusion protein of any one of claims 1 to 34, wherein the IL-2 domain comprises amino acid substitutions of E15K, K32E, R38E, and F42A or F42K.

50. The fusion protein of any one of claims 1 to 34, wherein the IL-2 domain comprises amino acid substitutions at positions R38, F42, and K76.

51. The fusion protein of any one of claims 1 to 34, wherein the IL-2 domain comprises amino acid substitutions of R38E, F42A or F42K, and K76E.

52. The fusion protein of any one of claims 1 to 34, wherein the IL-2 domain comprises amino acid substitutions at positions E15, R38, F42, and K76.

53. The fusion protein of any one of claims 1 to 34, wherein the IL-2 domain comprises amino acid substitutions of E15K, R38E, F42A or F42K, and K76E.

54. The fusion protein of any one of claims 1 to 53, wherein the interleukin-2 domain comprises a disulfide bond formed between two different amino acid residues from positions N30 to L80.

55. The fusion protein of any one of claims 1 to 54, wherein the interleukin-2 domain comprises a disulfide bond formed between two different amino acid residues, each independently at K35, R38, F42, Y45, E62, V69, or L72.

56. The fusion protein of any one of claims 1 to 55, wherein the interleukin-2 domain comprises a disulfide bond formed between K35C and L72C, R38C and L72C, F42C and V69C, Y42C and L72C, or Y45C and E62C.

57. The fusion protein of any one of claims 1 to 56, wherein the interleukin-2 domain comprises a disulfide bond formed between F42C and V69C.

58. The fusion protein of any one of claims 1 to 57, wherein the interleukin-2 domain comprises a disulfide bond formed between F42C and V69C and an amino acid substitution of E15K, K32E, or K76E.

59. The fusion protein of claim 58, wherein the interleukin-2 domain comprises a

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disulfide bond formed between F42C and V69C and an amino acid substitution of E15K.

60. The fusion protein of claim 58, wherein the interleukin-2 domain comprises a disulfide bond formed between F42C and V69C and an amino acid substitution of K32E.

61. The fusion protein of claim 58, wherein the interleukin-2 domain comprises a disulfide bond formed between F42C and V69C and an amino acid substitution of K76E.

62. The fusion protein of claim 58, wherein the interleukin-2 domain comprises a disulfide bond formed between F42C and V69C and amino acid substitutions of E15K and K32E.

63. The fusion protein of claim 58, wherein the interleukin-2 domain comprises a disulfide bond formed between F42C and V69C and amino acid substitutions of E15K and K76E.

64. The fusion protein of claim 1, wherein the IL-2 domain comprises an amino acid sequence of any one of SEQ ID NOs: 6 to 37, 42 to 81, 255, and 256.

65. The fusion protein of claim 1, wherein the IL-2 domain comprises an amino acid sequence of SEQ ID NO: 42, 50, 52, 56, 58, 60, 68, 70, 80, 255, or 256.

66. The fusion protein of claim 1, wherein the IL-2 domain comprises an amino acid sequence of SEQ ID NO: 56, 60, 68, 70, 80, 255, or 256.

67. The fusion protein of any one of claims 1 to 66, wherein the PD-1 binding domain comprises:

(i) a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115;

(ii) a CDRL1 of SEQ ID NO: 120, a CDRL2 of LAS, a CDRL3 of SEQ ID NO: 121, a CDRH1 of SEQ ID NO: 122, a CDRH2 of SEQ ID NO: 123, and a CDRH3 of SEQ ID NO: 124;

(iii) a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL3 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID

NO: 133;

(iv) a CDRL1 of SEQ ID NO: 138, a CDRL2 of TAT, a CDRL3 of SEQ ID NO: 139, a CDRH1 of SEQ ID NO: 140, a CDRH2 of SEQ ID NO: 141, and a CDRH3 of SEQ ID NO: 142;

(v) a CDRL1 of SEQ ID NO: 147, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 148, a CDRH1 of SEQ ID NO: 149, a CDRH2 of SEQ ID NO: 150, and a CDRH3 of SEQ ID NO: 151;

