(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 March 2019 (28.03.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/060888 A1

(51) International Patent Classification:
A61K 39/395 (2006.01) *C07K 16/18* (2006.01)
A61K 38/16 (2006.01) *C07K 16/28* (2006.01)
A61K 38/17 (2006.01) *A61P 35/04* (2006.01)
C07K 14/705 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/US2018/052639

(22) International Filing Date:
25 September 2018 (25.09.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/562,511 25 September 2017 (25.09.2017) US

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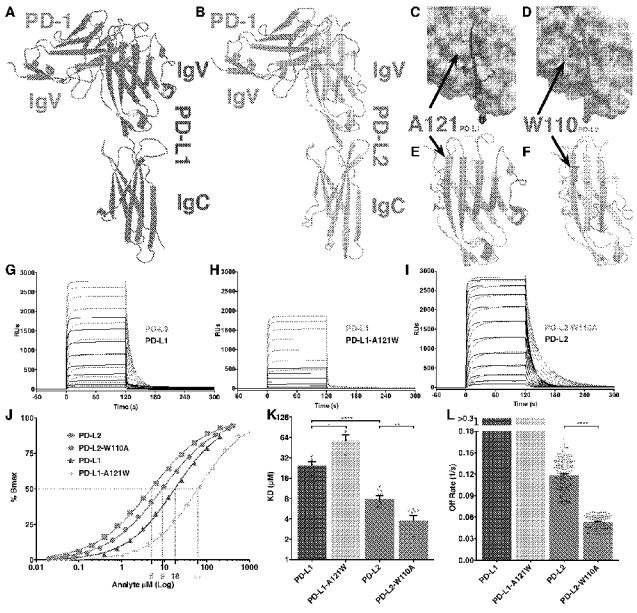
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

(54) Title: HETERODIMERIC-FC-FUSION PROTEINS



FIGs. 1A-1L

(57) Abstract: The present invention relates to immunomodulatory agents and methods of modulating a subject's T cell immune response. The immunomodulatory compositions described herein include heterodimeric-fusion proteins and peptides designed to mimic members of the B7 family of cell-surface protein ligands, which bind to and induce signal transduction through the CD28 family of receptors and others.

[Continued on next page]

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TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

HETERODIMERIC-FC-FUSION PROTEINS

[0001] This application claims the priority benefit of U.S. Provisional Patent Application Serial No. 62/562,511, filed September 25, 2017, which is hereby incorporated by reference in its entirety.

[0002] This invention was made with government support under grant numbers AR069515 and GM007308 awarded by the National Institutes of Health. The government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] The present invention relates to immunomodulatory agents and methods of modulating a subject's immune response. The immunomodulatory compositions described herein include heterodimeric-fusion proteins and peptides designed to mimic members of the B7 family of cell-surface protein ligands, which bind to and induce signal transduction through the CD28 family of receptors and others.

BACKGROUND OF THE INVENTION

[0004] Stimulating a patient's own immune response to recognize and target tumor cells is an attractive approach for cancer therapy, and many studies have demonstrated effectiveness of immunotherapy to induce the immune response. However, despite primary anti-tumor immune responses, functional, effector anti-Tumor T cells responses have been weak at best.

[0005] The programmed cell death-1 receptor (PD-1) is a receptor on T-cells that inhibits signaling downstream of the T cell Receptor (TCR) as well as other T cell co-receptors. Therefore, signal transduction initiated via its ligands, PD-L1 or PD-L2 (programmed cell death 1 ligand 1 and 2), usually provides a suppressive or inhibitory signal to the T cell that results in decreased T cell proliferation or other inhibition of T cell functions. Since the PD-1/PD-L1/PD-L2 axis is a critical immune checkpoint that tips immune responses towards tolerance, the PD-1/PD-L1 receptor ligand pair has been heavily targeted in cancer immunotherapy with monoclonal antibody therapies aimed to block their interaction.

[0006] PD-L2 has a 2 to 4 fold stronger affinity for PD-1 than does PD-L1, but both ligands bind with relatively weak KDs (~2-20 μM). Other than monoclonal antibodies, the only PD-1 drug to reach clinical trials is AMP-224 (aka GSK-2661380, PD-L2-Ig, or B7-DC-Ig), which is a fusion protein consisting of the extracellular domain of PD-L2 attached to an IgG Fc. This drug showed preclinical efficacy in murine tumor models. One mode of action that has been

reported is an alteration of the T regulatory cell (Tregs) repertoire by depletion of PD-1-high expressing Tregs. There is a need for additional immunomodulatory compositions and methods suitable for the treatment of cancer and other T cell mediated diseases.

[0007] The present invention is directed at overcoming this and other deficiencies in the art.

SUMMARY OF THE INVENTION

[0008] A first aspect of the present invention is directed to an isolated polypeptide comprising an extracellular domain portion of PD-L2 comprising the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, wherein said extracellular domain portion of PD-L2 comprises an amino acid residue substitution at one or more positions 64, 66, and 110 of SEQ ID NO:1.

[0009] Another aspect of the present invention is directed to an isolated polypeptide comprising an extracellular domain portion of PD-L2 comprising the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, wherein X at position 110 of SEQ ID NO: 1 is an amino acid residue other than tryptophan.

[0010] Another aspect of the present invention is directed to an isolated polypeptide comprising an extracellular domain portion of PD-L2 comprising the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, wherein X at position 64 of SEQ ID NO: 1 is an amino acid residue other than asparagine.

[0011] Another aspect of the present invention is directed to an isolated polypeptide comprising an extracellular domain portion of PD-L2 comprising the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, wherein X at position 66 of SEQ ID NO: 1 is an amino acid residue other than threonine or serine.

[0012] Another aspect of the present invention is directed to a fusion polypeptide. The fusion polypeptide comprises the isolated variant PD-L2 polypeptide as described herein and a heterologous polypeptide domain coupled to said isolated polypeptide at its carboxy terminus.

[0013] Another aspect of the present invention is directed to a multimeric protein comprising two or more fusion polypeptides as described herein.

[0014] Another aspect of the present invention is directed to a method of modulating a subject's T-cell immune response. This method involves administering a pharmaceutical composition comprising the fusion polypeptide or multimeric protein of the present invention as described herein to the subject in an amount effective to modulate said subject's T-cell immune response.

[0015] Another aspect of the present invention is directed to a heterodimeric binding protein. The heterodimeric binding protein of the present invention comprises (i) a first fusion polypeptide comprising an extracellular domain portion of a first B7 ligand coupled to a heterologous polypeptide domain; and (ii) a second fusion polypeptide comprising an extracellular domain portion of a second B7 ligand coupled to heterologous polypeptide domain, wherein said first and second B7 ligands are different ligands and wherein said first and second fusion polypeptides are coupled together.

[0016] Another aspect of the present invention is directed to a method of modulating a subject's T-cell immune response. This method involves administering a heteromeric binding molecule as described herein or a pharmaceutical composition comprising said heteromeric binding molecule to the subject in an amount effective to modulate said subject's T-cell immune response.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIGs. 1A-1L show how PD-L2's tryptophan elbow hinders PD-1 binding. FIG. 1A is a ribbon rendering of human PD-L1 in complex with mouse PD-1 (3BIK). FIG. 1B is a ribbon rendering of human PD-L2 in complex with mouse PD-1 (3BP5 with human PD-L2 sequence threaded onto the mouse structure). FIGs. 1C-1D are a surface electrostatic rendering of PD-1's binding face with PD-Ligand key G-strand residues displayed as sticks. PD-L1's A121 corresponds to PD-L2's W110. FIGs. 1E and 1F are ribbon rendering of PD-Ligand IgV domains with beta strand lettering and the key G-strand Ala/Trp difference highlighted with arrows. FIGs. 1G-1I are surface plasmon resonance (SPR) sensorograms using 1600-3000 RUs of PD-1-biotin immobilized to an SA-dextran chip. Analytes were injected for 120s in a 1:2 serial diluted series of concentrations and the surface was regenerated with 15s of glycine pH 3.0. PD-L1 (205-3.2uM), PD-L2 (100-0.1uM), PD-L1-A121W (96-1.5uM), and PD-L2-W110A (100-0.1uM) monomeric analytes were injected. FIG. 1J shows PD-L1, PD-L2, PD-L1-A121W, and PD-L2-W110A binding curves normalized to their Bmax values. (fit: specific binding with hill slope). FIG. 1K shows steady state KD values determined using the 1:1 langmuir model from three or more independent experiments. FIG. 1L shows off-rate measurements made by

fitting to the dissociation curves in FIGs. 1G and 1I. (fit: dissociation - one phase exponential decay) The dissociation curves for PD-L1 and PD-L1-A121W are too rapid for accurate fitting and therefore their off-rates were determined to be greater than the upper limit of 0.5 (1/s) for the Biacore T200. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

[0018] FIGs. 2A-2G show how PD-L2's N64 latch glycan hinders PD-1 binding. FIG. 2A is a ribbon rendering of human PD-L2 (3BP5 with human PD-L2 sequence threaded onto the mouse structure) with its C' "Latch" region highlighted with sticks. FIG. 2B is a Coomassie stain of purified, monomeric PD-Ligands ran on a 12% PAGE SDS. The monomeric proteins were expressed in HEK293 cells and purified via c-terminal 6-His tags. The PD-L2-N64S mutant migrates in the gel with increased electrophoretic mobility as compared with wildtype protein, indicating that the N64 site is glycosylated. FIGs. 2C and 2D are surface plasmon resonance (SPR) sensorograms using 1600-3000 RUs of PD-1-biotin immobilized to an SA-dextran chip. Analytes were injected for 120s in a 1:2 serial diluted series of concentrations and the surface was regenerated with 15s of glycine pH 3.0. Analytes were injected at concentration ranges from ~200μM to ~0.05μM. FIG. 2E shows PD-L2-N64S, PD-L2-W110A, PD-L2, PD-L2-E71A, and PD-L1 binding curves normalized to their Bmax values. (fit: specific binding with hill slope). FIG. 2F shows steady state KD values determined using the 1:1 langmuir model from three or more independent experiments. FIG. 2G shows off-rate measurements made by fitting to the dissociation curves in FIGs 2C and 2D. (fit: dissociation - one phase exponential decay) The dissociation curve for PD-L1 is too rapid for accurate fitting and therefore their off-rates were determined to be greater than the upper limit of 0.5 (1/s) for the Biacore T200. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

[0019] FIGs. 3A-3H demonstrate the differential functional impact the above PD-Ligand mutants have on $CD4^+$ T cell proliferation/inhibition. FIG. 3A shows controls for a $CD4^+$ T cell blast proliferation assay. $CD4^+$ T cells were isolated via positive selection from a single healthy donor on 5 separate occasions. Cells were then blasted for 1-2 weeks using 40 IU/mL recombinant IL-2. 5×10^5 cells were stained with CFSE and then plated with 2μg/mL αCD28 antibodies in soluble form in wells that were coated overnight with 10μg/mL αCD3 antibodies (non-absorbed was washed away). Wells subjected to αCD3 coated wells demonstrated 80-90% proliferation as measured by CSFE dilution. FIGS. 3B-3G show representative CFSE histograms of CD4+ T cell blasts subjected to αCD3 and αCD28 as in panel A along with 0-12 μg/mL coated monomeric PD-ligands. PD-L1-A121W is unable to inhibit T cell proliferation, whereas PD-L2-W110A and PD-L2-N64S demonstrate enhanced inhibitory capabilities compared with wt PD-L2. FIG. 3H is a summary proliferation bar graph for the above experiment looking only

as the 9 μg/mL PD-ligand coating condition over multiple independent experiments. This assay correlates the improved binding of PD-L2-W110A and PD-L2-N64S as seen in FIGS. 1 & 2 with enhanced T cell inhibitory capabilities.

[0020] FIG. 4 demonstrates that PD-1 and its ligands may oligomerize on the cell membrane naturally as assayed using a flow cytometry based FRET technique. FIG. 4A shows a summary bar graph of FRET % for various CFP/YFP fusion constructs transiently transfected into suspension expi-CHO cells. B7-CFP/YFP fusion constructs were designed with native extracellular and transmembrane domains, but without native cytoplasmic domains. CFP and YFP co-transfected into these cells results in minimal bystander FRET, but transfection of a CFP-YFP fused construct exhibits ~100% FRET. CD8alpha and CD80 CFP/YFP fusion constructs were used as experimental positive controls since CD8alpha forms stable homodimers and CD80 is known to exist in a dynamic equilibrium between a monomeric and dimeric state in the context of a cell membrane. PD-Ligands also demonstrated FRET in both homo- and hetero-combinations, suggesting they may also exist in a dynamic oligomeric state on the cell surface. PD-1 also demonstrated strong FRET. These results may indicate a functional relevance for dimeric or multimeric states of members of the PD-1-axis and implicates engineered homo- and hetero- multimers for therapeutic investigation.

[0021] FIGs. 5A-5B show PD-Ligand Fc fusion and heterodimeric Fc fusion construct design and binding to PD-1. FIG. 5A (Left) shows the extracellular domain of human PD-L1 (#19-238) was fused to the hinge and Fc portion of the human IgG1 protein (#99-330) with a Ser-Gly linker (encoded by the Kpn2I restriction enzyme cut-site). The construct is C-terminally tagged with 6-His for alternative purification methods. The middle-left panel of FIG. 5A shows the extracellular domain of human PD-L2 (#21-242) fused to the hinge and Fc portion of the human IgG1 protein (#99-330) with a Ser-Gly linker (encoded by the Kpn2I restriction enzyme cut-site). The construct is C-terminally tagged with 6-His for alternative purification methods. In FIG. 5A (middle-right panel) shows the wild-type, hydrophobic, mirrored, front-to-front dimerization interface of the CH3 domain of a human IgG1 in its quaternary structure (1HZH) (upper panel). This dimerization interface is the one utilized for the PD-L1-Fc and PD-L2-Fc homodimerization. The lower panel of this figure shows a mutated form of the CH3 dimerization interface following the "knob-in-hole" technique, which favors heterodimerization. The T389W (Uniprot P01857 numbering: T249W) mutation is made on one construct in order to introduce a bulky residue. The Y438V, L391A, and T389S (Uniprot P01857 numbering: Y290V, L251A, T249S) mutations are made on the second construct in order to accommodate the bulky Trp protruding from the first construct. (1HZH with pymol generated mutagenesis). FIG. 5A,

right panel, is a schematic of the PD-L1/L2-heterodimeric Fc fusion construct made. The extracellular domain of human PD-L1 (#19-238) was fused to the hinge and Fc portion of the human IgG1 protein (#99-330) with a Ser-Gly linker (encoded by the Kpn2I restriction enzyme cut-site) along with a T389W mutation in the IgG1 CH3 domain. The extracellular domain of human PD-L2 (#21-242) was fused to the hinge and Fc portion of the human IgG1 protein (#99-330) with a Ser-Gly linker (encoded by the Kpn2I restriction enzyme cut-site) along with Y438V, L391A, and T389S mutations in the IgG1 CH3 domain. For this heterodimeric construct only the PD-L1 side was c-terminally tagged with 6-His. FIG. 5B is a graph showing PD-Ligand-Fc fusion constructs binding to PD-1. Data was generated from the steady-state binding levels of SPR sensorograms. ~200 RUs of PD-Ligand-Fc fusion protein constructs were immobilized to a protein A SPR sensor chip. Fc fusion proteins were captured by protein A chip, subsequently a known concentration of monomeric PD-1 was flowed over the surface. The surface was regenerated with glycine pH 1.5 and the process was repeated with a different concentration of PD-1 analyte injected. Binding curves were normalized to their Bmax values (fit: specific binding with hill slope). Reference binding curves for monomeric PD-L1 and PD-L2 are plotted from a separate experiment. Upon immobilization, the two arms of an Fc-fused protein should act independently and their expected affinity for their binding partner is similar to the monomeric affinity measured. The fact that the three PD-Ligand-Fc constructs bind to PD-1 with stronger affinity than expected may suggest that these two B7-family protein arms of the Fc fusion are not acting independently. When tethered together at their C-terminus by the IgG1 hinge, the B7 proteins may be in equilibrium with a stabilizing confirmation, one that stabilizes the two arms such that their PD-1 binding interfaces are fully available for receptor binding. This stabilizing conformation may resemble the PD-L1 dimerized crystal structures published (3BIS, 3FN3, 4Z18, and 5JDR).

[0022] FIGs. 6A-6E show T cell inhibition by adsorbed PD-Ligand Fc fusion constructs. FIG. 6A shows a dose response (27-0.03ug/mL) of PD-L1-Fc, PD-L2-Fc, and PD-L1/L2-heteroFc adsorbed along with 10μg/mL anti-CD3 monoclonal antibody overnight. On day two the wells were washed, and $5x10^5$ Jurkat T cells were added along with 2μg/mL anti-CD28 antibodies. After a 24 hour 37°C incubation, the supernatants were harvested and their IL-2 concentration was measured by standard curve ELISA. FIGs. 6B-6E are the same as FIG. 6A except only the 3ug/mL adsorbed condition was used, rather than a dose response dilution set. FIGs. 6D and 6E include PD-L2-W110A-Fc, PD-L1/L2-W110A-heteroFc, and CD80/PD-L2-HeteroFc as examples of the types of combinatorial Fc-fusions possible. FIGs. 6B and 6D used

Jurkat T cells. FIGs. 6C and 6E used primary T cells from 3 healthy donors. Primary T cells were isolated using a CD3 negative selection kit (StemCell Technologies).

[0023] FIGs. 7A-7B show PD-L1/L2-heteroFc constructs' ability to modulate immune responses in soluble form both *in vitro* and *in vivo*. FIG. 7A shows a PBMC-super-antigen activation assay in the presence or absence of Nivolumab (OPDIVO), human PD-L1-Fc, human PD-L2-Fc, and human PD-L1/L2-heteroFc. 1×10^5 human PBMCs were subjected to a range of SEB concentrations in the presence or absence of 20ug/mL Fc-fusion construct. Nivolumab was used at 20ug/mL as an activation control. An IL-2 standard curve ELISA was performed on the supernatant following a 3 day, 37°C incubation. Data from four healthy donors was normalized to the vehicle's 1ug/mL SEB condition. (fit: [Agonist] vs. response -- Variable slope (four parameters)). FIG. 7B MC38 syngeneic mouse tumor model response to anti-PD-1 therapy or murine versions of the PD-Ligand Fc-fusion biologics. Each mouse was subjected to four 200μg IP doses every four days beginning on day six post implantation. Stats represent 2way ANOVA analysis on day 19. All three constructs demonstrated decreased tumor growth in this model.

DETAILED DESCRIPTION OF THE INVENTION

[0024] The present disclosure is directed to immunomodulatory agents and methods for modulating a subject's T cell immune response. These immunomodulatory agents and methods are designed to mimic members of the B7 family of cell-surface protein ligands that bind to the CD28 family of receptors on T cells to regulate the immune response. In one embodiment, the compositions and methods described herein are designed to enhance a subject's T cell immune response. In another embodiment, the compositions and methods described herein are designed to suppress a subject's T cell immune response.

Immunomodulatory Agents:

[0025] A first aspect of the present invention is directed to an isolated variant PD-L2 protein or active polypeptide thereof having higher binding affinity for PD-1 than the wildtype PD-L2 protein. PD-L2 (programmed cell death 1 ligand 2) is one of two ligands that bind PD-1. Human PD-L2 has the amino acid sequence of SEQ ID NO: 1 as shown below, where X at position 64 is an asparagine (N) residue, X at position 66 is a threonine (T) residue, and X at position 110 is a tryptophan (W) residue. The extracellular domain of PD-L2 comprises amino acid residues 21-203 of SEQ ID NO: 1 (amino acid residues 1-20 comprise signal peptide). Within this extracellular domain, the IgV-like domain comprises amino acid residues 21-118 of

SEQ ID NO: 1, and the IgC-like domain comprises amino acid residues 122-203 of SEQ ID NO: 1.

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         10         20         30         40         50
MIFLLLMLSL ELQLHQIAAL FTVTVPKELY IIEHGSNVTL ECNFDTGSHV
         60         70         80         90        100
NLGAITASLQ KVENDNSPHR ERATLLEEQL PLGKASFHIP QVQVRDEGQY
        110        120        130        140        150
QCIIIYGVAW DYKYLTLKVK ASYRKINTHI LKVPETDEVE LTCQATGYPL
        160        170        180        190        200
AEVSWPNVSV PANTSHSRTP EGLYQVTSVL RLKPPPGRNF SCVFWNTHVR
        210        220        230        240        250
ELTLASIDLQ SQMEPRTHPT WLLHIFIPFC IIAFIFIATV IALRKQLCQK
        260        270
LYSSKDTTKR PVTTTKREVN SAI
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[0026] In a first embodiment, the isolated PD-L2 variant protein or polypeptide as described herein comprises an extracellular domain portion of PD-L2 comprising the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, and at least one amino acid residue variation. The at least one amino acid residue variation is at position 64, 66, or 110 of SEQ ID NO: 1. In one embodiment, the PD-L2 variant protein or polypeptide comprises two or more amino acid residue variations at positions 64, 66, and/or 110 of SEQ ID NO: 1. In one embodiment, the at least one amino acid residue variation includes a substitution at position 110 of SEQ ID NO: 1, where X at position 110 is an amino acid residue other than tryptophan. In one embodiment, the at least one amino acid residue variation includes a substitution at position 64 of SEQ ID NO: 1, where X at position 64 is an amino acid residue other than asparagine. In one embodiment, the at least one amino acid residue variation includes a substitution at position 66 of SEQ ID NO: 1, where X at position 66 is an amino acid residue other than threonine or serine. In one embodiment, the at least one amino acid residue variation includes any two or more of the aforementioned substitutions, i.e., a substitution at position 110 of SEQ ID NO: 1, where X at position 110 is an amino acid residue other than tryptophan, a substitution at position 64 of SEQ ID NO: 1, where X at position 64 is an amino acid residue other than asparagine, and a substitution at position 66 of SEQ ID NO: 1, where X at position 66 is an amino acid residue other than threonine or serine. In another embodiment, the isolated PD-L2 variant polypeptide comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, and at least one amino acid residue variation selected from: a substitution at position 110 of SEQ ID NO: 1, where X at position 110 is an amino acid residue other than tryptophan, a substitution at position 64 of SEQ ID NO: 1, where X at position 64 is an amino acid residue other than asparagine, and a substitution at

position 66 of SEQ ID NO: 1, where X at position 66 is an amino acid residue other than threonine or serine.