(vi) a CDRL1 of SEQ ID NO: 156, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 157, a CDRH1 of SEQ ID NO: 158, a CDRH2 of SEQ ID NO: 159, and a CDRH3 of SEQ ID NO: 160;

(vii) a CDRL1 of SEQ ID NO: 165, a CDRL2 of YAF, a CDRL3 of SEQ ID NO: 166, a CDRH1 of SEQ ID NO: 167, a CDRH2 of SEQ ID NO: 168, and a CDRH3 of SEQ ID NO: 169;

(viii) a CDRL1 of SEQ ID NO: 174, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 175, a CDRH1 of SEQ ID NO: 176, a CDRH2 of SEQ ID NO: 177, and a CDRH3 of SEQ ID NO: 178;

(ix) a CDRL1 of SEQ ID NO: 183, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 184, a CDRH1 of SEQ ID NO: 185, a CDRH2 of SEQ ID NO: 186, and a CDRH3 of SEQ ID NO: 187; or

(x) a CDRL1 of SEQ ID NO: 192, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 193, a CDRH1 of SEQ ID NO: 194, a CDRH2 of SEQ ID NO: 195, and a CDRH3 of SEQ ID NO: 196.

68. The fusion protein of any one of clams 1 to 67, wherein the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 111, a CDRL2 of DAS, a CDRL3 of SEQ ID NO: 112, a CDRH1 of SEQ ID NO: 113, a CDRH2 of SEQ ID NO: 114, and a CDRH3 of SEQ ID NO: 115.

69. The fusion protein of any one of clams 1 to 67, wherein the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 120, a CDRL2 of LAS, a CDRL3 of SEQ ID NO: 121, a CDRH1 of SEQ ID NO: 122, a CDRH2 of SEQ ID NO: 123, and a CDRH3 of SEQ ID NO: 124.

70. The fusion protein of any one of clams 1 to 67, wherein the PD-1 binding domain

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comprises a CDRL1 of SEQ ID NO: 129, a CDRL2 of KVS, a CDRL3 of SEQ ID NO: 130, a CDRH1 of SEQ ID NO: 131, a CDRH2 of SEQ ID NO: 132, and a CDRH3 of SEQ ID NO: 133.

71. The fusion protein of any one of clams 1 to 67, wherein the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 138, a CDRL2 of TAT, a CDRL3 of SEQ ID NO: 139, a CDRH1 of SEQ ID NO: 140, a CDRH2 of SEQ ID NO: 141, and a CDRH3 of SEQ ID NO: 142.

72. The fusion protein of any one of clams 1 to 67, wherein the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 147, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 148, a CDRH1 of SEQ ID NO: 149, a CDRH2 of SEQ ID NO: 150, and a CDRH3 of SEQ ID NO: 151.

73. The fusion protein of any one of clams 1 to 67, wherein the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 156, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 157, a CDRH1 of SEQ ID NO: 158, a CDRH2 of SEQ ID NO: 159, and a CDRH3 of SEQ ID NO: 160.

74. The fusion protein of any one of clams 1 to 67, wherein the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 165, a CDRL2 of YAF, a CDRL3 of SEQ ID NO: 166, a CDRH1 of SEQ ID NO: 167, a CDRH2 of SEQ ID NO: 168, and a CDRH3 of SEQ ID NO: 169.

75. The fusion protein of any one of clams 1 to 67, wherein the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 174, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 175, a CDRH1 of SEQ ID NO: 176, a CDRH2 of SEQ ID NO: 177, and a CDRH3 of SEQ ID NO: 178.

76. The fusion protein of any one of clams 1 to 67, wherein the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 183, a CDRL2 of WAS, a CDRL3 of SEQ ID NO: 184, a CDRH1 of SEQ ID NO: 185, a CDRH2 of SEQ ID NO: 186, and a CDRH3 of SEQ ID NO: 187.

77. The fusion protein of any one of clams 1 to 67, wherein the PD-1 binding domain comprises a CDRL1 of SEQ ID NO: 192, a CDRL2 of AAS, a CDRL3 of SEQ ID NO: 193, a CDRH1 of SEQ ID NO: 194, a CDRH2 of SEQ ID NO: 195, and a CDRH3 of SEQ ID NO: 196.