[0027] In one embodiment the isolated PD-L2 variant protein or polypeptide comprises a fragment of the IgV domain of PD-L2. This PD-L2 variant polypeptide is shorter in length than the polypeptide comprising amino acid residues 21-118, *i.e.*, it is 96 amino acid residues or less, and retains its ability to bind to PD-1. In one embodiment, the isolated PD-L2 variant fragment contains an amino acid residue other than tryptophan at the position corresponding to position 110 of SEQ ID NO: 1 and comprises a stretch of surrounding amino acid residues to comprise a fragment having a length of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95 or 96 amino acid residues. In another embodiment, the isolated PD-L2 variant fragment contains an amino acid residue other than asparagine at the position corresponding to position 64 of SEQ ID NO: 1 and comprises a stretch of surrounding amino acid residues to comprise a fragment having a length of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95 or 96 amino acid residues. In another embodiment, the isolated PD-L2 variant fragment contains an amino acid residue other than threonine or serine at the position corresponding to position 66 of SEQ ID NO: 1 and comprises a stretch of surrounding amino acid residues to comprise a fragment having a length of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95 or 96 amino acid residues. In another embodiment, the isolated PD-L2 variant fragment is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95 or 96 amino acid residues in length and contains at any two or more amino acid residue variations selected from: a substitution of the tryptophan residue at the position corresponding to position 110 of SEQ ID NO: 1, a substitution of the asparagine residue at the position corresponding to position 64 of SEQ ID NO: 1, and a substitution of the threonine residue at the position corresponding to position 66 of SEQ ID NO: 1.

[0028] In another embodiment, the isolated PD-L2 variant protein or polypeptide as described herein comprises the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1. In one embodiment, this PD-L2 variant polypeptide contains an amino acid residue other than tryptophan at the position corresponding to position 110 of SEQ ID NO: 1. In one embodiment, this PD-L2 variant polypeptide contains an amino acid residue other than asparagine at the position corresponding to position 64 of SEQ ID NO: 1. In one embodiment, this PD-L2 variant polypeptide contains an

amino acid residue other than threonine or serine at the position corresponding to position 66 of SEQ ID NO: 1. In another embodiment, the isolated PD-L2 variant polypeptide comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1, and comprises two or more amino acid residue variations selected from: a substitution of the tryptophan residue at the position corresponding to position 110 of SEQ ID NO: 1, a substitution of the asparagine residue at the position corresponding to position 64 of SEQ ID NO: 1, and a substitution of the threonine residue at the position corresponding to position 66 of SEQ ID NO: 1 with an amino acid residue other than serine.

[0029] In one embodiment the isolated PD-L2 variant protein or polypeptide comprises portions of the IgV and IgC domains of PD-L2, but is less than the full domain of one or both of the IgV and IgC domains. This PD-L2 variant polypeptide is shorter in length than the polypeptide comprising amino acid residues 21-203, *i.e.*, it is less than or equal to180 amino acid residues in length. In one embodiment, this isolated PD-L2 variant comprises a variant amino acid residue at the position corresponding to position 110 of SEQ ID NO: 1, *i.e.*, an amino acid residue other than tryptophan, and retains its ability to bind to PD-1. In another embodiment, this isolated PD-L2 variant comprises a variant amino acid residue at the position corresponding to position 64 of SEQ ID NO: 1, *i.e.*, an amino acid residue other than asparagine. In another embodiment, this isolated PD-L2 variant comprises a variant amino acid residue at the position corresponding to position 66 of SEQ ID NO: 1, *i.e.*, an amino acid residue other than threonine or serine. The isolated PD-L2 variant fragment comprising one or more of the aforementioned variant amino acid residues further comprises a stretch of contiguous surrounding amino acid residues from the IgV and IgC domains to comprise a fragment having a length of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, or 180 amino acid residues.

[0030] In another embodiment, the isolated PD-L2 variant protein or polypeptide as described herein comprises the amino acid sequence of amino acid residues 1-203 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 1-203 of SEQ ID NO: 1. In another embodiment, the isolated PD-L2 variant polypeptide comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 1-203 of SEQ ID NO: 1. In one embodiment, this isolated PD-L2 variant comprises a variant amino acid residue at the position corresponding to position 110 of SEQ ID NO: 1, *i.e.*, an amino acid residue other than tryptophan, and retains its ability to bind to PD-1. In another

embodiment, this isolated PD-L2 variant comprises a variant amino acid residue at the position corresponding to position 64 of SEQ ID NO: 1, *i.e.*, an amino acid residue other than asparagine. In another embodiment, this isolated PD-L2 variant comprises a variant amino acid residue at the position corresponding to position 66 of SEQ ID NO: 1, *i.e.*, an amino acid residue other than threonine or serine. In another embodiment, the isolated PD-L2 variant polypeptide comprises two or more amino acid residue variations selected from: a substitution of the tryptophan residue at the position corresponding to position 110 of SEQ ID NO: 1, a substitution of the asparagine residue at the position corresponding to position 64 of SEQ ID NO: 1, and a substitution of the threonine residue at the position corresponding to position 66 of SEQ ID NO: 1 with an amino acid residue other than serine.

[0031] In another embodiment, the isolated PD-L2 variant protein or polypeptide as described herein comprises the amino acid sequence of amino acid residues 1-273 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 1-273 of SEQ ID NO: 1. In another embodiment, the isolated PD-L2 variant polypeptide comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 1-273 of SEQ ID NO: 1. In one embodiment, this isolated PD-L2 variant comprises a variant amino acid residue at the position corresponding to position 110 of SEQ ID NO: 1, *i.e.*, an amino acid residue other than tryptophan, and retains its ability to bind to PD-1. In another embodiment, this isolated PD-L2 variant comprises a variant amino acid residue at the position corresponding to position 64 of SEQ ID NO: 1, *i.e.*, an amino acid residue other than asparagine. In another embodiment, this isolated PD-L2 variant comprises a variant amino acid residue at the position corresponding to position 66 of SEQ ID NO: 1, *i.e.*, an amino acid residue other than threonine or serine. In another embodiment, this isolated PD-L2 variant polypeptide comprises two or more amino acid residue variations selected from: a substitution of the tryptophan residue at the position corresponding to position 110 of SEQ ID NO: 1, a substitution of the asparagine residue at the position corresponding to position 64 of SEQ ID NO: 1, and a substitution of the threonine residue at the position corresponding to position 66 of SEQ ID NO: 1.

[0032] The isolated PD-L2 variant protein or polypeptide thereof as described herein binds to PD-1 with a higher affinity than wildtype PD-L2. As described herein PD-L2 binding affinity for PD-1 is modulated, at least in part, by the amino acid residue at position 110 of SEQ ID NO: 1. In particular, the inventors have discovered that substitution of the tryptophan residue at position 110 of SEQ ID NO: 1, which is present in the wildtype PD-L2, enhances the binding affinity of PD-L2 for PD-1. Accordingly, the substitution of the amino acid residue at position

110 of SEQ ID NO: 1 in any of the isolated PD-L2 variants described herein with any amino acid residue other than tryptophan increases the binding affinity of PD-L2 or a polypeptide thereof to PD-1. In one embodiment, the amino acid residue at position 110 of SEQ ID NO: 1 is an alanine residue. The inventors have also found that substitution of the asparagine residue at the position corresponding to position 64 of SEQ ID NO: 1 with any other amino acid residue removes glycosylation at this site, thereby increasing the binding affinity of PD-L2 or a polypeptide there of to PD-1. Accordingly, substitution of the amino acid residue at position 64 of SEQ ID NO:1 in any of the isolated PD-L2 variants described herein with any amino acid residue other than asparagine increases the binding affinity of PD-L2 or a polypeptide thereof to PD-1. Likewise, a substitution of the threonine residue at the position corresponding to position 66 of SEQ ID NO: 1 with an amino acid residue other than serine to remove the glycosylation at this site will also increase the binding affinity of PD-L2 or a polypeptide thereof to PD-1. Accordingly, substitution of the amino acid residue at position 66 of SEQ ID NO:1 in any of the isolated PD-L2 variants described herein with any amino acid residue other than threonine or serine increases the binding affinity of PD-L2 or a polypeptide thereof to PD-1.

[0033] Another aspect of the present invention is directed to a fusion polypeptide. The fusion polypeptide comprises the isolated variant PD-L2 protein or polypeptide as described *supra*, and a heterologous polypeptide domain coupled to the isolated variant PD-L2 polypeptide at its carboxy terminus.

[0034] The heterologous polypeptide domain coupled to the isolated variant PD-L2 polypeptide or other B7 ligands as described herein can include, but is not limited to, one or more epitopes (e.g., FLAG) or a tag sequences (e.g., His_6, and the like) to allow for the detection and/or isolation of the fusion polypeptides; autoimmune antigens; a targeting domain that directs the fusion polypeptide to the immune tissue where T cell modulation is desired; a polypeptide or peptide which increases stability of the variant PD-L2 polypeptide, such as an immunoglobulin constant region (e.g., an Fc domain); a half life-extending sequence comprising a combination of two or more (e.g., 2, 5, 10, 15, 20, 25, etc) naturally occurring or non-naturally occurring charged and/or uncharged amino acids (e.g., serine, glycine, glutamic or aspartic acid); and/or a dimerization or multimerization domain.

[0035] In one embodiment, the heterologous polypeptide domain of the fusion polypeptide comprising the variant PD-L2 polypeptide described *supra* is a dimerization or multimerization domain. The dimerization or multimerization domain functions to dimerize or multimerize two or more fusion proteins either covalently or non-covalently. In one embodiment, dimerization domain includes, without limitation, a domain containing at least one

cysteine that is capable of forming an intermolecular disulfide bond with a cysteine on the partner fusion protein. The dimerization domain can contain one or more cysteine residues such that disulfide bond(s) can form between the partner fusion proteins. In one embodiment, dimerization domains contain one, two or three to about ten cysteine residues. In one embodiment embodiment, the dimerization domain is the hinge region of an immunoglobulin. Other exemplary dimerization domains include, without limitation, coiled coils, acid patches, zinc fingers, calcium hands, and leucine zippers (e.g., from jun and/or fos) (see U.S. Pat. No. 5,932,448 which is incorporated by reference in its entirety).

[0036] In one embodiment, the dimerization domain of the fusion polypeptide described herein is an Fc domain of an immunoglobulin heavy chain. In addition to serving as a dimerization domain the Fc domain can also act as a targeting domain, as it is capable of binding to Fc receptors expressed on diseased cells, and a stabilizing domain.