78. The fusion protein of any one of claims 1 to 77, wherein the half-life-extension domain comprises a fragment crystallizable (Fc) domain.

79. The fusion protein of claim 78, comprising an IL-2 domain, an intact anti-PD-1

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

80. The fusion protein of claim 78 or 79, 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 *N*-terminus of the first IL-2 domain is connected to the *C*-terminus of the first heavy chain directly or *via* the first peptide linker, and the *N*-terminus of the second IL-2 domain is connected to the *C*-terminus of the second heavy chain directly or *via* the second peptide linker.

81. The fusion protein of claim 79 or 80, wherein the intact anti-PD-1 antibody is a human or humanized antibody.

82. The fusion protein of any one of claims 79 to 81 , wherein the intact anti-PD-1 antibody is an IgA, IgD, IgE, IgG, or IgM antibody.

83. The fusion protein of any one of claims 79 to 82, wherein the intact anti-PD-1 antibody is an IgG antibody.

84. The fusion protein of any one of claims 79 to 83, wherein the intact anti-PD-1 antibody is an IgG1 or IgG4 antibody.

85. The fusion protein of any one of claims 79 to 84, wherein the intact anti-PD-1 antibody is an IgG1 antibody.

86. The fusion protein of any one of claims 79 to 84, wherein the intact anti-PD-1 antibody is an IgG4 antibody.

87. The fusion protein of any one of claims 79 to 86, wherein the intact anti-PD-1 antibody is in a knobs-in-holes configuration.

88. The fusion protein of claim 79 or 80, comprising:

(i) a light chain variable region of SEQ ID NO: 116 and a heavy chain variable region of SEQ ID NO: 117;

(ii) a light chain variable region of SEQ ID NO: 125 and a heavy chain variable region of SEQ ID NO: 126;

(iii) a light chain variable region of SEQ ID NO: 134 and a heavy chain variable region of SEQ ID NO: 135;

(iv) a light chain variable region of SEQ ID NO: 143 and a heavy chain variable region of SEQ ID NO: 144;

(v) a light chain variable region of SEQ ID NO: 152 and a heavy chain variable region of SEQ ID NO: 153;

(vi) a light chain variable region of SEQ ID NO: 161 and a heavy chain variable region of SEQ ID NO: 162;

(vii) a light chain variable region of SEQ ID NO: 170 and a heavy chain variable region of SEQ ID NO: 171;

(viii) a light chain variable region of SEQ ID NO: 179 and a heavy chain variable region of SEQ ID NO: 180;

(ix) a light chain variable region of SEQ ID NO: 188 and a heavy chain variable region of SEQ ID NO: 189; or

(x) a light chain variable region of SEQ ID NO: 197 and a heavy chain variable region of SEQ ID NO: 198.

89. The fusion protein of claim 78 or 79, wherein the fusion protein comprises (i) SEQ ID NO: 136; (ii) SEQ ID NO: 248; and (iii) SEQ ID NO: 249, 250, 251, 252, 253, or 254.

90. The fusion protein of claim 78 or 79, wherein the fusion protein comprises (i) SEQ ID NO: 136; (ii) SEQ ID NO: 257; and (iii) SEQ ID NO: 258, 259, 260, 261, 262, or 263.

91. The fusion protein of claim 78 or 79, wherein the fusion protein comprises (i) SEQ ID NO: 136; (ii) SEQ ID NO: 264; and (iii) SEQ ID NO: 202, 265, 266, 267, 268, or 269.

92. The fusion protein of claim 78 or 79, wherein the fusion protein comprises (i) SEQ ID NO: 118; (ii) SEQ ID NO: 201; and (iii) SEQ ID NO: 203 or 204.

93. The fusion protein of any one of claims 78 to 80, where in the fusion protein comprises (i) SEQ ID NO: 118 and (ii) SEQ ID NO: 205.