[0037] As known in the art, the term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain. The "Fc region" (also known as the “fragment crystallizable” or “tail” region) may be a native sequence Fc region or a variant Fc region. The Fc region of an immunoglobulin generally comprises two constant domains, C_H2 and C_H3. Typically, an Fc region includes a C_H2 and a C_H3 domain and can include at least a portion of the hinge domain, but does not usually include the entire C_H1 domain Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM. In one embodiment, the Fc domain is derived from a human immunoglobulin. In one embodiment, the Fc domain is derived from human IgG1 (UniProt P01857) including the C_H2 and C_H3 regions. The amino acid sequence of human IgG1 Fc region containing the hinge domain (residues 99-110; shown as underlined), CH2 (residues 111-223; shown in bold) and CH3 (residues 224-330; shown as double underlined) regions is shown below as SEQ ID NO: 4. In one embodiment, the Fc domain comprises amino acid residues 99-330 of SEQ ID NO: 4.

```
          10          20          30          40          50
ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
          60          70          80          90         100
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP
         110         120         130         140         150
KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS
         160         170         180         190         200
HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
         210         220         230         240         250
EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC
         260         270         280         290         300
LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
         310         320         330
QQGNVFSCSV MHEALHNHYT QKSLSLSPGK (SEQ ID NO: 4)
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[0038] Depending on the application, the Fc region from species other than human, for example, mouse or rat, may be used. The immunoglobulin Fc region used as a fusion partner in the fusion polypeptides describe herein generally may be from any mammalian species. Where it is undesirable to elicit an immune response in the host cell or animal against the Fc region, the Fc region may be derived from the same species as the host cell or animal. For example, a human immunoglobulin Fc region can be used when the host animal or cell is human; likewise, a murine immunoglobulin Fc region can be used where the host animal or cell will be a mouse.

[0039] The Fc-region or domain of the fusion polypeptides described herein may impart non-antigen binding functions to the polypeptide, termed "effector functions", such as activated complement binding, antibody-dependent cell cytotoxicity (ADCC), and other functions mediated through the binding of subregions of this dimeric structure with immune cell surface receptors, Fc-receptors. Certain natural and synthetic variants of the Fc-region polypeptides sequences with altered effector functions that are suitable for use in the fusion polypeptides described herein include the subclass variants; *e.g.,* IgGi, IgG2i, IgG3i, IgG24; and mutant polypeptides as described in *e.g.* U.S. Patent No. 5,624,821 to Winter, U.S. Patent No. 6,528,624 to Idusogie, U.S. Patent No. 7,183,387 to Presta, and U.S. Patent No. 7,317,091 to Lazar et al., which are hereby incorporated by reference in their entirety.

[0040] The PD-L2 and other fusion proteins and polypeptides described herein can be made by fusing the heterologous polypeptide domain to either the N-terminus or at the C-terminus of the PD-L2 polypeptide. In one embodiment, the heterologous polypeptide domain is coupled to the C-terminus of the PD-L2 polypeptide described herein. Heterologous polypeptide domains can be fused either directly to the PD-L2 polypeptide, either chemically or by recombinant expression from a single polynucleotide, or they may be joined via a linker or adapter molecule. A peptidyl linker or adapter molecule can be one or more amino acid residues, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, or 9 residues, or from about 10 to 50 amino acid residues, *e.g.*, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50 residues. A linker or adapter molecule can also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of the fused moieties. The peptide linker can be comprised of naturally occurring amino acids. Suitable peptide linkers include short peptides that are rich in glycine, serine, and glutamate amino acid residues, for example, linkers comprising: SG; GGGS (SEQ ID NO: 9); GSGEGEGSEGSG (SEQ ID NO: 10); and GGSEGEGSEGGS (SEQ ID NO: 11). Alternatively, the linker can be comprised of modified or non-naturally occurring amino acid residues.

[0041] Non-peptide linkers can also be used to couple domains of the fusion polypeptides described herein. For example, alkyl linkers such as -NH-(CH_2)S-C(O)-. These alkyl linkers may further be substituted by any non-sterically hindering group such as lower alkyl (e.g., C_1 - C_6) lower acyl, halogen (e.g., Cl, Br), CN, NH2, phenyl, etc. An exemplary non-peptide linker is a PEG linker which has a molecular weight of 100 to 5000 kD, preferably 100 to 500 kD.

[0042] In one embodiment, the PD-L2 fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 5 as shown below. This exemplary fusion polypeptide comprises the extracellular domain of the PD-L2 variant polypeptide as described *supra, i.e.*, the IgV domain and IgC domain (amino acid residues 21-203 of SEQ ID NO:1) containing one or more amino acid residue substitutions, coupled to a human IgG Fc portion. The PD-L2 portion of the fusion polypeptide of SEQ ID NO: 5 shown below is underlined, while the human IgG Fc portion is shown with a double underline. Optionally, the PD-L2 fusion polypeptide of SEQ ID NO: 5 further comprises a His-tag at its C-terminus.

LFTVTVPKELYIIEHGSNVTLECNFDTGSHVNLGAITASLQKVE**X**D**X**SPHRERATLLEEQ
LPLGKASFHIPQVQVRDEGQYQCIIIYGVA**X**DYKYLTLKVKASYRKINTHILKVPETDEV
ELTCQATGYPLAEVSWPNVSVPANTSHSRTPEGLYQVTSVLRLKPPPGRNFSCVFWNTH
VRELTLASIDLQSQMEPRTHPTSGEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK (SEQ ID NO: 5)

[0043] The PD-L2 portion of the fusion polypeptide of SEQ ID NO: 5 (shown above) contains one or more amino acid residue substitutions. In one embodiment, the amino acid substitution occurs at position 91 of SEQ ID NO: 5. The amino acid residue at position 91 of SEQ ID NO: 5 corresponds to the tryptophan at position 110 of SEQ ID NO: 1. Thus, in one embodiment, the amino acid residue at position 91 of SEQ ID NO: 5 (shown as X) is an amino acid residue other than tryptophan. In one embodiment, X at amino acid position 91 in SEQ ID NO: 5 is an alanine residue. In another embodiment, the amino acid substitution occurs at position 45 of SEQ ID NO: 5. The amino acid residue at position 45 of SEQ ID NO: 5 corresponds to the asparagine at position 64 of SEQ ID NO: 1. Thus, in one embodiment, the amino acid residue at position 45 of SEQ ID NO: 5 (shown as X) is an amino acid residue other

than asparagine. In one embodiment, X at position 45 in SEQ ID NO: 5 is a serine residue. In another embodiment, the amino acid substitution occurs at position 47 of SEQ ID NO: 5. The amino acid residue at position 47 of SEQ ID NO: 5 corresponds to the threonine at position 66 of SEQ ID NO: 1. Thus, in one embodiment, the amino acid residue at position 47 of SEQ ID NO: 5 (shown as X) is an amino acid residue other than threonine or serine. In one embodiment, the PD-L2 fusion polypeptide of SEQ ID NO: 5 contains one amino acid substitution (i.e., at one of positions 45, 47, or 91). In another embodiment, the PD-L2 fusion polypeptide of SEQ ID NO: 5 contains any two of the aforementioned amino acid substitutions. In another embodiment, the PD-L2 fusion polypeptide of SEQ ID NO: 5 comprises all three of the aforementioned amino acid substitutions.

[0044] Another embodiment of the present invention is directed to a multimeric protein comprising two or more PD-L2 fusion polypeptides as described herein coupled together. As used herein, the term "multimeric" refers to the stable association of two or more polypeptide chains either covalently, *e.g.*, by means of a disulfide bond, polypeptide bond, or a crosslinking agent, or non-covalently, e.g., by hydrophobic interaction. The term multimer is intended to encompass both homomultimers, wherein the subunits are the same, as well as, heteromultimers, wherein the subunits are different.

[0045] In one embodiment, two fusion proteins are linked to form dimers, i.e., heterodimers or homodimers. As described above, the two fusion proteins may associate covalently or non-covalently. In one embodiment, two PD-L2 fusion polypeptides, where the PD-L2 portion of the fusion polypeptides is a variant polypeptide as described herein, are coupled together to form a homodimer. In another embodiment, the PD-L2 fusion polypeptide as described herein (comprising a variant PD-L2 portion) is coupled together with a non-PD-L2 fusion polypeptide, *e.g.*, coupled to a PD-L1 fusion polypeptide, to form a heterodimer.

[0046] Another aspect of the present disclosure is directed to isolated nucleic acid molecules or polynucleotides encoding the PD-L2 variant protein or polypeptide as described herein, and isolated nucleic acid molecules or polynucleotides encoding the PD-L2 variant fusion polypeptides or proteins comprising the same.

[0047] In one embodiment, the nucleic acid molecule encoding a variant human PD-L2 protein or polypeptide has the nucleotide sequence of SEQ ID NO: 12 (shown below), where NNN (at positions 271, 272, and 273) is the codon encoding a tryptophan (W) residue at amino acid position 110 of the PD-L2 protein (SEQ ID NO:1). In one embodiment, the isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO: 12, or a fragment thereof, where NNN at positions 271, 272, and 273 encodes an amino acid residue other than

tryptophan, i.e., NNN is not TGG. In one embodiment, NNN is GCG, GCA, GCC, or GCT encoding an alanine residue.

[0048] In another embodiment, the nucleic acid molecule encoding a variant human PD-L2 protein or polypeptide has the nucleotide sequence of SEQ ID NO: 12 (shown below), where NNN (at positions 133, 134, 135 of SEQ ID NO: 12) is the codon encoding the asparagine (N) residue at amino acid position 64 of the PD-L2 protein (SEQ ID NO:1). In one embodiment, the isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO: 12, or a fragment thereof, where NNN at positions 133, 134, 135 encodes an amino acid residue other than asparagine, *i.e.*, where NNN is not AAT or AAC. In one embodiment, NNN is TCT, TCC, TCA, TCG, AGC, or AGT, encoding a serine residue.

[0049] In another embodiment, the nucleic acid molecule encoding a variant human PD-L2 protein or polypeptide has the nucleotide sequence of SEQ ID NO: 12 (shown below), where NNN (at positions 139, 140, 141) is the codon encoding a threonine (T) residue at amino acid position 66 of the PD-L2 protein (SEQ ID NO:1). In one embodiment, the isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO: 12, or a fragment thereof, where NNN at positions 139, 140, 141 encodes an amino acid residue other than threonine or serine, *i.e.*, NNN at positions 139, 140, and 141 is not TCT, TCC, TCA, TCG, AGT, AGC, ACT, ACC, ACA, or ACG.

CTGTTCACCGTGACAGTGCCCAAGGAGCTGTACATCATCGAGCACGGCTCTAACGTG
ACCCTGGAGTGCAATTTCGACACAGGCAGCCACGTGAACCTGGGCGCCATCACCGC
CAGCCTGCAGAAGGTGGAG**NNN**GAT**NNN**TCCCCTCACCGGGAGAGAGCCACACTGC
TGGAGGAGCAGCTGCCACTGGGCAAGGCCAGCTTTCACATCCCACAGGTGCAGGTG
AGGGACGAGGGACAGTACCAGTGCATCATCATCTATGGCGTGGCC**NNN**GATTACAA
GTATCTGACACTGAAGGTGAAGGCCTCCTACCGCAAGATCAACACCCACATCCTGA
AGGTGCCTGAGACAGACGAGGTGGAGCTGACCTGTCAGGCCACAGGCTATCCACTG
GCCGAGGTGTCTTGGCCCAACGTGAGCGTGCCTGCCAATACCAGCCACTCCCGGAC
ACCAGAGGGCCTGTATCAGGTGACCTCCGTGCTGAGGCTGAAGCCACCTCCAGGAC
GCAACTTCTCTTGCGTGTTCTGGAATACCCACGTGCGGGAGCTGACACTGGCCTCCA
TCGATCTGCAGTCTCAGATGGAGCCAAGGACCCACCCTACA

[0050] Suitable polynucleotide fragments of the variant PD-L2 nucleic acid molecule include fragments of SEQ ID NO: 12 encoding the extracellular domain, *i.e.*, amino acid residues 21-203 of the PD-L2 protein (SEQ ID NO: 1) or fragments thereof. Other suitable polynucleotide fragments of the variant PD-L2 nucleic acid molecule include fragments encoding the IgV domain of the extracellular domain, *i.e.*, amino acid residues 21-118 of the PD-L2 protein (SEQ ID NO: 1) or fragments thereof.

[0051] The nucleic acid molecules described herein include isolated polynucleotides, portions of expression vectors or portions of linear DNA sequences, including linear DNA

sequences used for *in vitro* transcription/translation, and vectors compatible with prokaryotic, eukaryotic or filamentous phage expression, secretion, and/or display of the variant PD-L2 protein or polypeptide and fusion polypeptides comprising said variant PD-L2 protein or polypeptide as described herein.

[0052] As described herein, an exemplary PD-L2 fusion polypeptide comprises the isolated variant PD-L2 protein or polypeptide as described *supra*, coupled to an Fc domain. A suitable nucleic acid molecule encoding that PD-L2/Fc fusion domain comprises the nucleotide sequence of SEQ ID NO: 12 or a polynucleotide fragment thereof, coupled to the nucleic acid molecule encoding the Fc domain. In one embodiment, the Fc domain is a human Fc domain. In one embodiment, the Fc domain is a human IgG Fc domain. In one embodiment, the Fc domain is a human IgG1 Fc domain encoded by the nucleotide sequence of SEQ ID NO: 13 as shown below.

GAGCCCAAGTCCTGCGACAAGACCCATACCTGTCCTCCTTGTCCCGCTCCTGAACTG
CTGGGCGGCCCTAGCGTGTTTCTGTTCCCCCCCAAGCCCAAGGACACCCTCATGATC
AGCAGGACACCCGAGGTCACCTGCGTCGTCGTGGACGTGTCCCACGAAGACCCCGA
GGTCAAGTTCAACTGGTACGTCGATGGCGTGGAGGTCCATAACGCCAAGACCAAGC
CCAGGGAGGAGCAGTACAACTCCACCTATAGGGTCGTGTCCGTGCTGACAGTCCTG
CACCAGGACTGGCTCAACGGCAAGGAGTATAAATGCAAGGTCAGCAATAAGGCCCT
CCCCGCCCCCATCGAGAAGACAATCTCCAAGGCTAAGGGCCAACCTAGGGAGCCTC
AAGTGTACACCCTGCCTCCTAGCAGAGACGAGCTGACCAAAAACCAGGTGAGCCTG
ACCTGTCTGGTGAAGGGCTTTTACCCCTCCGACATTGCCGTGGAGTGGGAGAGCAAC
GGCCAACCCGAAAACAACTACAAAACAACACCCCCTGTGCTGGACTCCGACGGCAG
CTTCTTCCTCTACTCCAAGCTGACCGTGGACAAAAGCAGGTGGCAGCAGGGCAACG
TCTTCAGCTGTTCCGTCATGCACGAGGCTCTGCACAACCACTACACCCAGAAAAGCC
TGTCCCTCAGCCCCGGCAAG (SEQ ID NO: 13).

[0053] In another embodiment, the nucleotide sequence encoding the human IgG1 Fc domain comprises one or more nucleotide base pair substitutions, insertions, or deletions such that the nucleotide sequence encodes a variant IgG1 Fc domain that favors heterodimerization with another Fc domain as discussed *supra*. Accordingly, in one embodiment, a variant of the human IgG1 Fc nucleotide sequence having SEQ ID NO: 13 is utilized in constructing a nucleic acid molecule encoding the fusion proteins described herein. For example, a suitable variant human IgG1 nucleic acid molecule is one that encodes an amino acid substitution of the threonine residue at the position corresponding to position 389 of the IgG1 full antibody (crystal structure IHZH). This variant nucleotide sequence is provided below as SEQ ID NO: 14.

GAGCCCAAGTCCTGCGACAAGACCCATACCTGTCCTCCTTGTCCCGCTCCTGAACTG
CTGGGCGGCCCTAGCGTGTTTCTGTTCCCCCCCAAGCCCAAGGACACCCTCATGATC
AGCAGGACACCCGAGGTCACCTGCGTCGTCGTGGACGTGTCCCACGAAGACCCCGA

GGTCAAGTTCAACTGGTACGTCGATGGCGTGGAGGTCCATAACGCCAAGACCAAGC
CCAGGGAGGAGCAGTACAACTCCACCTATAGGGTCGTGTCCGTGCTGACAGTCCTG
CACCAGGACTGGCTCAACGGCAAGGAGTATAAATGCAAGGTCAGCAATAAGGCCCT
CCCCGCCCCCATCGAGAAGACAATCTCCAAGGCTAAGGGCCAACCTAGGGAGCCTC
AAGTGTACACCCTGCCTCCTAGCAGAGACGAGCTGACCAAAAACCAGGTGAGCCTG
TGGTGTCTGGTGAAGGGCTTTTACCCCTCCGACATTGCCGTGGAGTGGGAGAGCAA
CGGCCAACCCGAAAACAACTACAAAACAACACCCCCTGTGCTGGACTCCGACGGCA
GCTTCTTCCTCTACTCCAAGCTGACCGTGGACAAAAGCAGGTGGCAGCAGGGCAAC
GTCTTCAGCTGTTCCGTCATGCACGAGGCTCTGCACAACCACTACACCCAGAAAAGC
CTGTCCCTCAGCCCCGGCAAG (SEQ ID NO: 14)

[0054] Another suitable variant human IgG1 nucleic acid molecule is one that encodes several amino acid substitutions that favor heterodimerization with an Fc domain encoded by the nucleotide sequence of SEQ ID NO: 14. These amino acid substitutions include a substitution of serine for threonine at the position corresponding to position 389 of the IgG1 full antibody (crystal structure IHZH); a substitution of alanine for leucine at the position corresponding to position 391 of the IgG1 full antibody (crystal structure IHZH); and a substitution of valine for tyrosine at the position corresponding to position 438 of the IgG1 full antibody (crystal structure IHZH). A suitable nucleotide sequence encoding an IgG1 Fc domain containing these amino acid substitutions is provided below as SEQ ID NO: 23.

GAGCCCAAGTCCTGCGACAAGACCCATACCTGTCCTCCTTGTCCCGCTCCTGAACTG
CTGGGCGGCCCTAGCGTGTTTCTGTTCCCCCCCAAGCCCAAGGACACCCTCATGATC
AGCAGGACACCCGAGGTCACCTGCGTCGTCGTGGACGTGTCCCACGAAGACCCCGA
GGTCAAGTTCAACTGGTACGTCGATGGCGTGGAGGTCCATAACGCCAAGACCAAGC
CCAGGGAGGAGCAGTACAACTCCACCTATAGGGTCGTGTCCGTGCTGACAGTCCTG
CACCAGGACTGGCTCAACGGCAAGGAGTATAAATGCAAGGTCAGCAATAAGGCCCT
CCCCGCCCCCATCGAGAAGACAATCTCCAAGGCTAAGGGCCAACCTAGGGAGCCTC
AAGTGTACACCCTGCCTCCTAGCAGAGACGAGCTGACCAAAAACCAGGTGAGCCTG
AGCTGT**GCG**GTGAAGGGCTTTTACCCCTCCGACATTGCCGTGGAGTGGGAGAGCAA
CGGCCAACCCGAAAACAACTACAAAACAACACCCCCTGTGCTGGACTCCGACGGCA
GCTTCTTCCTC**GTC**TCCAAGCTGACCGTGGACAAAAGCAGGTGGCAGCAGGGCAAC
GTCTTCAGCTGTTCCGTCATGCACGAGGCTCTGCACAACCACTACACCCAGAAAAGC
CTGTCCCTCAGCCCCGGCAAG (SEQ ID NO: 23)

[0055] The nucleic acid molecules of the present invention may be produced by chemical synthesis such as solid phase polynucleotide synthesis on an automated polynucleotide synthesizer and assembled into complete single or double stranded molecules. Alternatively, the polynucleotides of the invention may be produced by other techniques such a PCR followed by routine cloning. Techniques for producing or obtaining nucleic acid molecules of a given sequence are well known in the art.

[0056] The isolated nucleic acid molecules may comprise at least one non-coding sequence, such as a promoter or enhancer sequence, intron, polyadenylation signal, a cis sequence facilitating RepA binding, and the like. The polynucleotide sequences may also comprise additional sequences encoding additional amino acids that encode for example a marker or a tag sequence such as a histidine tag or an HA tag to facilitate purification or detection of the protein.

[0057] Another embodiment of the disclosure is directed to a vector comprising at least one polynucleotide as described herein. Such vectors may be plasmid vectors, viral vectors, vectors for baculovirus expression, transposon based vectors or any other vector suitable for introduction of the polynucleotides described herein into a given organism or genetic background by any means.

[0058] Another embodiment of the disclosure is directed to one or more expression vectors comprising the polynucleotides encoding the variant PD-L2 protein or polypeptide or a fusion protein comprising the variant PD-L2 protein or polypeptide as described herein. The polynucleotide sequences encoding the variant PD-L2 protein or polypeptide or a fusion protein comprising the variant PD-L2 protein or polypeptide disclosed herein are combined with sequences of promoter, signal peptide, translation initiation, 3′ untranslated region, polyadenylation, and transcription termination to form one or more expression vector constructs.

[0059] The promoter sequence of the expression construct is one that is suitable for driving expression of the variant PD-L2 polypeptide or fusion polypeptide. Suitable promoter sequences include, without limitation, the elongation factor 1-alpha promoter (EF1a) promoter, a phosphoglycerate kinase-1 promoter (PGK) promoter, a cytomegalovirus immediate early gene promoter (CMV), a chimeric liver-specific promoter (LSP) a cytomegalovirus enhancer/chicken beta-actin promoter (CAG), a tetracycline responsive promoter (TRE), a transthyretin promoter (TTR), a simian virus 40 promoter (SV40) and a CK6 promoter. Other promoters suitable for driving gene expression in mammalian cells that are known in the art are also suitable for incorporation into the expression constructs disclosed herein.

[0060] The expression construct can further encode a signal peptide that enhances protein expression. In one embodiment, the signal peptide comprises the amino acid sequence of DIATMRPTWAWWLFLVLLLALWAPARG (SEQ ID NO: 15). This signal peptide can be encoded by one of three nucleotide sequences shown below as SEQ ID NOs. 16, 17, and 18. Accordingly, the expression construct as described herein can further include any one of SEQ ID NOs: 16, 17, or 18 to enhance expression.

GATATTGCAACCATGAGACCTACCTGGGCTTGGTGGTTGTTCCTCGTGCTGCTGCTG GCTCTGTGGGCACCCGCTCGCGGT (SEQ ID NO: 16)

GACATCGCAACTATGAGACCTACCTGGGCTTGGTGGCTGTTCCTGGTGCTGCTGCTG GCTCTGTGGGCTCCTGCTCGGGGG (SEQ ID NO: 17)

GACATCGCTACTATGAGACCTACCTGGGCATGGTGGCTGTTCCTGGTGCTGCTGCTG GCTCTGTGGGCTCCCGCAAGGGGC (SEQ ID NO: 18)

[0061] The expression construct can further encode a linker sequence. The linker sequence can encode an amino acid sequence that spatially separates and/or links the one or more components of the expression construct.

[0062] Another aspect of the present invention is directed to a host cell comprising the vectors described herein. The variant PD-L2 polypeptide and PD-L2 fusion polypeptides described herein can be optionally produced by a cell line, a mixed cell line, an immortalized cell or clonal population of immortalized cells, as well known in the art (*see e.g.*, Ausubel et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, N.Y. (1987-2001); Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, N.Y. (1989); Harlow and Lane, Antibodies, a Laboratory Manual, Cold Spring Harbor, N.Y. (1989); Colligan et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, N.Y., (1997-2001), which are hereby incorporated by reference in their entirety).

[0063] The host cell chosen for expression may be of mammalian origin, *e.g.*, selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, HepG2, SP2/0, HeLa, myeloma, lymphoma, cells. Alternatively, the host cell may be of yeast, insect, or plant origin, or may be any derivative, immortalized or transformed cell thereof. Alternatively, the host cell may be selected from a species or organism incapable of glycosylating polypeptides, *e.g.*, a prokaryotic cell or organism, such as BL21, BL21(DE3), BL21-GOLD(DE3), XL1-Blue, JM109, HMS174, HMS174(DE3), and any of the natural or engineered *E. coli spp, Klebsiella*spp., or *Pseudomonas* spp strains.

[0064] Another aspect of the present invention is directed to a heterodimeric binding protein. This heterodimeric binding protein comprises (i) a first fusion polypeptide comprising an extracellular domain portion of a first B7 ligand coupled to a heterologous polypeptide domain; and (ii) a second fusion polypeptide comprising an extracellular domain portion of a second B7 ligand coupled to a heterologous polypeptide domain. In accordance with this aspect of the disclosure, the first and second B7 ligands of this heterodimeric binding protein are different ligands and are coupled together.

[0065] A "B7 ligand" as used herein refers to any member of the B7 family of immunoregulatory ligands. The B7 family comprises a family of structurally related, cell surface

protein ligands that bind to receptors on lymphocytes, *i.e.*, the CD28 family of receptors, to regulate immune responses. Known members of the B7 family which are suitable for inclusion in the heterodimeric binding protein of the present invention are provided in Table 1 below. The amino acid sequences of these B7 ligands are known in the art and provided in the Table below along with the extracellular domain information for each B7 ligand. The first B7 ligand and second B7 ligand of the heterodimeric binding protein of the present invention include any of these provided in Table 1. In one embodiment, the extracellular domain portion of the first or second B7 ligand includes the extracellular domain in its entirety or a fragment thereof. Alternatively, the extracellular domain portion of the first or second B7 ligand comprises the Ig-like V-type domain, or a fragment thereof. In another embodiment, the extracellular domain portion of the first or second B7 ligand comprises the Ig-like C-type domain, or a fragment thereof.

Table 1: B7 Ligands of the Heterodimeric Binding Protein

Name	Alternative Name	Binding Partner	EC Domain	UniProt Identifier	Amino Acid Sequence
B7.1	T-lymphocyte activation antigen CD80	CD28, CTLA-4, PD-L1	35-242 35-135 Ig-like V-type 145-230 Ig-like C2-type	P33681	MGHTRRQGTSPSKCPYLNFFQLL VLAGLSHFCSGVIHVTKEVKEVA TLSCGHNVSVEELAQTRIYWQKE KKMVLTMMSGDMNIWPEYKNR TIFDITNNLSIVILALRPSDEGTYE CVVLKYEKDAFKREHLAEVTLS VKADFPTPSISDFEIPTSNIRRIICS TSGGFPEPHLSWLENGEELNAIN TTVSQDPETELYAVSSKLDFNMT TNHSFMCLIKYGHLRVNQTFNW NTTKQEHFPDNLLPSWAITLISVN GIFVICCLTYCFAPRCRERRRNER LRRESVRPV **(SEQ ID NO: 3)**
B7.2	T-lymphocyte activation antigen CD86	CD28, CTLA-4	24-247 33-131 Ig-like V-type 150-225 Ig-like C2-type	P42081	MDPQCTMGLSNILFVMAFLLSG AAPLKIQAYFNETADLPCQFANS QNQSLSELVVFWQDQENLVLNE VYLGKEKFDSVHSKYMGRTSFD SDSWTLRLHNLQIKDKGLYQCII HHKKPTGMIRIHQMNSELSVLAN FSQPEIVPISNITENVYINLTCSSIH GYPEPKKMSVLLRTKNSTIEYDG VMQKSQDNVTELYDVSISLSVSF PDVTSNMTIFCILETDKTRLLSSP FSIELEDPQPPPDHIPWITAVLPTV IICVMVFCLILWKWKKKKRPRNS YKCGTNTMEREESEQTKKREKIH IPERSDEAQRVFKSSKTSSCDKSD

Name	Alternative Name	Binding Partner	EC Domain	UniProt Identifier	Amino Acid Sequence
					TCF **(SEQ ID NO: 24)**
B7-DC	PD-L2, CD273	PD-1	21-203 21-118 Ig-like V-type 122-203 Ig-like C-type	Q9BQ51	MIFLLLMLSLELQLHQIAALFTVT VPKELYIIEHGSNVTLECNFDTGS HVNLGAITASLQKVENDTSPHRE RATLLEEQLPLGKASFHIPQVQV RDEGQYQCIIIYGVAWDYKYLTL KVKASYRKINTHILKVPETDEVE LTCQATGYPLAEVSWPNVSVPA NTSHSRTPEGLYQVTSVLRLKPP PGRNFSCVFWNTHVRELTLASID LQSQMEPRTHPTWLLHIFIPFCIIA FIFIATVIALRKQLCQKLYSSKDT TKRPVTTTKREVNSAI **(SEQ ID NO: 25)**
B7-H1	PD-L1, CD274	PD-1, CD80	19-238 19-127 Ig-like V-type 133-225 Ig-like C2-type	Q9NZQ7	MRIFAVFIFMTYWHLLNAFTVTV PKDLYVVEYGSNMTIECKFPVEK QLDLAALIVYWEMEDKNIIQFVH GEEDLKVQHSSYRQRARLLKDQ LSLGNAALQITDVKLQDAGVYR CMISYGGADYKRITVKVNAPYN KINQRILVVDPVTSEHELTCQAE GYPKAEVIWTSSDHQVLSGKTTT TNSKREEKLFNVTSTLRINTTTNE IFYCTFRRLDPEENHTAELVIPEL PLAHPPNERTHLVILGAILLCLGV ALTFIFRLRKGRMMDVKKCGIQ DTNSKKQSDTHLEET **(SEQ ID NO: 2)**
B7-H2	inducible costimulator ligand (ICOS-L), CD275	ICOS	19-256 19-129 Ig-like V-type 141-227 Ig-like C2 type	O75144	MRLGSPGLLFLLFSSLRADTQEK EVRAMVGSDVELSCACPEGSRF DLNDVYVYWQTSESKTVVTYHI PQNSSLENVDSRYRNRALMSPA GMLRGDFSLRLFNVTPQDEQKF HCLVLSQSLGFQEVLSVEVTLHV AANFSVPVVSAPHSPSQDELTFT CTSINGYPRPNVYWINKTDNSLL DQALQNDTVFLNMRGLYDVVSV LRIARTPSVNIGCCIENVLLQQNL TVGSQTGNDIGERDKITENPVST GEKNAATWSILAVLCLLVVVAV AIGWVCRDRCLQHSYAGAWAV SPETELTGHV **(SEQ ID NO: 26)**
B7-H3	CD276		29-466 29-139 Ig-like V-type 1	Q5ZPR3	MLRRRGSPGMGVHVGAALGAL WFCLTGALEVQVPEDPVVALVG TDATLCCSFSPEPGFSLAQLNLIW QLTDTKQLVHSFAEGQDQGSAY ANRTALFPDLLAQGNASLRLQR

Name	Alternative Name	Binding Partner	EC Domain	UniProt Identifier	Amino Acid Sequence
			145-238 Ig-like C2-type 243-357 Ig-like V-type2 363-456 Ig-like C2-type 2		VRVADEGSFTCFVSIRDFGSAAV SLQVAAPYSKPSMTLEPNKDLRP GDTVTITCSSYQGYPEAEVFWQD GQGVPLTGNVTTSQMANEQGLF DVHSILRVVLGANGTYSCLVRNP VLQQDAHSSVTITPQRSPTGAVE VQVPEDPVVALVGTDATLRCSFS PEPGFSLAQLNLIWQLTDTKQLV HSFTEGRDQGSAYANRTALFPDL LAQGNASLRLQRVRVADEGSFT CFVSIRDFGSAAVSLQVAAPYSK PSMTLEPNKDLRPGDTVTITCSSY RGYPEAEVFWQDGQGVPLTGNV TTSQMANEQGLFDVHSVLRVVL GANGTYSCLVRNPVLQQDAHGS VTITGQPMTFPPEALWVTVGLSV CLIALLVALAFVCWRKIKQSCEE ENAGAEDQDGEGEGSKTALQPL KHSDSKEDDGQEIA **(SEQ ID NO: 27)**
B7-H4	VTCN1 (V-set domain-containing T-cell activation inhibitor 1)		35-241 35-146 Ig-like V-type 1 153-241 Ig-like V-type 2	Q7Z7D3	MASLGQILFWSIISIIIILAGAIALII GFGISGRHSITVTTVASAGNIGED GILSCTFEPDIKLSDIVIQWLKEG VLGLVHEFKEGKDELSEQDEMF RGRTAVFADQVIVGNASLRLKN VQLTDAGTYKCYIITSKGKGNAN LEYKTGAFSMPEVNVDYNASSE TLRCEAPRWFPQPTVV WASQVDQGANFSEVSNTSFELNS ENVTMKVVSVLYNVTINNTYSC MIENDIAKATGDIKVTESEIKRRS HLQLLNSKASLCVSSFFAISWAL LPLSPYLMLK **(SEQ ID NO: 28)**
B7-H5	VISTA(V-type Ig domain-containing suppressor of T cell activation);		33-168 Ig-like V-type	Q9H7M9	MGVPTALEAGSWRWGSLLFALF LAASLGPVAAFKVATPYSLYVCP EGQNVTLTCRLLGPVDKGHDVT FYKTWYRSSRGEVQTCSERRPIR NLTFQDLHLHHGGHQAANTSHD LAQRHGLESASDHHGNFSITMRN LTLLDSGLYCCLVVEIRHHHSEH RVHGAMELQVQTGKDAPSNCV VYPSSSQDSENITAAALATGACIV GILCLPLILLLVYKQRQAASNRR AQELVRMDSNIQGIENPGFEASPP AQGIPEAKVRHPLSYVAQRQPSE SGRHLLSEPSTPLSPPGPGDVFFP SLDPVPDSPNFEVI **(SEQ ID NO: 29)**

Name	Alternative Name	Binding Partner	EC Domain	UniProt Identifier	Amino Acid Sequence
B7-H6	NCR3LG (Natural cytotoxicity triggering receptor 3 ligand 1)	NKp30	27-244 27-138 Ig-like V-type 143-244 Ig-like C1-type	Q68D85	MTWRAAASTCAALLILLWALTT EGDLKVEMMAGGTQITPLNDNV TIFCNIFYSQPLNITSMGITWFWK SLTFDKEVKVFEFFGDHQEAFRP GAIVSPWRLKSGDASLRLPGIQL EEAGEYRCEVVVTPLKAQGTVQ LEVVASPASRLLLDQVGMKENE DKYMCESSGFYPEAINITWEKQT QKFPHPIEISEDVITGPTIKNMDG TFNVTSCLKLNSSQEDPGTVYQC VVRHASLHTPLRSNFTLTAARHS LSETEKTDNFSIHWWPISFIGVGL VLLIVLIPWKKICNKSSSAYTPLK CILKHWNSFDTQTLKKEHLIFFCT RAWPSYQLQDGEAWPPEGSVNI NTIQQLDVFCRQEGKWSEVPYV QAFFALRDNPDLCQCCRIDPALL TVTSGKSIDDNSTKSEKQTPREHS DAVPDAPILPVSPIWEPPPATTST TPVLSSQPPTLLLPLQ **(SEQ ID NO: 30)**
B7-H7	HHLA2; HERV-H LTR-associating protein 2	CD28H	133-328 138-222 Ig-like C-type 235-328 Ig-like V-type	Q9UM44	MKAQTALSFFLILITSLSGSQGIFP LAFFIYVPMNEQIVIGRLDEDIILP SSFERGSEVVIHWKYQDSYKVHS YYKGSDHLESQDPRYANRTSLFY NEIQNGNASLFFRRVSLLDEGIYT CYVGTAIQVITNKVVLKVGVFLT PVMKYEKRNTNSFLICSVLSVYP RPIITWKMDNTPIS ENNMEETGSLDSFSINSPLNITGS NSSYECTIENSLLKQTWTGRWT MKDGLHKMQSEHVSLSCQPVND YFSPNQDFKVTWSRMKSGTFSV LAYYLSSSQNTIINESRFSWNKEL INQSDFSMNLMDLNLSDSGEYLC NISSDEYTLLTIHTVHVEPSQETA SHNKGLWILVPSAILAAFLLIWSV KCCRAQLEARRSRHPADGAQQE RCCVPPGERCPSAPDNGEENVPL SGKV **(SEQ ID NO: 31)**

[0066] In one embodiment, the heterodimeric binding molecule as described herein is composed of (i) a first fusion polypeptide comprising a PD-L1 extracellular domain portion coupled to the heterologous polypeptide domain, *i.e.*, a PD-L1 fusion polypeptide, and (ii) a

second fusion polypeptide comprising a PD-L2 extracellular domain portion coupled to the heterologous polypeptide domain, *i.e.*, a PD-L2 fusion polypeptide.

[0067] The PD-L1 fusion polypeptide of the heterodimeric binding molecule comprises a PD-L1 extracellular domain. In one embodiment, the PD-L1 extracellular domain is derived from the human PD-L1 protein. In another embodiment the PD-L1 extracellular domain is derived from a non-human mammalian PD-L1 protein.

[0068] The amino acid sequence of human PD-L1 is shown below as SEQ ID NO: 2 (UniProtKB Identifier Q9NZQ7).