94. The fusion protein of any one of claims 1 to 77, wherein the half-life-extension domain is an albumin binding domain

95. The fusion protein of claim 94, comprising an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and optionally first and second peptide linkers; wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the albumin binding domain directly or *via* the first peptide linker, and the *N*-terminus of the albumin binding domain is connected to the *C*-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

96. The fusion protein of claim 94, comprising 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 the *N*-terminus of the albumin binding domain directly or *via* the first peptide linker, and the *C*-terminus of the albumin binding domain is connected to the *N*-terminus of the PD-1 binding domain directly or *via* the second peptide linker.

97. The fusion protein of claim 94, comprising an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and optionally first and second peptide linkers; wherein the *N*-terminus of the IL-2 domain is connected to the *C*-terminus of the PD-1 binding domain directly or *via* the first peptide linker, and the *N*-terminus of the PD-1 binding domain is connected to the *C*-terminus of the albumin binding domain directly or *via* the second peptide linker.

98. The fusion protein of claim 94, comprising 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 the *N*-terminus of the PD-1 binding domain directly or *via* the first peptide linker, and the *C*-terminus of the PD-1 binding domain is connected to the *N*-terminus of the albumin binding domain directly or *via* the second peptide linker.

99. The fusion protein of claim 94, comprising an IL-2 domain, a PD-1 binding domain, an albumin binding domain, and optionally first and second peptide linkers; wherein the

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

100. The fusion protein of claim 94, comprising 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 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 albumin binding domain directly or *via* the second peptide linker.

101. The fusion protein of any one of claims 94 to 100, wherein the albumin binding domain is an antibody or a fragment thereof that binds to a human serum albumin.

102. The fusion protein of any one of claims 94 to 101, wherein the albumin binding domain is a single domain antibody.

103. The fusion protein of any one of claims 94 to 102, wherein the albumin binding domain is a V_HH single domain antibody.

104. The fusion protein of claim 102 or 103, wherein the single domain antibody comprises (i) CDR1 of SEQ ID NO: 83, CDR2 of SEQ ID NO: 84, and CDR3 of SEQ ID NO: 85; or (ii) CDR1 of SEQ ID NO: 91, CDR2 of SEQ ID NO: 92, and CDR3 of SEQ ID NO: 93.

105. The fusion protein of any one of claims 102 to 104, wherein the single domain antibody has an amino acid sequence of SEQ ID NO: 90 or 97.

106. A pharmaceutical composition comprising the fusion protein of any one of claims 1 to 105, and a pharmaceutically acceptable excipient.

107. The pharmaceutical composition of claim 106, wherein the pharmaceutical composition is in single dosage form.

108. The pharmaceutical composition of claim 106 or 107, wherein the pharmaceutical composition is a solid.

109. The pharmaceutical composition of claim 106 or 107, wherein the pharmaceutical composition is in a parenteral dosage form.

110. The pharmaceutical composition of claim 109, the pharmaceutical composition is in an intravenous dosage form.

111. The pharmaceutical composition of any one of claims 106, 107, 109, or 110, wherein the pharmaceutical composition is a solution.

112. 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 105 or the pharmaceutical composition of any one of claims 106 to 111.

113. The method of claim 112, wherein the proliferative disease is cancer.

114. The method of claim 112 or 113, wherein the proliferative disease is metastatic cancer.

115. 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 105 or the pharmaceutical composition of any one of claims 106 to 111.

116. The method of claim 115, wherein the cell is a cancerous cell.

117. A method of activating an immune effector cell, comprising contacting the cell with an effective amount of the fusion protein of any one of claims 1 to 105 or the pharmaceutical composition of any one of claims 106 to 111.