```
          10         20         30         40         50
MRIFAVFIFM TYWHLLNAFT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL
          60         70         80         90        100
AALIVYWEME DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ
         110        120        130        140        150
ITDVKLQDAG VYRCMISYGG ADYKRITVKV NAPYNKINQR ILVVDPVTSE
         160        170        180        190        200
HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL FNVTSTLRIN
         210        220        230        240        250
TTTNEIFYCT FRRLDPEENH TAELVIPELP LAHPPNERTH LVILGAILLC
         260        270        280        290
LGVALTFIFR LRKGRMMDVK KCGIQDTNSK KQSDTHLEET
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[0069] In one embodiment, the PD-L1 fusion polypeptide of the heterodimeric protein comprises an extracellular domain of human PD-L1 having the amino acid sequence of amino acid residues 19-127 of SEQ ID NO: 2 (*i.e.*, the IgV domain of the extracellular domain), or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 19-127 of SEQ ID NO: 2. In another embodiment, the extracellular domain of PD-L1 in the PD-L1 fusion polypeptide comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 19-127 of SEQ ID NO: 2.

[0070] In another embodiment, the PD-L1 fusion polypeptide of the heterodimeric protein comprises an extracellular domain of human PD-L1 having the amino acid sequence of amino acid residues 19-225 of SEQ ID NO: 2 (*i.e.*, the IgV and IgC domains of the extracellular domain), or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 19-225 of SEQ ID NO: 2. In another embodiment, the extracellular domain of PD-L1 in the PD-L1 fusion polypeptide comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 19-225 of SEQ ID NO: 2.

[0071] In another embodiment, the PD-L1 fusion polypeptide of the heterodimeric binding protein comprises a human PD-L1 extracellular domain fragment containing portions of the IgV and/or IgC domains of PD-L1, but the fragment is less than the full domains of one or

both the IgV and IgC domains. This PD-L1 extracellular domain fragment is shorter in length than the polypeptide comprising amino acid residues 19-225 of SEQ ID NO: 2, *i.e.*, it is less than or equal to 205 amino acids in length. In another embodiment, the PD-L1 extracellular domain fragment is shorter in length than the polypeptide comprising the amino acid residues 19-127 of SEQ ID NO: 2, *i.e.*, it is less than or equal to 107 amino acid residues in length. In accordance with this embodiment, the PD-L1 extracellular domain fragment of the PD-L1 fusion polypeptide comprises a fragment of the extracellular domain *(i.e.*, a fragment of residues 19-225 of SEQ ID NO: 2) that is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60., 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 200, or 205 amino acid residues in length.

[0072] The amino acid sequence of murine PD-L1 is shown below as SEQ ID NO: 19 (UniProtKB Identifier Q9EP73-1)

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          10         20         30         40         50
MRIFAGIIFT ACCHLLRAFT ITAPKDLYVV EYGSNVTMEC RFPVERELDL
          60         70         80         90        100
LALVVYWEKE DEQVIQFVAG EEDLKPQHSN FRGRASLPKD QLLKGNAALQ
         110        120        130        140        150
ITDVKLQDAG VYCCIISYGG ADYKRITLKV NAPYRKINQR ISVDPATSEH
         160        170        180        190        200
ELICQAEGYP EAEVIWTNSD HQPVSGKRSV TTSRTEGMLL NVTSSLRVNA
         210        220        230        240        250
TANDVFYCTF WRSQPGQNHT AELIIPELPA THPPQNRTHW VLLGSILLFL
         260        270        280        290
IVVSTVLLFL RKQVRMLDVE KCGVEDTSSK NRNDTQFEET
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[0073] In one embodiment, the PD-L1 fusion polypeptide of the heterodimeric protein comprises an extracellular domain of murine PD-L1 having the amino acid sequence of amino acid residues 19-127 of SEQ ID NO: 19, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 19-127 of SEQ ID NO: 19. In another embodiment, the extracellular domain of PD-L1 in the PD-L1 fusion polypeptide comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 19-127 of SEQ ID NO: 19.

[0074] In another embodiment, the PD-L1 fusion polypeptide of the heterodimeric protein comprises an extracellular domain of murine PD-L1 having the amino acid sequence of amino acid residues 19-224 of SEQ ID NO: 19, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 19-224 of SEQ ID NO: 19. In another embodiment, the extracellular domain of PD-L1 in the PD-L1 fusion polypeptide

comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 19-224 of SEQ ID NO: 19.

[0075] In another embodiment, the PD-L1 fusion polypeptide of the heterodimeric binding protein comprises a murine PD-L1 extracellular domain fragment containing portions of the IgV and/or IgC domains of PD-L1, but less than the full domains of one or both the IgV and IgC domains. This PD-L1 extracellular domain fragment is shorter in length than the polypeptide comprising amino acid residues 19-224 of SEQ ID NO: 19, *i.e.*, it is less than or equal to 204 amino acids in length. In another embodiment, the PD-L1 extracellular domain fragment is shorter in length than the polypeptide comprising the amino acid residues 19-127 of SEQ ID NO: 19, *i.e.*, it is less than or equal to 107 amino acid residues in length. In accordance with this embodiment, the PD-L1 extracellular domain fragment of the PD-L1 fusion polypeptide comprises a fragment of the extracellular domain (i.e., a fragment of residues 19-224 of SEQ ID NO: 19) that is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60., 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 200, or 205 amino acid residues in length.

[0076] The PD-L2 fusion polypeptide of the heterodimeric binding molecule comprises a PD-L2 extracellular domain polypeptide. In one embodiment, the PD-L2 extracellular domain polypeptide is derived from the human PD-L2 protein. In another embodiment the PD-L2 extracellular domain polypeptide is derived from a non-human mammalian PD-L2 protein.

[0077] The amino acid sequence of the human PD-L2 is provided herein as SEQ ID NO: 1, where X at position 110 of SEQ ID NO: 1 is a tryptophan. In one embodiment, the PD-L2 fusion polypeptide of the heterodimeric protein comprises an extracellular domain of human PD-L2 having the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, where X at position 110 of SEQ ID NO: 1 is any amino acid residue, X at position 64 of SEQ ID NO: 1 is any amino acid residue, and X at position 66 of SEQ ID NO: 1 is any amino acid residue. In another embodiment, the extracellular portion of the PD-L2 fusion polypeptide comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1. In one embodiment X at position 110 is a tryptophan residue. In another embodiment X at position 110 is a residue other than tryptophan. In another embodiment X at position 110 is an alanine residue. In another embodiment, X at position 110 is any residue that enhances the binding affinity of PD-L2 for PD-1. In another embodiment, X at

position 64 is an asparagine residue. In another embodiment, X at position 64 is a residue other than asparagine. In another embodiment, X at position 64 is a serine residue. In another embodiment, X at position 64 is any residue that enhances the binding affinity of PD-L2 for PD-1. In another embodiment, X at position 66 is a threonine residue. In another embodiment, X at position 66 is a residue other than threonine or serine. In another embodiment, X at position 66 is any residue that enhances the binding affinity of PD-L2 for PD-1. The PD-L2 extracellular domain portion of the fusion polypeptide comprising amino acid residues 21-118 of SEQ ID NO: 1 may contain any one, any two, or all three of the aforementioned substitutions at residues corresponding to residues 110, 64, and 66 of SEQ ID NO: 1.

[0078] In another embodiment, the PD-L2 fusion polypeptide of the heterodimeric protein comprises an extracellular domain of human PD-L2 having the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1, wherein X at position 110 of SEQ ID NO: 1 is any amino acid residue, X at position 64 of SEQ ID NO: 1 is any amino acid residue, and X at position 66 of SEQ ID NO: 1 is any amino acid residue. In another embodiment, the extracellular portion of the PD-L2 fusion polypeptide comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1. Within this PD-L2 extracellular domain polypeptide, X at the position corresponding to position 110 of SEQ ID NO: 1 is a tryptophan residue. In another embodiment X at the position corresponding to position 110 is an alanine residue. In another embodiment, X at the position corresponding to position 110 is any residue that enhances the binding affinity of PD-L2 for PD-1. In another embodiment, X at the position corresponding to position 64 of SEQ ID NO: 1 is an asparagine residue. In another embodiment, X at the position corresponding to position 64 is a residue other than asparagine. In another embodiment, X at the position corresponding to position 64 is a serine residue. In another embodiment, X at the position corresponding to position 64 is any residue that enhances the binding affinity of PD-L2 for PD-1. In another embodiment, X at the position corresponding to position 66 of SEQ ID NO: 1 is a threonine residue. In another embodiment, X at the position corresponding to position 66 is a residue other than threonine or serine. In another embodiment, X at the position corresponding to position 66 is any residue that enhances the binding affinity of PD-L2 for PD-1. The PD-L2 extracellular domain portion of the fusion polypeptide comprising amino acid residues 21-203 of SEQ ID NO: 1 may contain any one, any two, or all three of the aforementioned substitutions at residues corresponding to residues 110, 64, and 66 of SEQ ID NO: 1.

[0079] In another embodiment, the PD-L2 fusion polypeptide of the heterodimeric binding protein comprises a human PD-L2 extracellular domain fragment containing portions of the IgV and/or IgC domains of PD-L2, but less than the full domains of one or both the IgV and IgC domains. This PD-L2 variant polypeptide is shorter in length than the polypeptide comprising amino acid residues 21-203 of SEQ ID NO: 1, *i.e.*, it is less than or equal to180 amino acid residues in length. Alternatively, this PD-L2 variant polypeptide is shorter in length than the polypeptide comprising amino acid residues 21-118 of SEQ ID NO: 1, *i.e.*, it is less than or equal to 97 amino acid residues in length. In accordance with this embodiment, the PD-L2 extracellular domain fragment of the PD-L2 fusion polypeptide comprises a fragment of the extracellular domain that is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60., 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, or 180 amino acid residues in length. Within this PD-L2 extracellular domain fragment, X at the position corresponding to position 110 of SEQ ID NO: 1 is any amino acid residue, X at the position corresponding to position 64 of SEQ ID NO: 1 is any amino acid residue, and X at the position corresponding to position 66 of SEQ ID NO: 1 is any amino acid residue. In one embodiment X at the position corresponding to position 110 is a tryptophan residue. In another embodiment X at the position corresponding to position 110 is a residue other than tryptophan. In another embodiment X at the position corresponding to position 110 is an alanine residue. In another embodiment, X at the position corresponding to position 64 is an asparagine residue. In another embodiment, X at the position corresponding to position 64 is a residue other than asparagine. In another embodiment, X at the position corresponding to position 64 is a serine residue. In another embodiment, X at the position corresponding to position 66 is a threonine residue. In another embodiment, X at the position corresponding to position 66 is a residue other than threonine or serine residue. The PD-L2 extracellular domain portion of the fusion polypeptide comprising amino acid residues 21-203 of SEQ ID NO: 1 may contain any one, any two, or all three of the aforementioned substitutions at residues corresponding to residues 110, 64, and 66 of SEQ ID NO: 1.

[0080] In another embodiment, the PD-L2 fusion polypeptide comprises a murine PD-L2 fusion polypeptide. The amino acid sequence of the murine PD-L2 is provided below as SEQ ID NO: 20 (UnitProKB Identifier Q9WUL5-1).

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        10         20         30         40         50
MLLLLPILNL SLQLHPVAAL FTVTAPKEVY TVDVGSSVSL ECDFDRRECT
        60         70         80         90        100
ELEGIRASLQ KVENDTSLQS ERATLLEEQL PLGKALFHIP SVQVRDSGQY
       110        120        130        140        150
RCLVICGAAW DYKYLTVKVK ASYMRIDTRI LEVPGTGEVQ LTCQARGYPL
       160        170        180        190        200
AEVSWQNVSV PANTSHIRTP EGLYQVTSVL RLKPQPSRNF SCMFWNAHMK
       210        220        230        240
ELTSAIIDPL SRMEPKVPRT WPLHVFIPAC TIALIFLAIV IIQRKRI
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[0081] In one embodiment, the PD-L2 fusion polypeptide of the heterodimeric protein comprises an extracellular domain of murine PD-L2 having the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 20, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 20. In another embodiment, the extracellular portion of the PD-L2 fusion polypeptide comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 20.

[0082] In another embodiment, the PD-L2 fusion polypeptide of the heterodimeric protein comprises an extracellular domain of murine PD-L2 having the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 20, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 20. In another embodiment, the extracellular portion of the PD-L2 fusion polypeptide comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 20.

[0083] In another embodiment, the PD-L2 fusion polypeptide of the heterodimeric binding protein comprises a murine PD-L2 extracellular domain fragment containing portions of the IgV and/or IgC domains of PD-L2, but less than the full domains of one or both the IgV and IgC domains, *i.e.*, a smaller extracellular derived polypeptide than the polypeptide comprising amino acid residues 21-118 or 21-203 of SEQ ID NO: 20. In accordance with this embodiment, the PD-L2 extracellular domain fragment of the PD-L2 fusion polypeptide comprises a fragment of the extracellular domain (i.e., a fragment of residues 21-203 of SEQ ID NO: 20) that is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60., 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, or 180 amino acid residues in length.

[0084] The fusion polypeptides of the heterodimeric proteins described herein each comprise a heterologous polypeptide domain. Suitable heterologous polypeptide domains are described *supra*, e.g., tag domain, dimerization or multimerization domains, targeting domains,

stabilization domains, etc. Heterologous polypeptide domains can be fused directly to the B7 polypeptide, either chemically or by recombinant expression from a single polynucleotide. Alternatively, the heterologous polypeptide domain can be coupled to the B7 polypeptide via a linker or adapter molecule. Suitable linkers and adapter molecules are described *supra.*

[0085] In one embodiment, the heterologous polypeptide domain of each fusion polypeptide in the heterodimeric binding protein as described herein comprises an immunoglobulin Fc domain. Suitable Fc domains are described *supra.* In one embodiment, the Fc domain is a human Fc domain, e.g., a human IgG Fc domain. In one embodiment, the Fc domain is a human IgG Fc domain engineered to comprise one or more amino acid insertions, deletions, or substitutions that facilitate or favor heterodimerization with another Fc domain. Methods of engineering Fc domains to favor heterodimerization are known in the art, *see e.g.*, Ha et al., "Immunoglobulin Fc Heterodimer Platform Technology: From Design to Applications in Therapeutic Antibodies and Proteins," *Frontiers in Immunology* 7:1-16 (2016), which is hereby incorporated by reference in its entirety.

[0086] One modification to the Fc region, which was utilized in constructing the heterodimeric proteins described herein, involves amino acid substitutions of partner Fc regions to create a "knobs-into-holes" interaction between Fc regions of partnering fusion polypeptides (*see* U.S. Patent No. 8,216,805 to Carter et al., which is hereby incorporated by reference in its entirety). In this approach a "knob" is introduced into the C_H3 domain of the Fc region of the first fusion polypeptide by substituting a small residue with a bulky one (e.g. substitute a threonine with a tryptophan residue). In the C_H3 domain of the Fc region of the second fusion polypeptide a "hole" is created to accommodate the "knob" of the Fc region of the first fusion polypeptide. This "hole" is created by replacing the closest neighboring amino acid residues to the knob with smaller amino acid residues.

[0087] Other modifications to the Fc regions of the fusion polypeptides described herein to favor heterodimerization include the charge-to-charge swap design, where symmetric charge pairs at the CH3-interface are converted to an asymmetric charge pair. This approach is often referred to as the DD-KK approach. Yet another modification to the Fc region of the fusion polypeptides to favor heterodimerization includes the charge-to-steric complementarity swap and long-rang electrostatic interaction design. These modifications are well know to those of skill in the art and suitable for incorporation in the Fc regions of the fusion polypeptides that form the heterodimeric proteins as described herein (*see e.g.,* Ha et al., "Immunoglobulin Fc Heterodimer Platform Technology: From Design to Applications in Therapeutic Antibodies and Proteins,"

Frontiers in Immunology 7:1-16 (2016), which is hereby incorporated by reference in its entirety).

[0088] An exemplary heterodimeric binding protein of the present disclosure comprises a PD-L1 fusion polypeptide comprising an extracellular domain of PD-L1 as described above coupled to an Fc domain, and a PD-L2 fusion polypeptide comprising an extracellular domain of PD-L2 as described above coupled to an Fc domain.

[0089] The amino acid sequence of an exemplary PD-L1 fusion polypeptide (PD-L1-aFc) is provided below as SEQ ID NO:6. In this fusion polypeptide, the extracellular domain of human PD-L1 (residues 1-220 of SEQ ID NO: 6) is coupled to an Fc domain (residues 222-454 of SEQ ID NO: 6). The Fc region of this exemplary PD-L1 fusion polypeptide comprises an amino acid substitution of the threonine residue at the position corresponding to position 389 of the full IgG1 heavy chain (crystal structure IHZH) (residue 373 of SEQ ID NO: 6) to a bulky tryptophan residue to favor heterodimerization with the second fusion polypeptide of the heterodimeric protein.

PD-L1-aFc (Component 1 of 2 of human PD-L1/L2-heteroFc): SEQ ID NO: 6

FTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQ
HSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYN
KINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTS
TLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERSGEPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSL**W**CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKHHHHHH

[0090] The amino acid sequence of an exemplary PD-L2 fusion polypeptide (PD-L2-bFc) is provided below as SEQ ID NO: 7. This fusion polypeptide comprises an extracellular domain of human PD-L2 (residues 1-201 of SEQ ID NO: 7) coupled to an Fc domain (residues 204-435 of SEQ ID NO: 7). In this exemplary PD-L2 fusion polypeptide, X at position 91 of SEQ ID NO: 7 (corresponding to position 110 of SEQ ID NO: 1) is any amino acid residue. In one embodiment X at position 91 of SEQ ID NO: 7 is a tryptophan residue. In another embodiment X at position 91 of SEQ ID NO: 7 is an alanine residue. In another embodiment, X at position 91 of SEQ ID NO: 7 is any amino acid residue that enhances the binding affinity of PD-L2 for PD-1. In another embodiment, X at position 45 of SEQ ID NO: 7 (corresponding to amino acid residue position 64 of SEQ ID NO: 1) is any amino acid. In one embodiment X at position 45 of SEQ ID NO: 7 is an asparagine residue. In another embodiment X at position 45 of SEQ ID NO: 7 is a serine residue. In another embodiment, X at position 45 of SEQ ID NO: 7 is any amino acid residue that enhances the binding affinity of PD-L2 for PD-1. In another

embodiment, X at position 47 of SEQ ID NO: 7 (corresponding to amino acid residue position 66 of SEQ ID NO: 1) is any amino acid. In one embodiment X at position 47 of SEQ ID NO: 7 is a threonine residue. In another embodiment X at position 47 of SEQ ID NO: 7 is a residue other than threonine or serine. In another embodiment, X at position 47 of SEQ ID NO: 7 is any amino acid residue that enhances the binding affinity of PD-L2 for PD-1.