FIG. 1

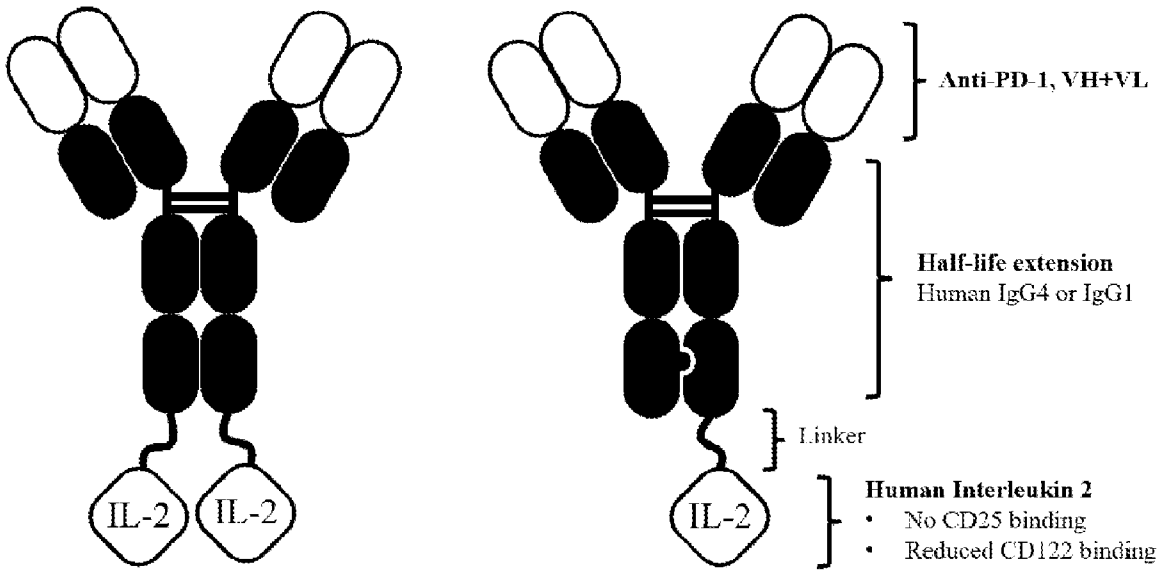

IL-2
IL-2
IL-2
Anti-PD-1, VH+VL
Half-life extension
Human IgG4 or IgG1
Linker
Human Interleukin 2
• No CD25 binding
• Reduced CD122 binding

FIG. 2

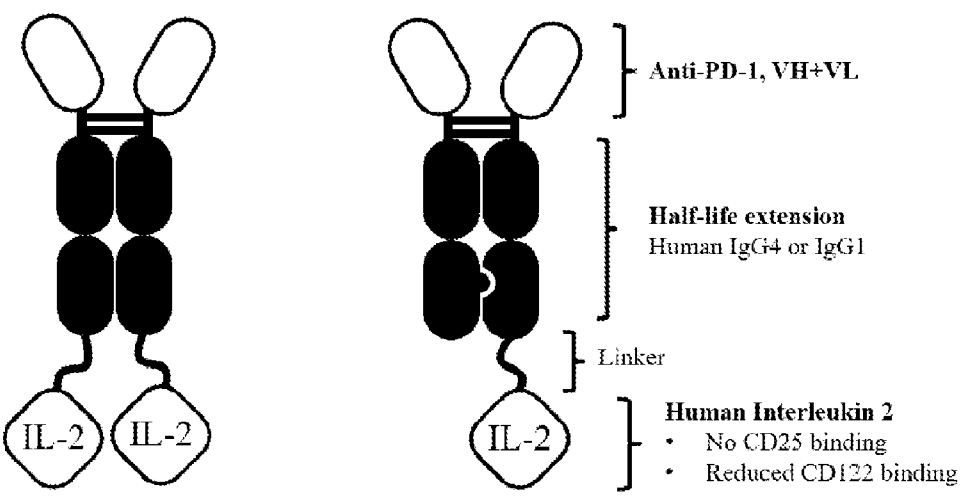

IL-2
IL-2
IL-2
Anti-PD-1, VH+VL
Half-life extension
Human IgG4 or IgG1
Linker
Human Interleukin 2
• No CD25 binding
• Reduced CD122 binding