PD-L2-bFc (Component 2 of 2 of PD-L1/L2-heteroFc and component 2 of 2 of CD80/PD-L2-heteroFc): SEQ ID NO: 7

LFTVTVPKELYIIEHGSNVTLECNFDTGSHVNLGAITASLQKVE**X**D**X**SPHRERATLLEEQ
LPLGKASFHIPQVQVRDEGQYQCIIIYGVA**X**DYKYLTLKVKASYRKINTHILKVPETDEV
ELTCQATGYPLAEVSWPNVSVPANTSHSRTPEGLYQVTSVLRLKPPPGRNFSCVFWNTH
VRELTLASIDLQSQMEPRTHPTSGEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL**S**C**A**V
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL**V**SKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK

[0091] The Fc region of the exemplary PD-L2 fusion polypeptide (SEQ ID NO: 7) comprises several amino acid substitutions that favor heterodimerization with the first PD-L1 fusion polypeptide as described above. These substitutions include a substitution of threonine with serine at the position corresponding to position 389 of the full IgG1 antibody heavy chain (crystal structure IHZH) (residue 354 of SEQ ID NO: 7); a substitution of leucine with alanine at the position corresponding to position 391 of the IgG1 full antibody heavy chain (crystal structure IHZH) (residue 356 of SEQ ID NO: 7); and a substitution of tyrosine with at the position corresponding to position 438 of the IgG full antibody heavy chain (crystal structure IHZH) (residue 395 of SEQ ID NO: 7).

[0092] As described herein, the heterodimeric PD-L1/PD-L2 protein of the present disclosure, when present in a soluble form, activates T-cell immune response. Therefore, another embodiment of the present disclosure is directed to a pharmaceutical composition comprising the heterodimeric PD-L1/PD-L2 protein. Suitable components of this and other pharmaceutical compositions of the present disclosure are described herein. As described in more detail *infra*, pharmaceutical compositions comprising the heterodimeric PD-L1/PD-L2 proteins are suitable for use in methods of modulating the T-cell response in a subject. In particular, the PD-L1/PD-L2 heterodimeric protein as described herein is suitable for inducing a T cell response in a subject in need thereof. For example, the PD-L1/PD-L2 heterodimeric protein as described herein, is suitable for inducing a T cell response in a subject having a tumor and depressed anti-tumor T cell response. Administration of the PD-L1/PD-L2 heterodimeric

protein construct described herein to this subject will enhance the subject's anti-tumor T cell response.

[0093] In another embodiment, the heterodimeric binding molecule as described herein is composed of (i) a first fusion polypeptide comprising a murine PD-L1 extracellular domain portion coupled to a heterologous polypeptide domain, *i.e.*, a murine PD-L1 fusion polypeptide, and (ii) a second fusion polypeptide comprising a murine PD-L2 extracellular domain portion coupled to a heterologous polypeptide domain, *i.e.*, a murine PD-L2 fusion polypeptide.

[0094] The amino acid sequence of an exemplary PD-L1 fusion polypeptide (PD-L1-aFc) is provided below as SEQ ID NO: 21. This PD-L1 fusion polypeptide comprises an extracellular domain of murine PD-L1 (residues 1-221 of SEQ ID NO: 21) coupled to an Fc domain (residues 224-450 of SEQ ID NO: 21). The Fc region of this exemplary PD-L1 fusion polypeptide comprises an amino acid substitution of the threonine residue at the position 369 of SEQ ID NO: 221 with a bulky tryptophan residue (underlined) to favor heterodimerization with the second fusion polypeptide of the heterodimeric protein.

PD-L1-aFc (Component 1 of 2 of murine PD-L1/L2-heteroFc): SEQ ID NO: 21

FTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYWEKEDEQVIQFVAGEEDLKPQ
HSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCCIISYGGADYKRITLKVNAPYRK
INQRISVDPATSEHELICQAEGYPEAEVIWTNSDHQPVSGKRSVTTSRTEGMLLNVTSSL
RVNATANDVFYCTFWRSQPGQNHTAELIIPELPATHPPQNRTHSGVPRDCGCKPCICTVP
EVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQ
FNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKE
QMAKDKVSL**W**CMITDFFPEDITVEWQWNGQPAENYKNTQPIMNTNGSYFVYSKLNVQ
KSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGKHHHHHH

[0095] The amino acid sequence of an exemplary murine PD-L2 fusion polypeptide (PD-L2-bFc) is provided below as SEQ ID NO: 22. This PD-L2 fusion polypeptide comprises an extracellular domain of murine PD-L2 (residues 1-201 of SEQ ID NO: 22) coupled to an Fc domain (residues 204-430 of SEQ ID NO: 22).

PD-L2-bFc (Component 2 of 2 murine L1/L2-heteroFc): SEQ ID NO: 22

LFTVTAPKEVYTVDVGSSVSLECDFDRRECTELEGIRASLQKVENDTSLQSERATLLEEQ
LPLGKALFHIPSVQVRDSGQYRCLVICGAAWDYKYLTVKVKASYMRIDTRILEVPGTGE
VQLTCQARGYPLAEVSWQNVSVPANTSHIRTPEGLYQVTSVLRLKPQPSRNFSCMFWN
AHMKELTSAIIDPLSRMEPKVPRTSGVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLT
PKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLN
GKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSL**S**C**A**ITDFFPED
ITVEWQWNGQPAENYKNTQPIMNTNGSYFV**V**SKLNVQKSNWEAGNTFTCSVLHEGLH
NHHTEKSLSHSPGK

[0096] The Fc region of the exemplary murine PD-L2 fusion polypeptide (SEQ ID NO: 22) comprises several amino acid substitutions that favor heterodimerization with the first PD-L1 fusion polypeptide as described above. These substitutions include a substitution of threonine with a serine residue at position 349 of SEQ ID NO: 22; a substitution of leucine with an alanine residue at position 351 of SEQ ID NO: 22; and a substitution of tyrosine with a valine residue at position 390 of SEQ ID NO: 22.

[0097] In another embodiment, the heterodimeric binding molecule as described herein is composed of (i) a first fusion polypeptide comprising a CD80 extracellular domain portion coupled to a heterologous polypeptide domain, *i.e.*, a CD80 fusion polypeptide, and (ii) a second fusion polypeptide comprising a PD-L2 extracellular domain portion coupled to a heterologous polypeptide domain, *i.e.*, a PD-L2 fusion polypeptide.

[0098] The CD80 fusion polypeptide of the heterodimeric binding molecule comprises a CD80 extracellular domain coupled to a heterologous polypeptide domain. In one embodiment, the CD80 extracellular domain is derived from the human CD80 protein. In another embodiment the CD80 extracellular domain is derived from a non-human mammalian CD80 protein.