FIG. 3

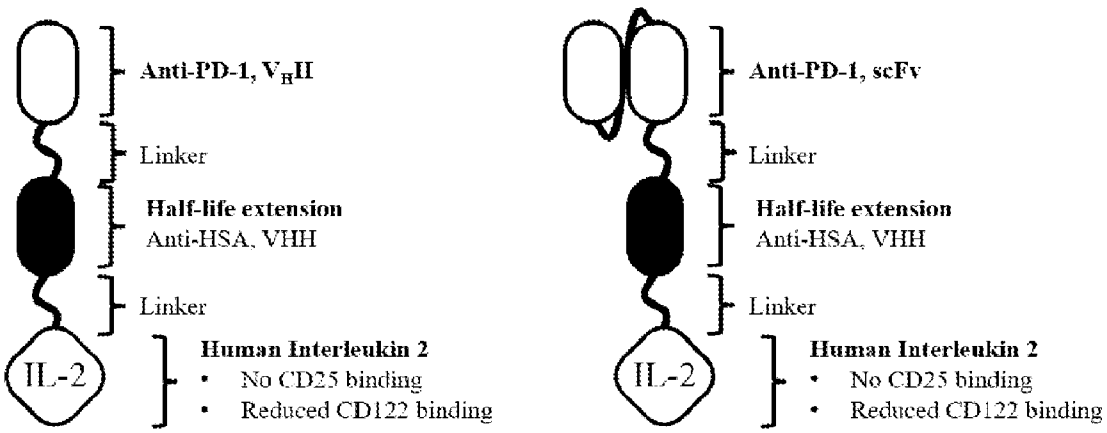

Anti-PD-1, V_HH
Linker
Half-life extension
Anti-HSA, VHH
Linker
IL-2
Human Interleukin 2
• No CD25 binding
• Reduced CD122 binding
Anti-PD-1, scFv
Linker
Half-life extension
Anti-HSA, VHH
Linker
IL-2
Human Interleukin 2
• No CD25 binding
• Reduced CD122 binding

FIG. 4

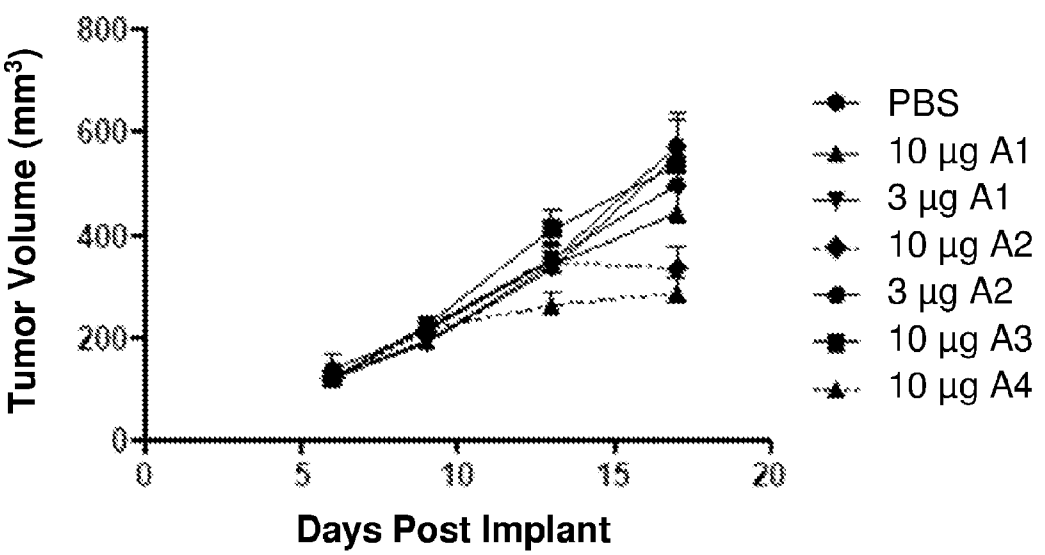
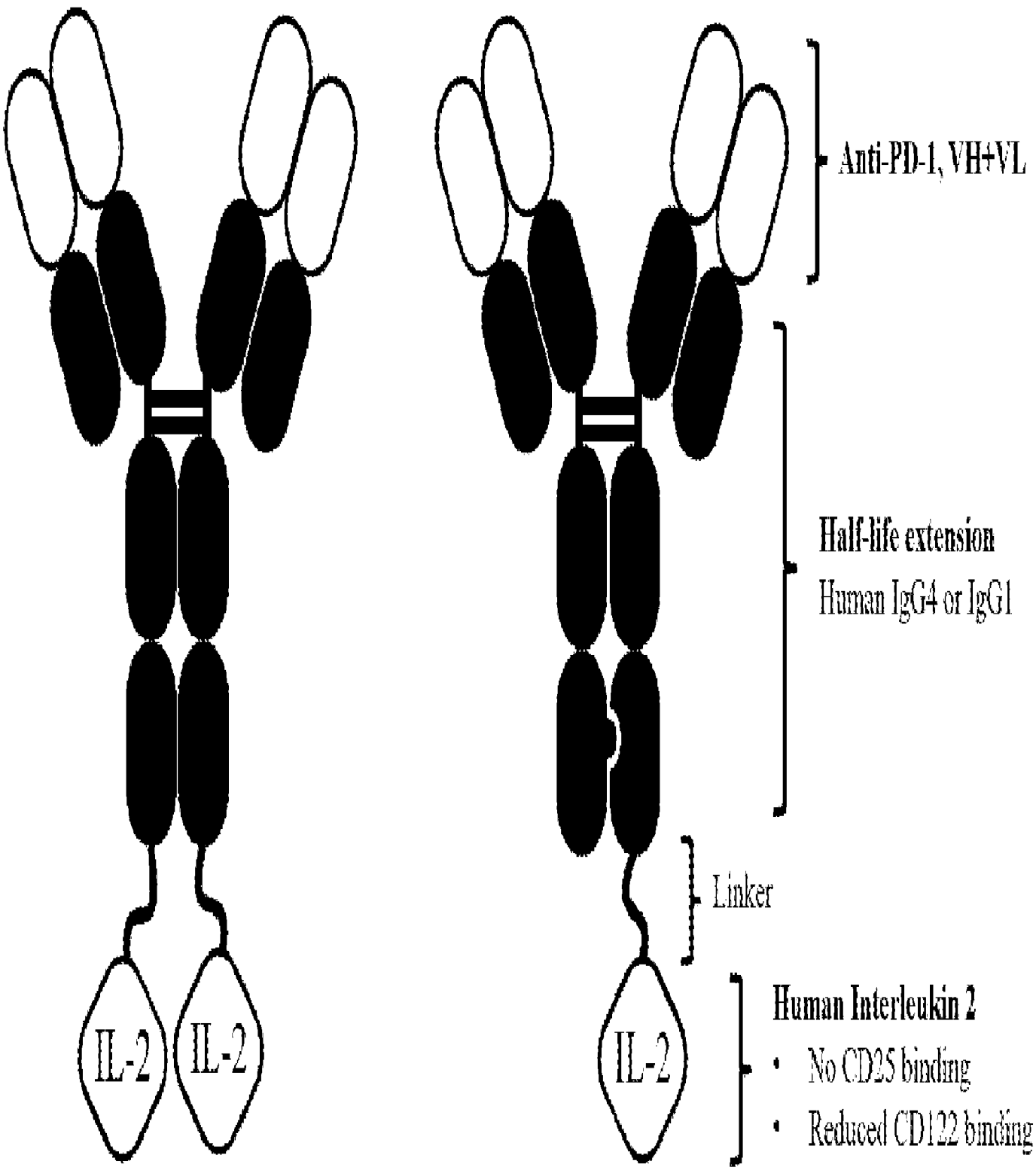

Tumor Volume (mm3)
800
600
400
200
0
0
5
10
15
20
Days Post Implant
PBS
10 µg A1
3 µg A1
10 µg A2
3 µg A2
10 µg A3
10 µg A4

FIG. 1


IL-2
IL-2
IL-2
Anti-PD-1, VH+VL
Half-life extension
Human IgG4 or IgG1
Linker
Human Interleukin 2
• No CD25 binding
• Reduced CD122 binding