[0099] The amino acid sequence of human CD80 (also known as Human T-lymphocyte activation antigen; UniProt identifier: P33681-1) is shown below as SEQ ID NO: 3.

```
         10         20         30         40         50
MGHTRRQGTS PSKCPYLNFF QLLVLAGLSH FCSGVIHVTK EVKEVATLSC
        60         70         80         90        100
GHNVSVEELA QTRIYWQKEK KMVLTMMSGD MNIWPEYKNR TIFDITNNLS
       110        120        130        140        150
IVILALRPSD EGTYECVVLK YEKDAFKREH LAEVTLSVKA DFPTPSISDF
       160        170        180        190        200
EIPTSNIRRI ICSTSGGFPE PHLSWLENGE ELNAINTTVS QDPETELYAV
       210        220        230        240        250
SSKLDFNMTT NHSFMCLIKY GHLRVNQTFN WNTTKQEHFP DNLLPSWAIT
       260        270        280
LISVNGIFVI CCLTYCFAPR CRERRRNERL RRESVRPV
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[0100] In one embodiment, the CD80 fusion polypeptide of the heterodimeric binding protein comprises an extracellular domain of CD80 having the amino acid sequence of amino acid residues 35-135 of SEQ ID NO: 3, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 35-135 of SEQ ID NO: 3 (*i.e.*, the IgV region of the extracellular domain). In another embodiment, the extracellular domain of CD80 in the CD80 fusion polypeptide comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 35-135 of SEQ ID NO: 3. As described *supra*, the extracellular domain of

CD80 or fragment thereof is coupled to a heterologous polypeptide domain to create the CD80 fusion polypeptide of the heterodimeric binding protein.

[0101] In another embodiment, the CD80 fusion polypeptide of the heterodimeric binding protein comprises an extracellular domain of CD80 having the amino acid sequence of amino acid residues 35-230 of SEQ ID NO: 3 (*i.e.*, the IgV and IgC regions of the extracellular domain), or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 35-230 of SEQ ID NO: 3. In another embodiment, the extracellular domain of CD80 in the CD80 fusion polypeptide comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 35-230 of SEQ ID NO: 3.

[0102] In another embodiment, the CD80 fusion polypeptide of the heterodimeric binding protein comprises a CD80 extracellular domain fragment containing portions of the IgV and/or IgC domains of CD80 but less than the full domains of one or both the IgV and IgC domains. This CD80 extracellular domain fragment is shorter in length than the polypeptide comprising amino acid residues 35-230 of SEQ ID NO: 3, *i.e.*, it is less than or equal to 194 amino acid residues. In another embodiment, the CD80 extracellular domain fragment is shorter in length than the polypeptide comprising amino acid residues 35-135 of SEQ ID NO: 3, *i.e.*, it is less than or equal to 99 amino acid residues. In accordance with this embodiment, the CD80 extracellular domain fragment of the CD80 fusion polypeptide comprises a fragment of the extracellular domain (i.e., a fragment of residues 35-230 of SEQ ID NO: 3) that is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60., 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, or 190 amino acid residues in length.

[0103] The PD-L2 fusion polypeptide of the heterodimeric binding molecule comprises a PD-L2 extracellular domain. In one embodiment, the PD-L2 extracellular domain is derived from the human PD-L2 protein. In another embodiment the PD-L2 extracellular domain is derived from a non-human mammalian PD-L2 protein. The amino acid sequence of the human PD-L2 is provided herein as SEQ ID NO: 1, where X at position 110 of SEQ ID NO: 1 is a tryptophan residue, X at position 64 of SEQ ID NO:1 is an asparagine residue, and X at position 66 of SEQ ID NO: 1 is a threonine residue.

[0104] In one embodiment, the PD-L2 fusion polypeptide of the heterodimeric protein comprises an extracellular domain of PD-L2 having the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, wherein X at the positions corresponding to positions 64, 66, and 110 of SEQ ID NO: 1 are any amino acid

residue. In another embodiment, the extracellular portion PD-L2 of the PD-L2 fusion polypeptide comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1. In one embodiment X at the position corresponding to position 110 of SEQ ID NO: 1 is a tryptophan residue. In another embodiment X at the position corresponding to position 110 is an alanine residue. In another embodiment, X at the position corresponding to position 110 is any residue that enhances the binding affinity of PD-L2 for PD-1. In one embodiment X at the position corresponding to position 64 of SEQ ID NO: 1 is an asparagine residue. In another embodiment X at the position corresponding to position 64 is a serine residue. In another embodiment, X at the position corresponding to position 64 is any residue that enhances the binding affinity of PD-L2 for PD-1. In one embodiment X at the position corresponding to position 66 of SEQ ID NO: 1 is a threonine residue. In another embodiment X at the position corresponding to position 66 is any amino acid residue other than threonine or serine. In another embodiment, X at the position corresponding to position 66 is any residue that enhances the binding affinity of PD-L2 for PD-1.

[0105] In another embodiment, the PD-L2 fusion polypeptide of the heterodimeric protein comprises an extracellular domain of PD-L2 having the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1, wherein X at positions corresponding to positions 64, 66, 110 of SEQ ID NO: 1 are any amino acid residue. In another embodiment, the extracellular portion PD-L2 of the PD-L2 fusion polypeptide comprises an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1. In one embodiment X at the position corresponding to position 110 of SEQ ID NO: 1 is a tryptophan residue. In another embodiment X at the position corresponding to position 110 is an alanine residue. In another embodiment, X at the position corresponding to position 110 is any residue that enhances the binding affinity of PD-L2 for PD-1. In one embodiment X at the position corresponding to position 64 of SEQ ID NO: 1 is an asparagine residue. In another embodiment X at the position corresponding to position 64 is a serine residue. In another embodiment, X at the position corresponding to position 64 is any residue that enhances the binding affinity of PD-L2 for PD-1. In one embodiment X at the position corresponding to position 66 of SEQ ID NO: 1 is a threonine residue. In another embodiment X at the position corresponding to position 66 is an amino acid residue other than threonine or serine. In another

embodiment, X at the position corresponding to position 66 is any residue that enhances the binding affinity of PD-L2 for PD-1.

[0106] In another embodiment, the PD-L2 fusion polypeptide of the heterodimeric binding protein comprises a PD-L2 extracellular domain fragment containing portions of the IgV and/or IgC domains of PD-L2, but less than the full domains of one or both the IgV and IgC domains, *i.e.*, a smaller extracellular derived polypeptide than the polypeptide comprising amino acid residues 21-118 or 21-203 of SEQ ID NO: 1. In accordance with this embodiment, the PD-L2 extracellular domain fragment of the PD-L2 fusion polypeptide comprises a fragment of the extracellular domain (i.e., a fragment of residues 21-203 of SEQ ID NO: 1) that is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60., 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, or 180 amino acid residues in length.

[0107] The fusion polypeptides of the heterodimeric proteins described herein each comprise a heterologous polypeptide domain. Suitable heterologous polypeptide domains are described *supra*, e.g., tag domain, dimerization or multimerization domains, targeting domains, stabilization domains, etc.

[0108] In one embodiment, the fusion polypeptides of the heterodimeric proteins as described herein each comprise an immunoglobulin Fc domain. Suitable Fc domains are described *supra*. In one embodiment, the Fc domain is a human IgG Fc domain. In one embodiment, the Fc domain is a human IgG Fc domain engineered to comprise one or more amino acid substitutions that facilitate or favor heterodimerization with another Fc domain as described *supra*.

[0109] The amino acid sequence of an exemplary CD80 fusion polypeptide (CD80-aFc) of a heteromeric binding protein of the present disclosure is provided below as SEQ ID NO: 8. This exemplary CD80 fusion polypeptide comprises an extracellular domain of CD80 (residues 1-208 of SEQ ID NO: 8) coupled to an Fc domain (residues 211-442 of SEQ ID NO: 8). The Fc region of this exemplary CD80 fusion polypeptide comprises an amino acid substitution of the threonine residue at the position corresponding to position 389 of the IgG1 full antibody heavy chain (crystal structure IHZH) (residue 361 of SEQ ID NO: 8) with a bulky tryptophan residue to favor heterodimerization with the second fusion polypeptide of the heterodimeric protein.

CD80-aFc (Component 1 of 2 of CD80/PD-L2-heteroFc): SEQ ID NO: 8

VIHVTKEVKEVATLSCGHNVSVEELAQTRIYWQKEKKMVLTMMSGDMNIWPEYKNRTIFDITNN
LSIVILALRPSDEGTYECVVLKYEKDAFKREHLAEVTLSVKADFPTPSISDFEIPTSNIRRIICSTSGGF
PEPHLSWLENGEELNAINTTVSQDPETELYAVSSKLDFNMTTNHSFMCLIKYGHLRVNQTFNWN
TTKQEHFPDNSGEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE

KTISKAKGQPREPQVYTLPPSRDELTKNQVSL**W**CLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKHHHHHH

[0110] An exemplary PD-L2 fusion polypeptide comprising an extracellular domain of PD-L2 coupled to an Fc domain comprises the amino acid sequence of SEQ ID NO: 7 as shown *infra*, where X at positions 45, 47, and 91 of SEQ ID NO: 7 (corresponding to positions 64, 66, and 110 of SEQ ID NO: 1, respectively) are any amino acid residue. In one embodiment X at position 91 of SEQ ID NO: 7 is a tryptophan residue. In another embodiment X at position 91 of SEQ ID NO: 7 is an alanine residue, or any amino acid residue other than a tryptophan residue. In another embodiment, X at position 91 of SEQ ID NO: 7 is any amino acid residue that enhances the binding affinity of PD-L2 for PD-1. In one embodiment X at position 45 of SEQ ID NO: 7 is an asparagine residue. In another embodiment X at position 45 of SEQ ID NO: 7 is a serine residue, or any amino acid residue other than an asparagine residue. In another embodiment X at position 47 of SEQ ID NO: 7 is threonine residue. In another embodiment X at position 47 of SEQ ID NO: 7 is any amino acid residue other than a threonine or serine residue.

[0111] The Fc region of the exemplary PD-L2 fusion polypeptide (*i.e.*, residues 204-435 of SEQ ID NO: 7) comprises several amino acid substitutions that favor heterodimerization with the CD80 fusion polypeptide as described above. These substitutions include a substitution of serine for threonine at the position corresponding to position 389 of the IgG1 full antibody heavy chain (crystal structure IHZH) (residue 354 of SEQ ID NO: 7); a substitution of alanine for leucine at the position corresponding to position 391 of the IgG1 full antibody heavy chain (crystal structure IHZH) (residue 356 of SEQ ID NO: 7); and a substitution of valine for tyrosine at the position corresponding to position 438 of the IgG full antibody heavy chain (crystal structure IHZH) (residue 395 of SEQ ID NO: 7).

Pharmaceutical Compositions:

[0112] Pharmaceutical compositions comprising the immunomodulatory agents described herein, *i.e.*, the isolated variant PD-L2 protein or polypeptide, fusion proteins comprising the variant PD-L2 protein or polypeptide, homodimer and heterodimer binding proteins comprising the variant PD-L2 protein or polypeptide, and heterodimer binding proteins comprising first and second B7 fusion polypeptides, are within the scope of the present disclosure. Such pharmaceutical compositions comprise a therapeutically effective amount of one or more immunomodulatory agents as described herein, in admixture with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the

mode of administration. Acceptable formulation agents preferably are nontoxic to recipients at the dosages and concentrations employed.

[0113] The pharmaceutical composition can contain formulation agent(s) for modifying, maintaining, or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. Suitable formulation agents include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine, or lysine), antimicrobials, antioxidants (such as ascorbic acid, sodium sulfite, methionine or sodium hydrogen-sulfite), buffers (such as borate, bicarbonate, Tris-HCl, histidine, citrates, phosphates, or other organic acids), bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)), complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin, or hydroxypropyl-beta-cyclodextrin), fillers, monosaccharides, disaccharides, and other carbohydrates (such as glucose, mannose, or dextrins), proteins (such as serum albumin, gelatin, or immunoglobulins), coloring, flavoring and diluting agents, emulsifying agents, hydrophilic polymers (such as polyvinylpyrrolidone), low molecular weight polypeptides, salt-forming counterions (such as sodium), preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, or hydrogen peroxide), solvents (such as glycerin, propylene glycol, or polyethylene glycol), sugar alcohols (such as mannitol or sorbitol), suspending agents, surfactants or wetting agents (such as pluronics; PEG; sorbitan esters; polysorbates such as polysorbate 20 or polysorbate 80; triton; tromethamine; lecithin; cholesterol or tyloxapal), stability enhancing agents (such as sucrose or sorbitol), tonicity enhancing agents (such as alkali metal halides - preferably sodium or potassium chloride - or mannitol sorbitol), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants (*see, e.g.*, Remington's Pharmaceutical Sciences (18th Ed., A.R. Gennaro, ed., Mack Publishing Company 1990), and subsequent editions of the same, which are hereby incorporated by reference in their entirety for any purpose).

[0114] The optimal pharmaceutical composition will be determined by a skilled artisan depending upon, for example, the intended route of administration, delivery format, and desired dosage (*see, e.g.*, Remington's Pharmaceutical Sciences (18th Ed., A.R. Gennaro, ed., Mack Publishing Company 1990), which is hereby incorporated by reference in its entirety). Such compositions can influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the immunomodulatory agents described herein.

[0115] The primary vehicle or carrier in a pharmaceutical composition can be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier for injection can be

water, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise histidine or Tris buffer of about pH 6.0-8.5, which can further include sorbitol or a suitable substitute. In one embodiment of the present invention, pharmaceutical compositions comprising the immunomodulatory agents can be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (*see, e.g.*, Remington's Pharmaceutical Sciences (18th Ed., A.R. Gennaro, ed., Mack Publishing Company 1990), which is hereby incorporated by reference in its entirety) in the form of an aqueous solution.

[0116] The pharmaceutical compositions can be selected for parenteral delivery. Alternatively, the compositions can be selected for inhalation or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the skill of the art. The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 6 to about 8.

[0117] When parenteral administration is contemplated, the therapeutic compositions for use in this invention can be in the form of a pyrogen-free, parenterally acceptable, aqueous solution comprising the desired immunomodulatory fusion polypeptide or heterodimeric binding protein, in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which an immunomodulatory agent is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads, or liposomes, that provides for the controlled or sustained release of the product which can then be delivered via a depot injection. Hyaluronic acid can also be used, and this can have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

[0118] In one embodiment, a pharmaceutical composition can be formulated for inhalation. For example, the pharmaceutical composition can be formulated as a dry powder for inhalation. Inhalation solutions can also be formulated with a propellant for aerosol delivery. In yet another embodiment, solutions can be nebulized. Pulmonary administration is further

described in International Publication No. WO94/20069, which describes the pulmonary delivery of chemically modified proteins.

[0119] It is also contemplated that certain formulations can be administered orally. In one embodiment of the present invention, formulations that are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule can be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders can also be employed.

[0120] Another pharmaceutical composition can involve an effective quantity of an immunomodulatory agent as described herein in a mixture with non-toxic excipients that are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or another appropriate vehicle, solutions can be prepared in unit-dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

[0121] Additional pharmaceutical compositions will be evident to those skilled in the art, including formulations involving the immunomodulatory agents described herein, in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art (*see, e.g.*, International Publication No. WO93/15722, which describes the controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions, and Wischke & Schwendeman, *Int. J. Pharm.* 364: 298-327 (2008), and Freiberg & Zhu, *Int. J. Pharm.* 282: 1-18 (2004), which discuss microsphere/microparticle preparation and use, which are hereby incorporated by reference in their entirety).

[0122] Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, *e.g.* films, or microcapsules. Sustained release matrices can include polyesters, hydrogels, polylactides (*see e.g.*, U.S. Patent No. 3,773,919 and European Patent No. 0 058 481, which are hereby incorporated by reference in their entirety), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., *Biopolymers* 22: 547-56 (1983) which is hereby incorporated by reference in its entirety), poly(2-hydroxyethyl-methacrylate) (Langer et al., *J. Biomed. Mater. Res.* 15: 167-277 (1981) and Langer, *Chem.*

Tech. 12: 98-105 (1982), which are hereby incorporated by reference in their entirety), ethylene vinyl acetate or poly-D(-)-3-hydroxybutyric acid (European Patent No. 0 133 988, which is hereby incorporated by reference in its entirety). Sustained-release compositions can also include liposomes, which can be prepared by any of several methods known in the art. *See e.g.*, Epstein et al, *Proc. Natl. Acad. Sci. U.S.A.* 82: 3688-92 (1985) and European Patent Nos. EP0036676, EP0088046, and EP0143949, which are hereby incorporated by reference in their entirety.

[0123] The pharmaceutical composition to be used for in *vivo* administration typically should be sterile. This can be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method can be conducted either prior to, or following, lyophilization and reconstitution. The composition for parenteral administration can be stored in lyophilized form or in a solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle. The parenteral composition can be diluted into parenteral acceptable diluents (e.g., saline and 5% Dextrose).

[0124] Once the pharmaceutical composition has been formulated, it can be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. Such formulations can be stored either in a ready-to-use form or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

Methods of Use:

[0125] Another aspect of the present invention is directed to methods of modulating a subject's T-cell immune response. This method involves administering an immunomodulatory agent as described herein, or pharmaceutical compositions containing the same, to the subject in an amount effective to modulate said subject's T-cell immune response.

[0126] Some of the immunomodulatory agents provided herein are generally useful *in vivo* and *ex vivo* as immune response-stimulating therapeutics. In general, the disclosed immunomodulatory agents, *i.e.*, the isolated variant PD-L2 protein or polypeptide, fusion proteins comprising the variant PD-L2 protein or polypeptide, homodimer and heterodimer binding proteins comprising the variant PD-L2 protein or polypeptide, and heterodimer binding proteins comprising first and second B7 fusion polypeptides, are useful for treating a subject having or being predisposed to any disease or disorder to which the subject's immune system mounts an immune response. The ability of immunomodulatory agents to inhibit or reduce PD-1

signal transduction enables a more robust immune response to be possible. The disclosed compositions are useful to stimulate or enhance immune responses involving T cells

[0127] The disclosed immunomodulatory agents are useful for stimulating or enhancing an immune response in a subject having a tumor, in particular a subject having a tumor and a depressed anti-tumor T cell response, by administering to a subject an amount of an immunomodulatory agent effective to stimulate an anti-tumor T cell response in the subject. The types of tumors that may be treated with the provided compositions and methods include, but are not limited to, the following: bladder, brain, breast, cervical, colorectal, esophageal, kidney, liver, lung, nasopharyngeal, pancreatic, prostate, skin, stomach, uterine, ovarian, testicular, and hematologic.

[0128] Since the immunomodulatory agents described herein are generally useful *in vivo* and *ex vivo* as immune response-stimulating therapeutics, these compositions are useful for treating infections in which T cell exhaustion or T cell anergy has occurred causing the infection to remain with the host over a prolonged period of time. Exemplary infections to be treated are chronic infections cause by a hepatitis virus, a human immunodeficiency virus (HIV), a human T-lymphotrophic virus (HTLV), a herpes virus, an Epstein-Barr virus, or a human papilloma virus. It will be appreciated that other infections can also be treated using the immunomodulatory agents. The disclosed compositions are also useful as part of a vaccine. In a preferred embodiment, the type of disease to be treated or prevented is a chronic infectious disease caused by a bacterium, virus, protozoan, helminth, or other microbial pathogen that enters intracellularly and is attacked, *i.e.*, by cytotoxic T lymphocytes.

[0129] Chronic infections in human and animal models are associated with a failure of the host immune response to generate and sustain functional CD8+ and CD4+ T-cell populations, which also results in poor antibody responses to neutralize infectivity. This loss of function is referred to as T cell exhaustion. T cell anergy is a tolerance mechanism in which the lymphocyte is intrinsically functionally inactivated following an antigen encounter, but remains alive for an extended period of time in a hyporesponsive state. One method for treating chronic infection is to revitalize exhausted T cells or to reverse T cell exhaustion in a subject as well as overcoming T cell anergy. Reversal of T cell exhaustion can be achieved by interfering with the interaction between PD-1 and its ligands PD-L1 (B7-H1) and PD-L2 (PD-L2). Acute, often lethal, effects of pathogens can be mediated by toxins or other factors that fail to elicit a sufficient immune response prior to the damage caused by the toxin. This may be overcome by interfering with the interaction between PD-1 and its ligands, allowing for a more effective, rapid immune response.

[0130] Since viral infections are cleared primarily by T-cells, an increase in T-cell activity is therapeutically useful in situations where more rapid or thorough clearance of an infective viral agent would be beneficial to an animal or human subject. Thus, the immunomodulatory agents can be administered for the treatment of local or systemic viral infections, including, but not limited to, immunodeficiency (e.g., HIV), papilloma (e.g., HPV), herpes (e.g., HSV), encephalitis, influenza (e.g., human influenza virus A), and common cold (e.g., human rhinovirus) viral infections. For example, pharmaceutical formulations including the immunomodulatory agents can be administered topically to treat viral skin diseases such as herpes lesions or shingles, or genital warts. Pharmaceutical formulations of immunomodulatory compositions can also be administered to treat systemic viral diseases, including, but not limited to, AIDS, influenza, the common cold, or encephalitis.

[0131] Representative infections that can be treated, include but are not limited to infections cause by microorganisms including, but not limited to, *Actinomyces, Anabaena, Bacillus, Bacteroides, Bdellovibrio, Bordetella, Borrelia, Campylobacter, Caulobacter, Chlamydia, Chlorobium, Chromatium, Clostridium, Corynebacterium, Cytophaga, Deinococcus, Escherichia, Francisella, Halobacterium, Heliobacter, Haemophilus, Hemophilus influenza* type B (HIB), *Histoplasma, Hyphomicrobium, Legionella, Leishmania, Leptspirosis, Listeria, Meningococcus* A, B and C, *Methanobacterium, Micrococcus, Myobacterium, Mycoplasma, Myxococcus, Neisseria, Nitrobacter, Oscillatoria, Prochloron, Proteus, Pseudomonas, Phodospirillum, Rickettsia, Salmonella, Shigella, Spirillum, Spirochaeta, Staphylococcus, Streptococcus, Streptomyces, Sulfolobus, Thermoplasma, Thiobacillus*, and *Treponema, Vibrio, Yersinia, Cryptococcus neoformans, Histoplasma capsulatum, Candida albicans, Candida tropicalis, Nocardia asteroides, Rickettsia ricketsii, Rickettsia typhi, Mycoplasma pneumoniae, Chlamydial psittaci, Chlamydial trachomatis, Plasmodium falciparum, Plasmodium vivax, Trypanosoma brucei, Entamoeba histolytica, Toxoplasma gondii, Trichomonas vaginalis* and *Schistosoma mansoni.*

[0132] Some of the immunomodulatory agents provided herein are generally useful *in vivo* and *ex vivo* as immune response-suppressing therapeutics. These immunomodulatory agents are useful for treating a subject having or being predisposed to any disease or disorder to which the subject's immune system mounts an exaggerated or unwanted immune response, *e.g.*, an autoimmune condition. Autoimmune conditions that can be treated with such immunomodulatory agents of the invention include, without limitation, lupus erythematosus; Wiskott-Aldrich syndrome; autoimmune lymphoproliferative syndrome; myasthenia gravis; rheumatoid arthritis (RA); lupus nephritis; multiple sclerosis; systemic lupus erythematosis,

subacute cutaneous lupus erythematosus, cutaneous lupus erythematosus including chilblain lupus erythematosus, chronic arthritis, Sjogren's syndrome, autoimmune nephritis, autoimmune vasculitis, autoimmune hepatitis, autoimmune carditis, autoimmune encephalitis, autoimmune mediated hematological disease, inflammatory chronic rhinosinusitis, colitis, celiac disease, inflammatory bowel disease, Barrett's esophagus, and/or inflammatory gastritis.

[0133] Pharmaceutical compositions including immunomodulatory agents described herein are provided *supra.* Pharmaceutical compositions containing peptides or polypeptides may be for administration by parenteral (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), transdermal (either passively or using iontophoresis or electroporation), or transmucosal (nasal, vaginal, rectal, or sublingual) routes of administration. The compositions may also be administered using bioerodible inserts and may be delivered directly to an appropriate lymphoid tissue (e.g., spleen, lymph node, or mucosal-associated lymphoid tissue) or directly to an organ or tumor. The compositions can be formulated in dosage forms appropriate for each route of administration.

[0134] As used herein the term “effective amount” or “therapeutically effective amount” means a dosage sufficient to treat, inhibit, or alleviate one or more symptoms of the disorder being treated or to otherwise provide a desired pharmacologic and/or physiologic effect. The precise dosage will vary according to a variety of factors such as subject-dependent variables (e.g., age, immune system health, etc.), the disease, and the treatment being effected. Therapeutically effective amounts of immunomodulatory agents cause an immune response to be activated, enhanced, augmented, or sustained, and/or overcome or alleviate T cell exhaustion and/or T cell anergy, and/or activate monocytes, macrophages, dendritic cells and other antigen presenting cells (“APCs”).

[0135] In a preferred embodiment, the immunomodulatory agent is administered in a range of 0.1-20 mg/kg based on extrapolation from tumor modeling and bioavailability. A most preferred range is 5-20 mg of immunomodulatory agent/kg. Generally, for intravenous injection or infusion, dosage may be lower than when administered by an alternative route.

EXAMPLES

[0136] Examples are provided below to illustrate the present invention. These examples are not meant to constrain the present invention to any particular application or theory of operation

Example 1 – PD-L2 Variant Proteins and Polypeptides Having Enhanced PD-1 Binding

[0137] It is widely accepted that PD-L2 has a 2- to 6-fold stronger affinity for PD-1 than that of PD-L1. To date this affinity difference has been attributed to the conserved $W110_{PD\text{-}L2}$ residue since in the mPD-1/mPD-L2 (3BP5) complex crystal structure this Trp fits snuggly into a pocket on PD-1's front face (see Fig. 1D & 1F). The corresponding residue on PD-L1 $A121_{PD\text{-}L1}$ makes a comparatively flat contact with the PD-1 receptor (Fig. 1C & 1E). The data presented herein validates a ~4 fold affinity discrepancy between the two ligands (Fig. 1J &1K), but it contradicts $W110_{PD\text{-}L2}$'s reported roll. Instead of $W110_{PD\text{-}L2}$ contributing to enhanced binding affinity to PD-1, it was unexpectedly found that the $W110_{PD\text{-}L2}$ residue *hinders* PD-1 binding (Fig. 1I-1L). The $W110A_{PD\text{-}L2}$ mutation extends PD-L2's off-rate and thus improves its affinity for PD-1, while $A121W_{PD\text{-}L1}$ exerts the opposite effect on PD-L1 (Fig. 1H-1K). Thus, it was concluded that $W110_{PD\text{-}L2}$ acts as an elbow to prevent PD-L2 from binding to PD-1 too tightly.

[0138] Most receptor-ligand interactions in the immunological synapse are weak in order to provide a fine balance of stimulatory versus inhibitory inputs to T cells. The inventors propose that the evolutionary insertion of W110 into PD-L2 is an example of this evolutionary-honing to ensure that the PD-L2 displayed on APCs does not bind to and inhibit T cells via PD-1 too firmly. Thus incorporating the W110A mutation into biologics which harbor the PD-L2 extracellular domain should render them slightly more potent. This is exemplified by PD-L2-W110A's improved ability to inhibit T cell proliferation when adsorbed on plastic along with anti-CD-3 (Fig. 3).

[0139] Generally, N-linked glycosylation sites on extracellular domains of proteins are thought to help stabilize and solubilize the protein and rarely play a role in protein-protein interactions. However, the inventors found that removing the glycosylation site closest to PD-L2's PD-1 binding interface resulted in enhanced PD-1 binding (Fig. 2). The $N64S_{PD\text{-}L2}$ mutant exhibited a longer off-rate for PD-1 than its wild type counterpart (Fig. 2C & 2G). Moreover, the $N64S_{PD\text{-}L2}$ mutant is strikingly potent at inhibiting T cell proliferation (Fig. 3F & 3H). Biologics which harbor PD-L2's extracellular domain will be significantly more potent if they mutate the N64 glycosylation site.

Example 2 – PD-L1/PD-L2 Heterodimeric Fc-Fusion Proteins as Immunomodulatory Compositions for Modulating T-cell Immune Response

[0140] PD-L1 is known to bind to both PD-1 and CD80. PD-L2 is a known ligand for PD-1, but investigators have long suggested that it may have an elusive binding partner. Moreover, PD-L1 is fairly ubiquitously expressed, while PD-L2 is curiously restricted mainly to APCs. The data herein points to the possibility that the PD-Ligands may homo- or hetero-oligomerize naturally when restricted to the 2D mobility of a cell membrane (Fig. 4). While the matter is still up for debate, the possibility that PD-L1 and PD-L2 exert slightly different or complementary inputs to T cells remains a prospect. In order to investigate this, a series of Fc fusion proteins including a heterodimeric PD-L1/PD-L2 Fc fusion were constructed in order to force dimerization. The "knob-in-hole" technique of Fc heterodimerization was utilized and a restriction site linker was incorporated within the fusion protein so that different versions of PD-ligands and/or B7 proteins could be easily incorporated. Initially, a PD-L1-Fc (homodimer), a PD-L2-Fc (homodimer), and a PD-L1/L2-heteroFc were created and their affinity for PD-1 was tested along with their ability to inhibit activated T cells and modulate immune responses in vitro and in vivo (Figs. 5, 6 and 7).

[0141] All three of these B7-protein-Fc fusions had a stronger affinity for PD-1 than anticipated. Upon study of published human PD-L1 dimeric crystal structures it was hypothesized that all B7-proteins may be able to form a quaternarily stabilized confirmation in which their IgC domains form main-chain interactions that stabilize each other in a conformation that leaves their front-facing IgV domain binding sites exposed and stable. This stabilizing conformation may only be relevant in the context of these fusion proteins, which are tethered in a close, dimeric form due to the Fc region. However, preliminary evidence indicates that the PD-Ligands oligomerize naturally on a cell membrane, including in a hetero-combination with PD-L1 and PD-L2 (Fig. 4). Thus, a PD-L1/L2-heteroFc fusion protein may stably display a heterodimeric B7 combination that has a naturally occurring, biological effect.

[0142] It was determined that the PD-L1/L2-heteroFc harbored an improved ability to inhibit T cells when adsorbed along with activating anti-CD-3 as compared to PD-L1-Fc and PD-L2-Fc (Fig. 6). PD-L1/L2-heteroFc also displayed an improved ability to inhibit SEB activated PBMC reactions compared with PD-L1-Fc and PD-L2-Fc (Fig. 7). Murine versions of these three Fc fusion constructs also demonstrated an ability to decrease tumor burden in vivo in the MC38 syngeneic tumor model (Fig. 7).

[0143] Other heterodimeric B7 protein combinations are expected to form stabilizing conformations with novel phenotypes as well. One such construct is a CD80/PD-L2-heteroFc (Fig. 6). Some heterodimeric B7 fusion pairs will serve as immune-stimulating-anti-cancer biologics, while other combinations will be immune-inhibiting-autoimmune therapeutics. Additionally each combination can be used as a scientific tool to better understand the costimulatory and inhibitory T cell network, especially if evidence arises that certain heterodimeric pairs of B7's dimerize in the 2D plain of the plasma membrane/T cell synapse.

[0144] Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

WHAT IS CLAIMED IS:

1. A heterodimeric binding molecule comprising:

(i) a first fusion polypeptide comprising an extracellular domain portion of a first B7 ligand coupled to a heterologous polypeptide domain; and

(ii) a second fusion polypeptide comprising an extracellular domain portion of a second B7 ligand coupled to a heterologous polypeptide domain, wherein said first and second B7 ligands are different ligands and wherein said first and second fusion polypeptides are coupled together.

2. The heterodimeric binding molecule of claim 1, wherein the first fusion polypeptide comprises a PD-L1 extracellular domain portion coupled to the heterologous polypeptide domain, and the second fusion polypeptide comprises a PD-L2 extracellular domain portion coupled to the heterologous polypeptide domain.

3. The heterodimeric binding molecule of claim 2, wherein the PD-L1 extracellular domain portion of the first fusion polypeptide comprises the amino acid sequence of amino acid residues 19-127 of SEQ ID NO: 2, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 19-127 of SEQ ID NO: 2.

4. The heterodimeric binding molecule of claim 2, wherein the PD-L1 extracellular domain portion of the first fusion polypeptide comprises the amino acid sequence of amino acid residues 19-225 of SEQ ID NO: 2, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 19-225 of SEQ ID NO: 2.

5. The heterodimeric binding molecule of claim 2, wherein the PD-L2 extracellular domain portion of the second fusion polypeptide comprises the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, wherein X at positions 64, 66, and 110 of SEQ ID NO: 1 is any amino acid residue.

6. The heterodimeric binding molecule of claim 2, wherein the PD-L2 extracellular domain portion of the second fusion polypeptide comprises the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1, wherein X at positions 64, 66 and 110 of SEQ ID NO: 1 is any amino acid residue.

7. The heterodimeric binding molecule of claim 5 or claim 6, wherein X at position 110 of SEQ ID NO: 1 is a tryptophan.

8 The heterodimeric binding molecule of claim 5 or claim 6, wherein X at position 110 of SEQ ID NO: 1 is any amino acid residue except tryptophan.

9. The heterodimeric binding molecule of claim 5 or claim 6, wherein X at position 64 of SEQ ID NO: 1 is an asparagine.

10. The heterodimeric binding molecule of claim 5 or claim 6, wherein X at position 64 of SEQ ID NO: 1 is any amino acid residue except asparagine.

11. The heterodimeric binding molecule of claim 5 or claim 6, wherein X at position 66 of SEQ ID NO: 1 is a threonine.

12. The heterodimeric binding molecule of claim 5 or claim 6, wherein X at position 66 of SEQ ID NO: 1 is any amino acid residue except threonine or serine.

13. The heterodimeric binding molecule of claim 1, wherein the first fusion polypeptide comprises a CD80 extracellular domain portion coupled to the heterologous polypeptide domain, and the second fusion polypeptide comprises a PD-L2 extracellular domain portion coupled to the heterologous polypeptide domain.

14. The heterodimeric binding molecule of claim 13, wherein the CD80 extracellular domain portion of the first fusion polypeptide comprises the amino acid sequence of amino acid residues 35-135 of SEQ ID NO: 3, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 35-135 of SEQ ID NO: 3.

15. The heterodimeric binding molecule of claim 13, wherein the CD80 extracellular domain portion of the first fusion polypeptide comprises the amino acid sequence of amino acid residues 35-230 of SEQ ID NO: 3, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 35-230 of SEQ ID NO: 3.

16. The heterodimeric binding molecule of claim 13, wherein the PD-L2 extracellular domain portion of the second fusion polypeptide comprises the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, wherein X at positions 64, 66, and 110 of SEQ ID NO: 1 is any amino acid residue.

17. The heterodimeric binding molecule of claim 13, wherein the PD-L2 extracellular domain portion of the second fusion polypeptide comprises the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1, wherein X at positions 64, 66, and 110 of SEQ ID NO: 1 is any amino acid residue.

18. The heterodimeric binding molecule of claim 16 or claim 17, wherein X at position 110 of SEQ ID NO: 1 is a tryptophan.

19. The heterodimeric binding molecule of claim 16 or claim 17, wherein X at position 110 of SEQ ID NO: 1 is any amino acid residue except tryptophan.

20. The heterodimeric binding molecule of claim 16 or claim 17, wherein X at position 64 of SEQ ID NO: 1 is an asparagine.

21. The heterodimeric binding molecule of claim 16 or claim 17, wherein X at position 64 of SEQ ID NO: 1 is any amino acid residue except asparagine.

22. The heterodimeric binding molecule of claim 16 or claim 17, wherein X at position 66 of SEQ ID NO: 1 is a threonine.

23. The heterodimeric binding molecule of claim 16 or claim 17, wherein X at position 66 of SEQ ID NO: 1 is any amino acid residue except threonine or serine.

24. The heterodimeric binding molecule of any of claims 1-23, wherein the heterologous polypeptide domain of each of the first and second fusion polypeptides of the heterodimeric binding molecule comprises a dimerization domain and said first and second fusion polypeptide are covalently coupled together via said dimerization domains.

25. The heterodimeric binding molecule of any of claims 1-23, wherein the heterologous polypeptide domain of each of the first and second fusion polypeptides of the heterodimeric binding molecule comprises a dimerization domain and said first and second fusion polypeptide are non-covalently coupled together via said dimerization domains.

26. The heterodimeric binding molecule of any one of claims 1-25, wherein the heterologous polypeptide domain of each of the first and second fusion polypeptides of the heterodimeric binding molecule comprises an immunoglobulin Fc domain.

27. The heterodimeric binding molecule of claim 26, wherein the immunoglobulin Fc domain of each first and second fusion polypeptides is a human IgG Fc domain.

28. The heterodimeric binding molecule of claim 26, wherein the immunoglobulin Fc domain comprises an amino acid sequence derived from the amino acid sequence of SEQ ID NO: 4.

29. The heterodimeric binding molecule of claim 26, wherein the immunoglobulin Fc domain of each first and second fusion polypeptide comprise one or more amino acid substitutions that favor heterodimerization between the Fc domain of the first and second fusion polypeptides.

30. The heterodimeric binding molecule of claim 1, wherein

(i) the first fusion polypeptide further comprises a linker portion coupling said extracellular domain portion of the first B7 ligand to the heterologous polypeptide domain, and

(ii) the second fusion polypeptide further comprises a linker portion coupling said extracellular domain portion of the second B7 ligand to the heterologous polypeptide domain.

31. A pharmaceutical composition comprising:

the heteromeric binding molecule of any one of claims 1-30 and

a pharmaceutically acceptable carrier.

32. A method of modulating a subject's T-cell immune response, said method comprising:

administering the pharmaceutical composition of claim 31 to the subject in an amount effective to modulate said subject's T-cell immune response.

33. The method of claim 32 further comprising:

selecting a subject having a tumor and a depressed anti-tumor T-cell response, wherein said administering enhances the subject's anti-tumor T-cell response.

34. The method of claim 32 further comprising:

selecting a subject having an autoimmune condition, wherein said administering depresses the subject's T-cell autoimmune response.

35. An isolated polypeptide comprising:

an extracellular domain portion of PD-L2 comprising the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-118 of SEQ ID NO: 1, wherein said extracellular domain portion of PD-L2 comprises an amino acid residue substitution at one or more positions 64, 66, and 110 of SEQ ID NO: 1.

36. The isolated polypeptide of claim 35, wherein said polypeptide comprises the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 21-203 of SEQ ID NO: 1.

37. The isolated polypeptide of claim 35, wherein said polypeptide comprises the amino acid sequence of amino acid residues 1-203 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 1-203 of SEQ ID NO: 1.

38. The isolated polypeptide of claim 35, wherein said polypeptide comprises the amino acid sequence of amino acid residues 1-273 of SEQ ID NO: 1, or an amino acid sequence having at least 85% sequence identity to the amino acid sequence of amino acid residues 1-273 of SEQ ID NO: 1.

39. The isolated polypeptide of any one of claims 35-38, wherein said amino acid residue substitution at position 64 of SEQ ID NO: 1 comprises a substitution of asparagine with another amino acid residue, said amino acid residue substitution at position 66 of SEQ ID NO: 1 comprises a substitution of threonine with an amino acid residue other than serine, and said amino acid residue substitution at position 110 of SEQ ID NO: 1 comprises a substitution of tryptophan with another amino acid residue.

40. The isolated polypeptide of claim 39, wherein the amino acid residue substitution at position 64 of SEQ ID NO: 1 is an asparagine to serine substitution.

41. The isolated polypeptide of claim 39, wherein the amino acid residue substitution at position 66 of SEQ ID NO: 1 is a threonine to any amino acid residue other than serine substitution.

42. The isolated polypeptide of claim 39, wherein the amino acid residue substitution at position 110 of SEQ ID NO: 1 is a tryptophan to alanine substitution.

43. A polynucleotide encoding the isolated polypeptide of any one of claims 35-42.

44. An expression vector comprising the polynucleotide of claim 43.

45. A host cell comprising the expression vector of claim 44.

46. A fusion polypeptide comprising:
the isolated polypeptide of any one of claims 35-42 and
a heterologous polypeptide domain coupled to said isolated polypeptide at its carboxy terminus.

47. The fusion polypeptide of claim 46, wherein the heterologous polypeptide domain comprises an immunoglobulin Fc domain.

48. The fusion polypeptide of claim 47, wherein the immunoglobulin Fc domain is an IgG Fc domain.

49. The fusion polypeptide of claim 47, wherein the immunoglobulin Fc domain is a human IgG Fc domain.

50. The fusion polypeptide of claim 47, wherein the immunoglobulin Fc domain is derived from the amino acid sequence of SEQ ID NO: 4.

51. The fusion polypeptide of claim 46 further comprising:
a linker portion coupling said isolated polypeptide and said heterologous polypeptide domain.

52. A multimeric protein comprising two or more fusion polypeptides of claim 46 coupled together.

53. The multimeric protein of claim 52, wherein said heterologous polypeptide domain of each of said two or more fusion polypeptides comprises a dimerization domain, and said two or more fusion polypeptides of the multimeric protein are covalently coupled via said dimerization domains.

54. The multimeric protein of claim 52, wherein said heterologous polypeptide domain of each of said two or more fusion polypeptides comprises a dimerization

domain, and said two or more fusion polypeptides of the multimeric protein are non-covalently coupled via said dimerization domains.

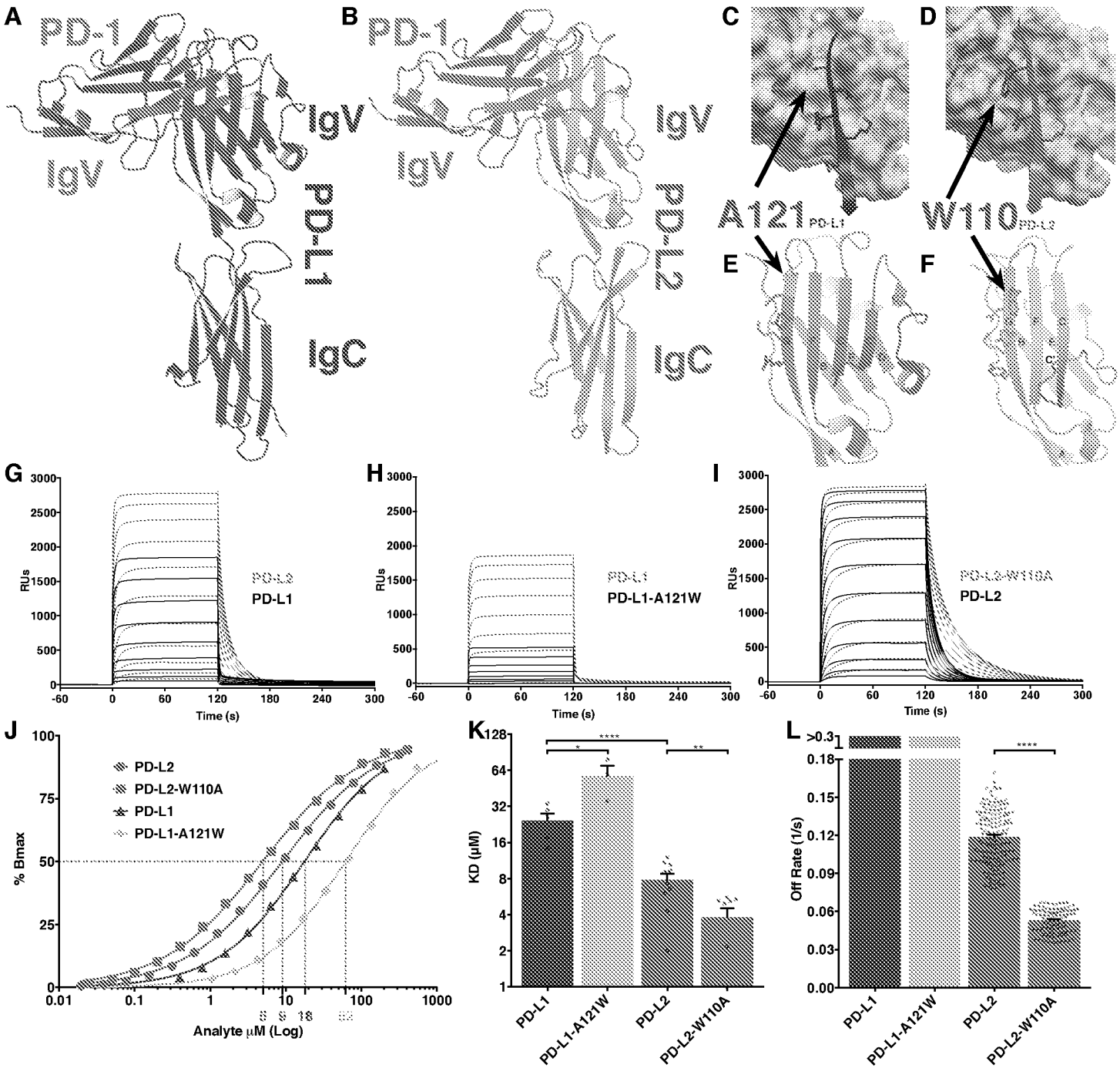
55. The multimeric protein of any one of claims 52-54, wherein said heterologous polypeptide domain of each of said two or more fusion polypeptides comprises an immunoglobulin Fc domain.

56. A pharmaceutical composition comprising:
the fusion polypeptide of claim 46 or the multimeric protein of claim 52 and
a pharmaceutically acceptable carrier.

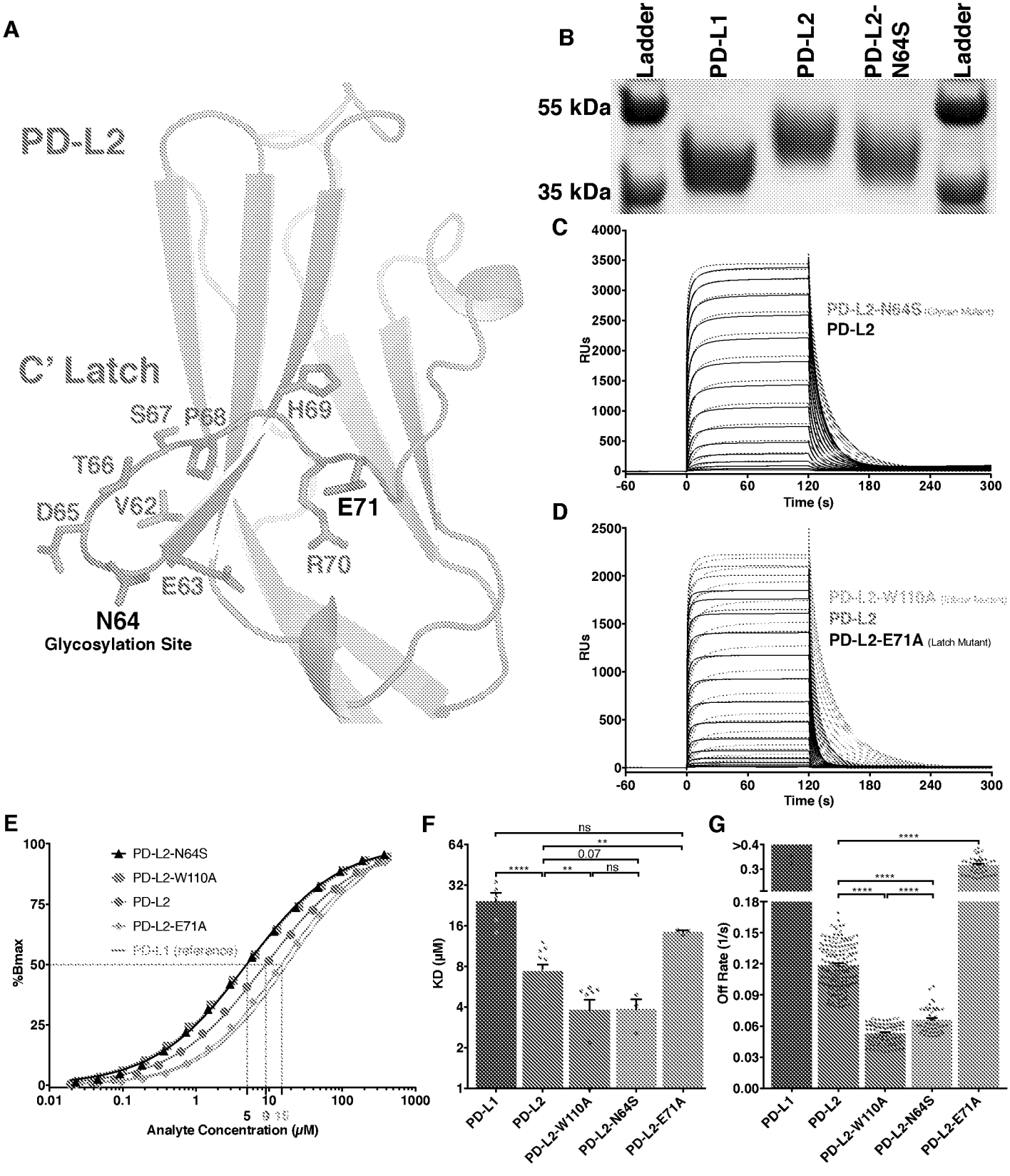
57. A method of modulating a subject's T-cell immune response, said method comprising:
administering the pharmaceutical composition of claim 56 to the subject in an amount effective to modulate said subject's T-cell immune response.

58. The method of claim 57 further comprising
selecting a subject having a tumor and a depressed anti-tumor T-cell response, wherein said administering enhances the subject's anti-tumor T-cell response.

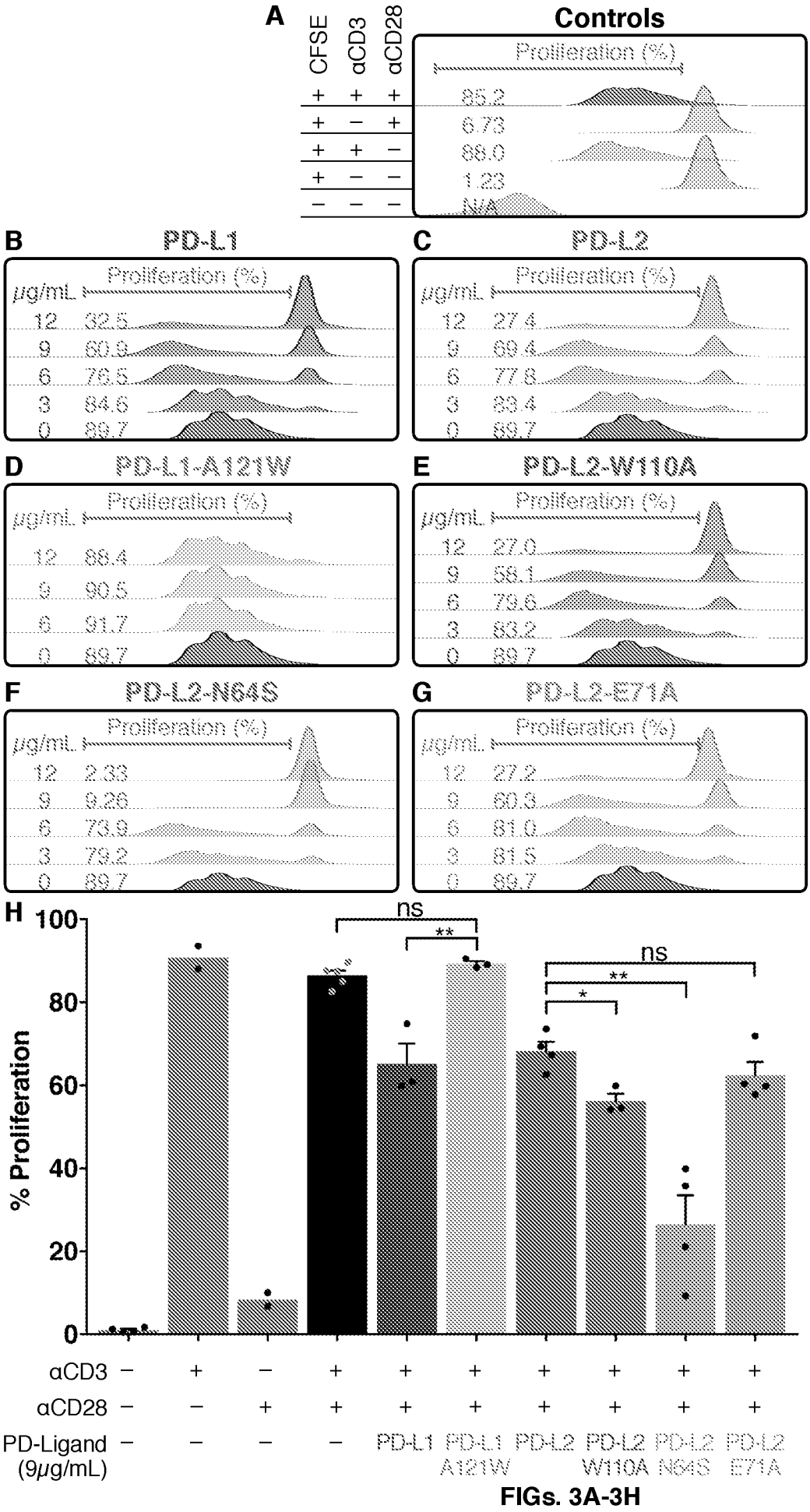
59. The method of claim 57 further comprising:
selecting a subject having an autoimmune condition, wherein said administering depresses the subject's T-cell autoimmune response.


A
PD-1
IgV
IgV
PD-L1
IgC
B
PD-1
IgV
IgV
PD-L2
IgC
C
A121 PD-L1
D
W110 PD-L2
E
F
G
RUs
PD-L2
PD-L1
Time (s)
H
RUs
PD-L1
PD-L1-A121W
Time (s)
I
RUs
PD-L2-W110A
PD-L2
Time (s)
J
PD-L2
PD-L2-W110A
PD-L1
PD-L1-A121W
% Bmax
Analyte μM (Log)
K
KD (μM)
PD-L1
PD-L1-A121W
PD-L2
PD-L2-W110A
L
Off Rate (1/s)
PD-L1
PD-L1-A121W
PD-L2
PD-L2-W110A

FIGs. 1A-1L


A
PD-L2
C' Latch
S67
P68
H69
T66
D65
V62
E71
R70
E63
N64
Glycosylation Site
B
Ladder
PD-L1
PD-L2
PD-L2-N64S
Ladder
55 kDa
35 kDa
C
RUs
Time (s)
PD-L2-N64S (Glycan Mutant)
PD-L2
D
RUs
Time (s)
PD-L2-W110A (Binder Mutant)
PD-L2
PD-L2-E71A (Latch Mutant)
E
%Bmax
Analyte Concentration (μM)
PD-L2-N64S
PD-L2-W110A
PD-L2
PD-L2-E71A
PD-L1 (reference)
F
KD (μM)
ns
**
0.07
****
**
ns
PD-L1
PD-L2
PD-L2-W110A
PD-L2-N64S
PD-L2-E71A
G
Off Rate (1/s)
****
****
****
PD-L1
PD-L2
PD-L2-W110A
PD-L2-N64S
PD-L2-E71A

FIGs. 2A-2G


A
Controls
CFSE
αCD3
αCD28
Proliferation (%)
85.2
6.73
88.0
1.23
N/A
B
PD-L1
C
PD-L2
D
PD-L1-A121W
E
PD-L2-W110A
F
PD-L2-N64S
G
PD-L2-E71A
μg/mL
12
9
6
3
0
32.5
60.9
76.5
84.6
89.7
27.4
69.4
77.8
83.4
89.7
88.4
90.5
91.7
89.7
27.0
58.1
79.6
83.2
89.7
2.33
9.26
73.9
79.2
89.7
27.2
60.3
81.0
81.5
89.7
H
% Proliferation
ns
**
ns
**
*
αCD3
αCD28
PD-Ligand (9μg/mL)
PD-L1
PD-L1 A121W
PD-L2
PD-L2 W110A
PD-L2 N64S
PD-L2 E71A

FIGs. 3A-3H

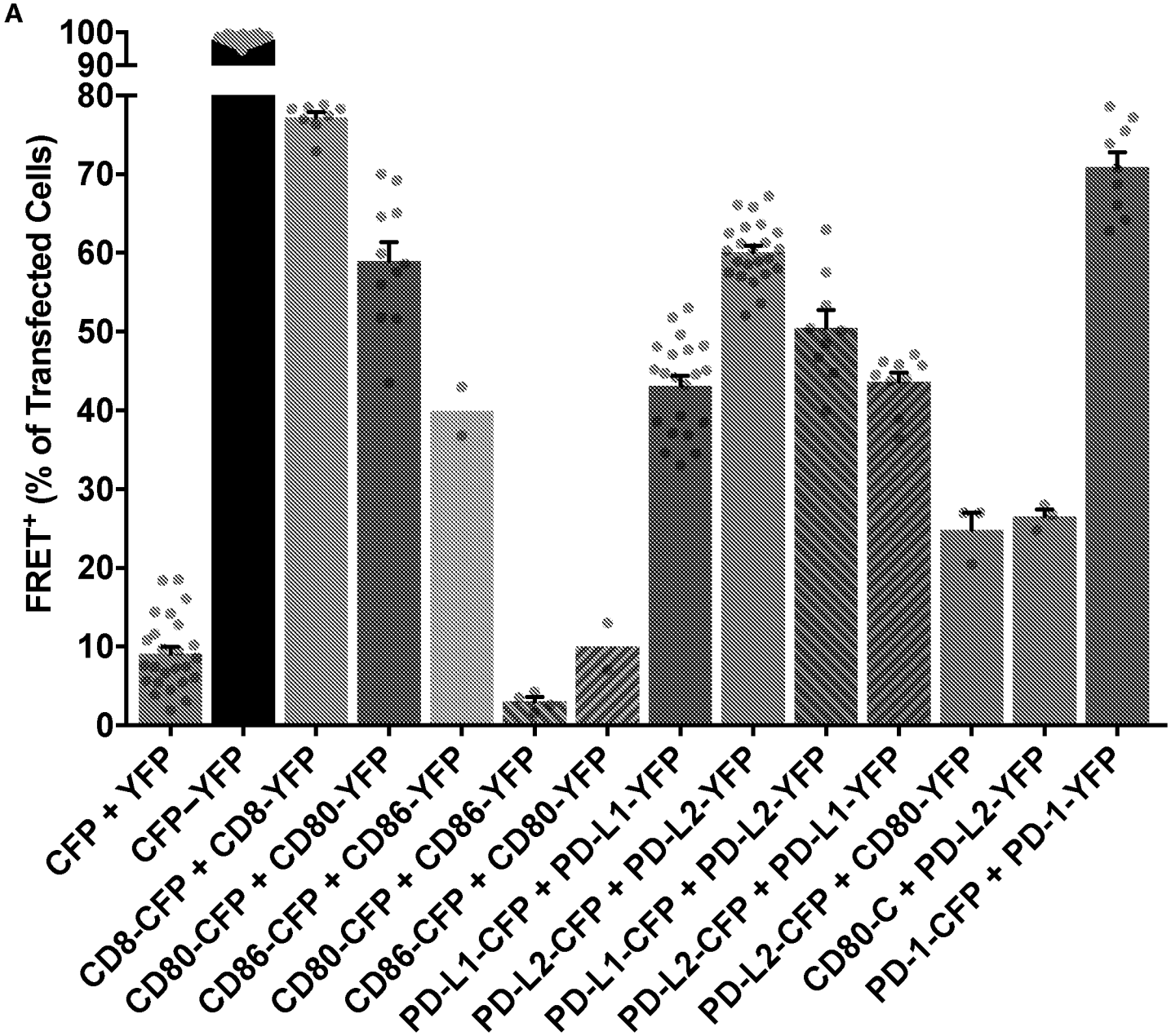
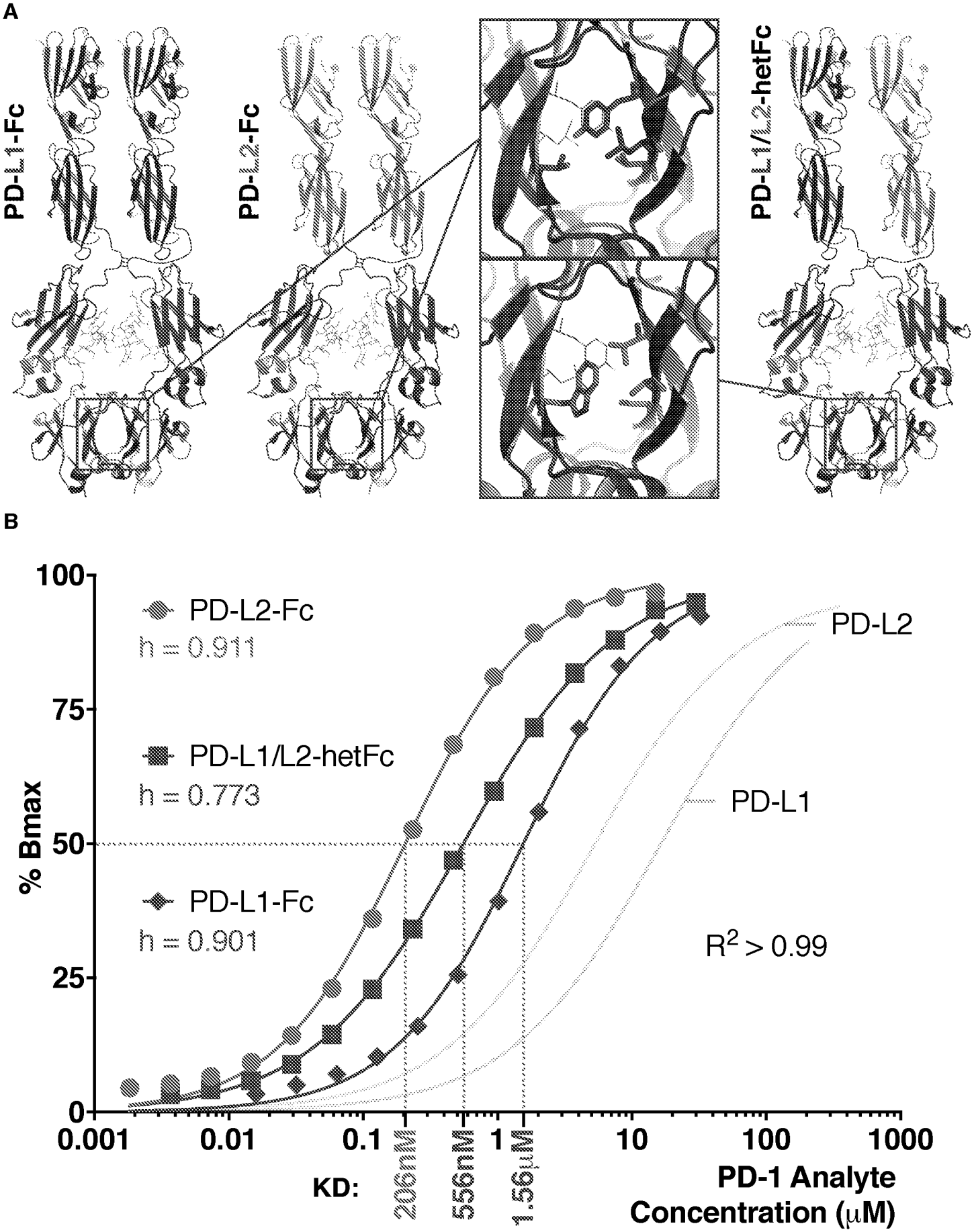
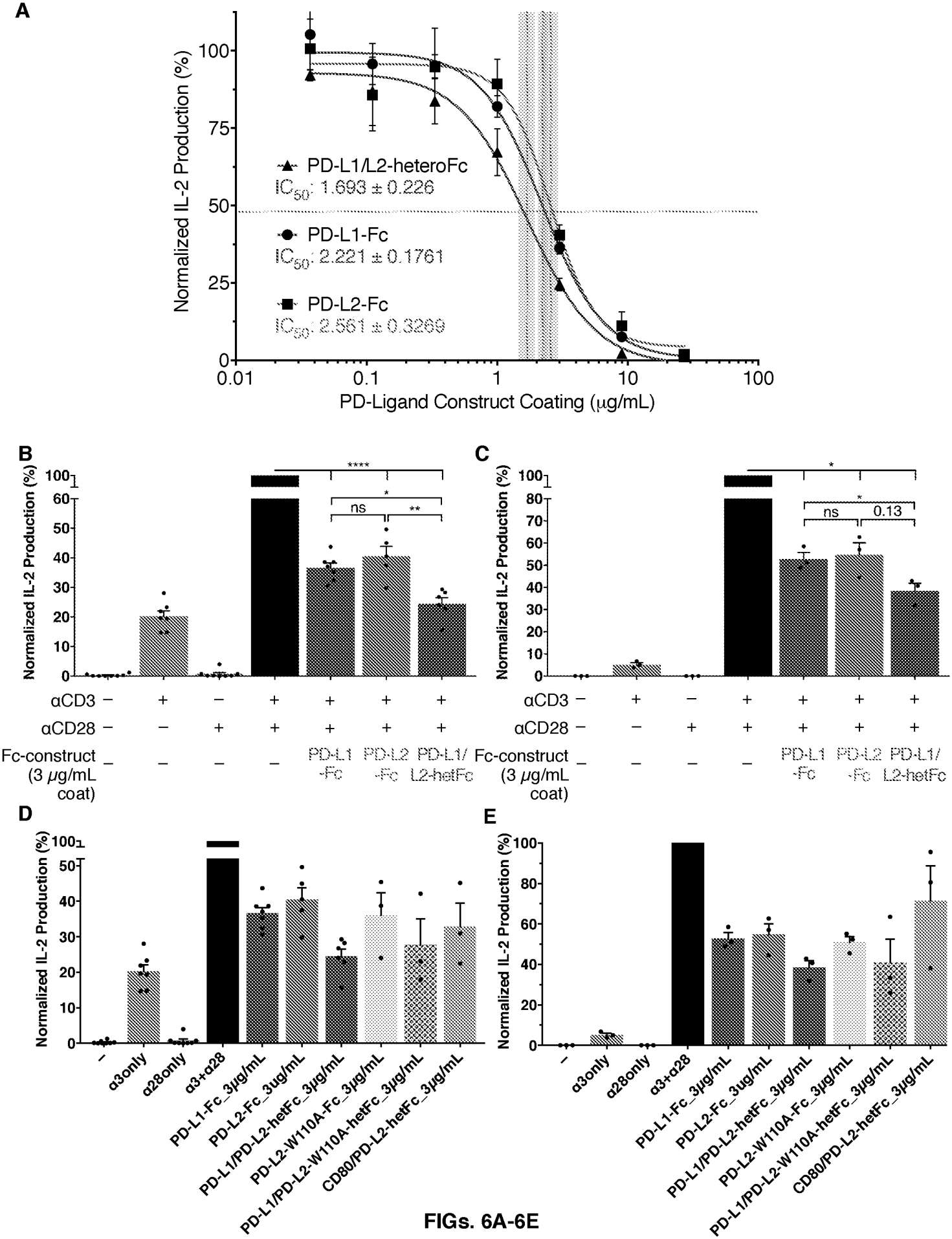

A
FRET+ (% of Transfected Cells)
100
90
80
70
60
50
40
30
20
10
0
CFP + YFP
CFP–YFP
CD8-CFP + CD8-YFP
CD80-CFP + CD80-YFP
CD86-CFP + CD86-YFP
CD80-CFP + CD86-YFP
CD86-CFP + CD80-YFP
PD-L1-CFP + PD-L1-YFP
PD-L2-CFP + PD-L2-YFP
PD-L1-CFP + PD-L2-YFP
PD-L2-CFP + PD-L1-YFP
PD-L2-CFP + CD80-YFP
CD80-C + PD-L2-YFP
PD-1-CFP + PD-1-YFP

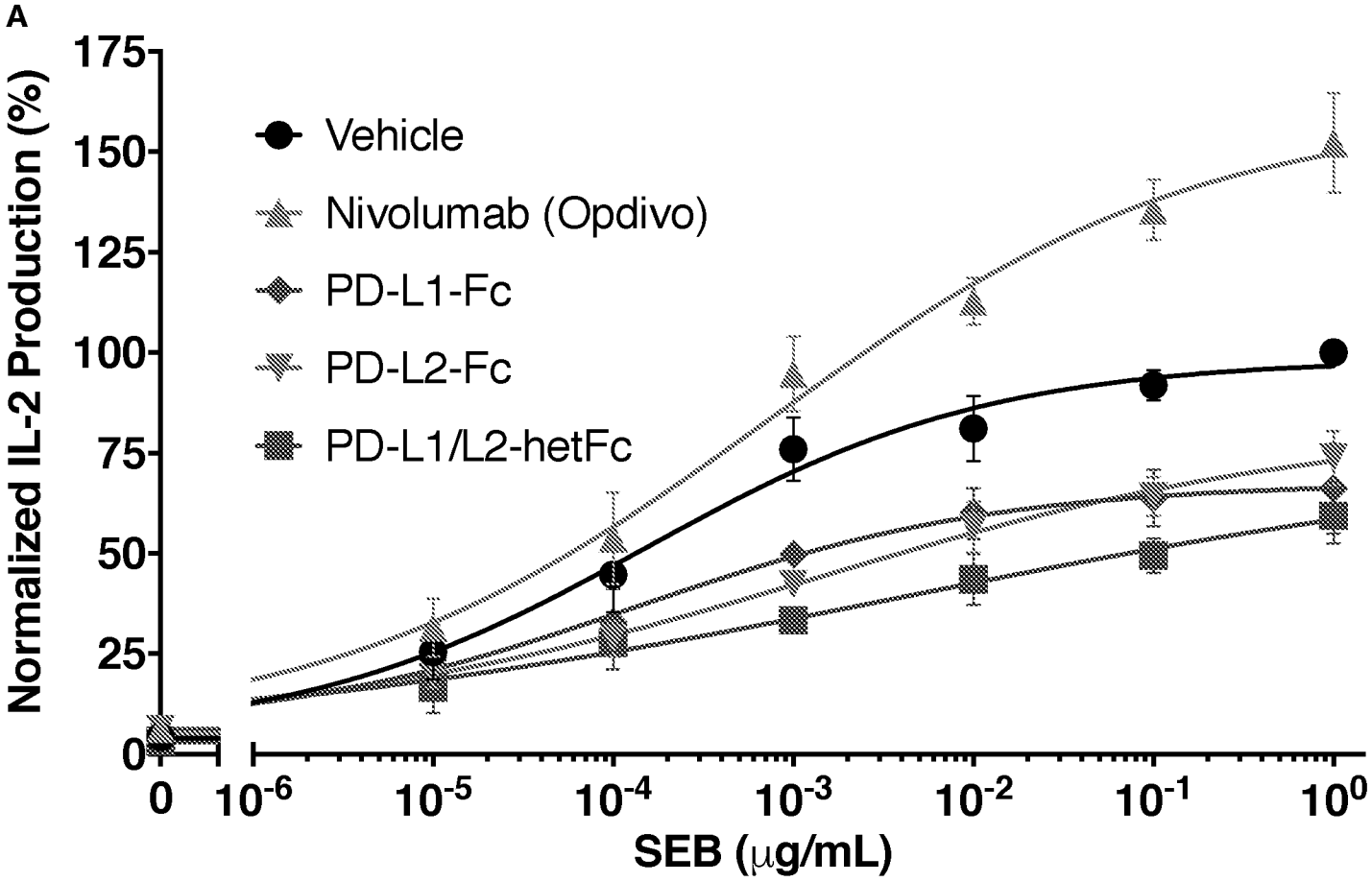
FIG. 4

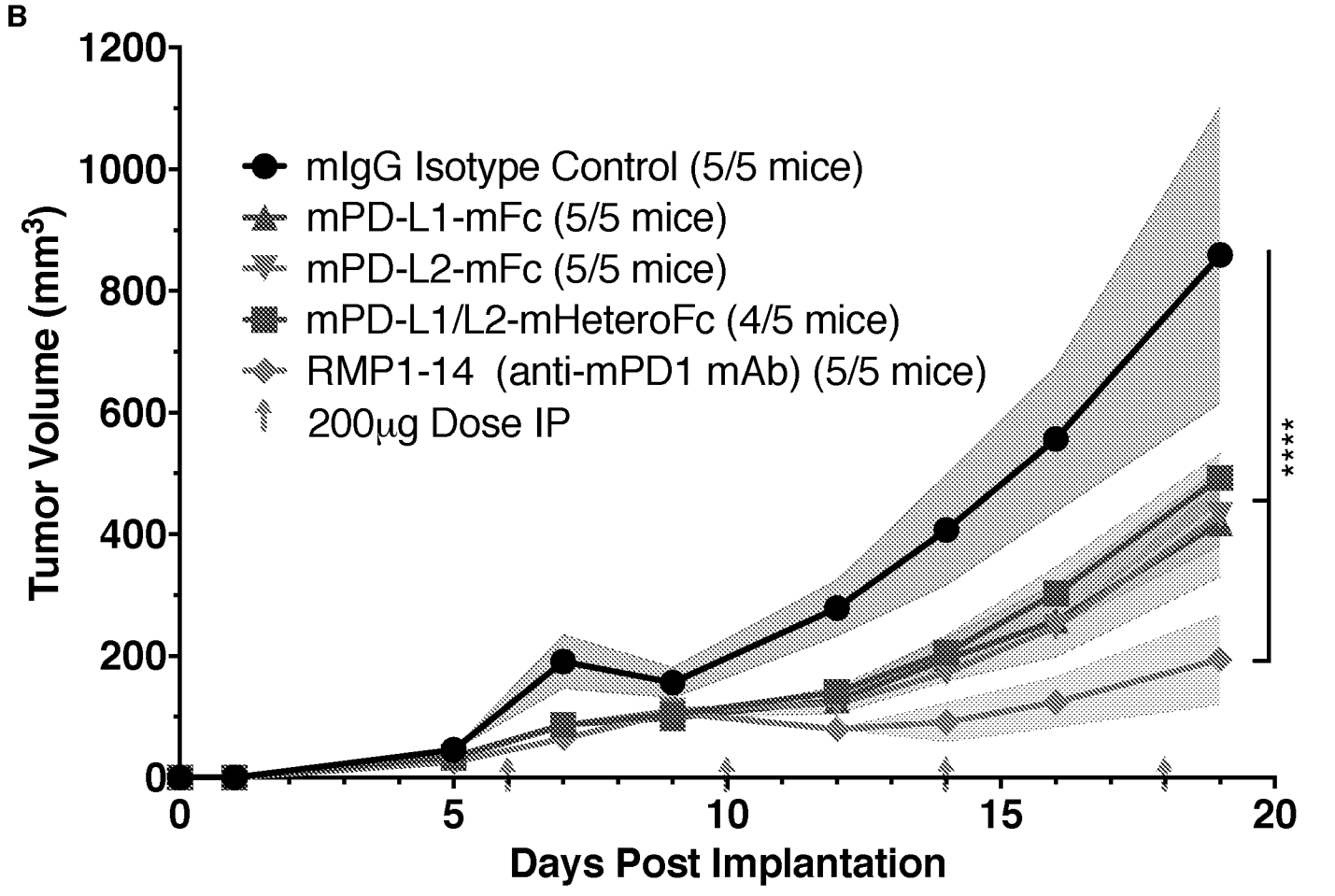

A
PD-L1-Fc
PD-L2-Fc
PD-L1/L2-hetFc
B
100
75
50
25
0
% Bmax
PD-L2-Fc
h = 0.911
PD-L1/L2-hetFc
h = 0.773
PD-L1-Fc
h = 0.901
PD-L2
PD-L1
R2 > 0.99
0.001
0.01
0.1
1
10
100
1000
KD:
206nM
556nM
1.56μM
PD-1 Analyte Concentration (μM)

FIGs. 5A-5B


A
Normalized IL-2 Production (%)
PD-L1/L2-heteroFc
IC50: 1.693 ± 0.226
PD-L1-Fc
IC50: 2.221 ± 0.1761
PD-L2-Fc
IC50: 2.561 ± 0.3269
PD-Ligand Construct Coating (μg/mL)
B
C
αCD3
αCD28
Fc-construct (3 μg/mL coat)
PD-L1 -Fc
PD-L2 -Fc
PD-L1/ L2-hetFc
D
E
–
α3only
α28only
α3+α28
PD-L1-Fc_3μg/mL
PD-L2-Fc_3ug/mL
PD-L1/PD-L2-hetFc_3μg/mL
PD-L2-W110A-Fc_3μg/mL
PD-L1/PD-L2-W110A-hetFc_3μg/mL
CD80/PD-L2-hetFc_3μg/mL

FIGs. 6A-6E


A
Normalized IL-2 Production (%)
175
150
125
100
75
50
25
0
Vehicle
Nivolumab (Opdivo)
PD-L1-Fc
PD-L2-Fc
PD-L1/L2-hetFc
0
10-6
10-5
10-4
10-3
10-2
10-1
100
SEB (μg/mL)


B
Tumor Volume (mm3)
1200
1000
800
600
400
200
0
mIgG Isotype Control (5/5 mice)
mPD-L1-mFc (5/5 mice)
mPD-L2-mFc (5/5 mice)
mPD-L1/L2-mHeteroFc (4/5 mice)
RMP1-14 (anti-mPD1 mAb) (5/5 mice)
200μg Dose IP
****
0
5
10
15
20
Days Post Implantation

FIGs. 7A-7B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/52639

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 39/395, 38/16, 38/17; C07K 14/705, 16/18, 16/28; A61P 35/04, 35/00 (2019.01)

CPC - C07K 14/705, 16/18, 16/28; A61P 35/04, 35/00; A61K 39/395, 38/16, 38/1774

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/0017199 A1 (LANGERMANN, S.) 17 January 2013; paragraphs [0017], [0061], [0073], [0103]-[0104], [0172], [0257], [0279]-[0280], [0282]-[0284], [0491].	1-3, 5, 9/5, 30
A	US 2012/0003220 A1 (CHEN, L.) 5 January 2012; paragraphs [0023], [0029], [0035]-[0037], [0040], [0079].	35
A	WO 02/00730 A2 (GENETICS INSTITUTE, INC. et al) 3 January 2002; page 7, lines 18-31; page 27, lines 1-11; page 34, lines 2-12.	35
A	US 2015/0361155 A1 (THOMAS JEFFERSON UNIVERSITY) 17 December 2015; paragraphs [0038], [0077], [0082], [0124], [0149].	35

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 January 2019 (15.01.2019)	07 FEB 2019

Name and mailing address of the ISA/	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/52639

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

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

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

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

3. ☒ Claims Nos.: 24-29, 31-34, 43-59
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 5 (in-part), 9 (in-part), 30, and 35 (in-part)

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

-***-Continued from Box No. III: Observations Where Unity of Invention is Lacking-***-

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

Groups I+, Claims 1-23, 30, 35-42, PD-L1 amino acid residues 19-127 of SEQ ID NO: 2 (ED of first fusion polypeptide), and PD-L2 amino acid residues 21-118 of SEQ ID NO: 1, with N64 (ED of second fusion polypeptide) are directed toward a heterodimeric binding molecule.

The heterodimeric binding molecule will be searched to the extent it encompasses PD-L1 amino acid residues 19-127 of SEQ ID NO: 2 (first exemplary ED of first fusion polypeptide), and PD-L2 amino acid residues 21-118 of SEQ ID NO: 1, with N64 (first exemplary ED of second fusion polypeptide). Applicant is invited to elect additional fusion polypeptide sequence(s), with specified SEQ ID NO: for each, or with specified substitution(s) at specified site(s) of a SEQ ID NO:, such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), to be searched. Additional fusion polypeptide sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1-3, 5 (in-part), 9 (in-part), 30, and 35 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass PD-L1 amino acid residues 19-127 of SEQ ID NO: 2 (ED of first fusion polypeptide), and PD-L2 amino acid residues 21-118 of SEQ ID NO: 1, with N64 (ED of second fusion polypeptide). Applicants must specify the claims that encompass any additionally elected fusion polypeptide sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be PD-L1 amino acid residues 19-225 of SEQ ID NO: 2 (ED of first fusion polypeptide).

No technical features are shared between the fusion polypeptide sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: a heterodimeric binding molecule comprising: (i) a first fusion polypeptide comprising an extracellular domain portion of a first B7 ligand coupled to a heterologous polypeptide domain; and (ii) a second fusion polypeptide comprising an extracellular domain portion of a second B7 ligand coupled to a heterologous polypeptide domain, wherein said first and second B7 ligands are different ligands and wherein said first and second fusion polypeptides are coupled together; these shared technical features are previously disclosed by WO 00/67788 A2 to Genetics Institute, Inc. (hereinafter 'Genetics').

Genetics discloses a dimeric binding molecule (a dimeric costimulatory (binding) molecule; page 3, lines 18-25; page 4, lines 1-4) comprising: (i) a first fusion polypeptide comprising an extracellular domain portion of a first B7 ligand (a first fusion polypeptide comprising an extracellular domain portion of a first B7 ligand; page 3, lines 30-31; page 4, lines 5-7) coupled to a heterologous polypeptide domain (coupled to a portion of an immunoglobulin molecule (a heterologous polypeptide domain); page 4, lines 5-7); and (ii) a second fusion polypeptide comprising an extracellular domain portion of a second B7 ligand (a second fusion polypeptide comprising an extracellular domain portion of a second B7 ligand; page 3, line 30 - page 4, lines 7) coupled to a heterologous polypeptide domain (coupled to a portion of an immunoglobulin molecule (a heterologous polypeptide domain); page 4, lines 5-7), wherein said first and second fusion polypeptides are coupled together (wherein said first and second fusion polypeptides are coupled together; page 25, lines 25-29). Genetics further discloses wherein the B7 molecule is a B7-1 molecule, or a B7-2 molecule (wherein the B7 molecule is a B7-1 molecule, or a B7-2 molecule; page 3, lines 30-31), and wherein B7-2 functions in primary immune response (wherein B7-2 functions in primary immune response; page 2, lines 4-5), while B7-1 prolongs T cell responses (B7-1 prolongs T cell responses; page 2, lines 5-7).

Genetics does not disclose a heterodimeric binding molecule; wherein said first and second B7 ligands are different ligands. However, it would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have provided dimeric costimulatory molecules comprising each of the B7 species disclosed by Genetics in order to provide both an immediate immune response stimulation, as well as prolongation of the stimulus in order to better enable the effective generation of an immune response to an administered antigen.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Genetics reference, unity of invention is lacking.