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(54) Title: COMPOSITIONS OF PROTEIN COMPLEXES AND METHODS OF USE THEREOF

(57) Abstract: Provided herein are protein complexes comprising a sensor domain and a therapeutic domain linked by a linker, and methods of use thereof. In aspects of the present disclosure, activity of the therapeutic domain comprises a dependence on sensor domain binding to target markers.

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COMPOSITIONS OF PROTEIN COMPLEXES AND METHODS OF USE THEREOF

SEQUENCE LISTING

[0001] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on November 14, 2022 is named 51177-046WO2_Sequence_Listing_11_14_22 and is 634,137 bytes in size.

BACKGROUND

[0002] Interleukin 2 (IL-2) is a potent cytokine that exhibits toxicity upon systemic administration. There is a need for a version of IL-2 that can be delivered systemically but can be regulated to exhibit therapeutic activity on an effective subset of T cells.

SUMMARY

[0003] In some aspects, the present disclosure provides for a complex comprising: (a) a therapeutic domain comprising an IL-2 peptide and (b) a sensor domain comprising an antibody, wherein said sensor domain is configured to bind PD-1 and IL-2 in a mutually exclusive manner. In some embodiments, the complex further comprises a linker linking the therapeutic domain to the sensor domain. In some embodiments, the sensor domain is configured: (i) to bind IL-2 in the absence of PD-1; and (ii) to not bind IL-2 in the presence of PD-1. In some embodiments, the antibody is an antibody fragment or an antibody derivative. In some embodiments, the sensor domain comprises a single dual binding antibody (DBA) configured to bind PD-1 and IL-2. In some embodiments, the DBA comprises a heavy chain CDR3 having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any one of SEQ ID NOs: 11-20, 154-156, 168-173, 114-119, 415, 421, 433, 439, 445, 451, 457, 463, 469, 475, 481, 487, 493, 499, 505, 511, 517, 523, 529, 535, 541, 547, 553, 559, 565, 571, 577, 583, 589, 595, 601, 607, 613, 619, 625, 631, 637, 643, 649, 655, 661, or 667. In some embodiments, the DBA comprises a heavy chain CDR1, CDR2, or CDR3 comprising a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any of the sequences recited in Table 3, Table 7, Table 8, or Table 19. In some embodiments, the DBA comprises a V_H or a V_L comprising a sequence having at least 80%, at

least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any of the sequences recited in Table 18. In some embodiments, the complex comprises an Fc domain. In some embodiments, the Fc domain is from IgG. In some embodiments, the Fc domain is homodimeric. In some embodiments, the Fc domain is heterodimeric. In some embodiments, the Fc domain comprises: (a) a first polypeptide comprising a knob mutation and (b) a second polypeptide comprising a hole mutation. In some embodiments, the knob mutation or the hole mutation comprises mutations of any one of following pairs of residues relative to IgG: 366 and 407, 405 and 394, or 407 and 366. In some embodiments, the knob mutation comprises an arginine residue, a phenylalanine residue, a tyrosine residue or a tryptophan residue and the hole mutation comprises an alanine residue, a serine residue, a threonine residue, or a valine residue. In some embodiments, the complex comprises a sensor domain comprising a full-length DBA, wherein the IL-2 peptide is linked to an N-terminus of a heavy chain of said full-length DBA or wherein the IL-2 peptide is linked to an N-terminus of a light chain of said full-length DBA. In some embodiments, the complex comprises a sensor domain comprising a full-length DBA, wherein the IL-2 peptide is linked to a C-terminus of a heavy chain of said full-length DBA. In some embodiments, the complex comprises: (a) a first polypeptide according to N-[IL-2]-[linker]-[V_H]-[C_H]-[hinge]-Fc-C; and a second polypeptide according to N-[V_L]-[C_L]-C, or (b) a first polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc-C; and a second polypeptide according to N-[IL-2]-[linker]-[V_L]-[C_L]-C, wherein N- denotes a peptide N-terminus, C- denotes a peptide C-terminus, [linker] denotes said linker, V_H indicates a heavy chain variable domain of said DBA, C_H indicates a heavy chain constant domain of an immunoglobulin, V_L denotes a light chain variable domain of said DBA, [hinge] denotes a hinge region of an immunoglobulin, Fc denotes an Fc region of an immunoglobulin, and C_L denotes a light chain constant domain of an immunoglobulin. In some embodiments, the complex comprises any one of AF003345, AF003243, AF003246, AF003247, AF003341, AF003644, AF003651, AF003657, or AF003934. In some embodiments, the complex comprises: (a) a first polypeptide according to N-[IL-2]-[linker]-[V_H]-[C_H]-[hinge]-Fc[knob]-C; a second polypeptide according to N-[V_L]-[C_L]-C; and a third polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc[hole]-C, or (b) a first polypeptide according to N-[IL-2]-[linker]-[V_H]-[C_H]-[hinge]-Fc[hole]-C; a second polypeptide according to N-[V_L]-[C_L]-C; and a third polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc[knob]-C, wherein N- denotes a peptide N-terminus, C- denotes a peptide C-terminus, [linker] denotes said linker, V_H indicates a heavy chain variable domain of said DBA, C_H

indicates a heavy chain constant domain of an immunoglobulin, V_L denotes a light chain variable domain of said DBA, [hinge] denotes a hinge region of an immunoglobulin, Fc[knob] denotes an Fc of an immunoglobulin comprising a knob mutation, Fc[hole] denotes an Fc region of an immunoglobulin comprising a hole mutation, and C_L denotes a light chain constant domain of an immunoglobulin. In some embodiments, the knob mutation or the hole mutation comprises mutations of any one of following pairs of residues relative to IgG: 366 and 407, 405 and 394, or 407 and 366. In some embodiments, the complex comprises any one of AF003229, AF003230, AF003232, AF003740, AF003747, AF003749, AF003753, AF003945, AF003947, AF003951, AF003952, AF003953, AF003955, AF003956, or AF003941. In some embodiments, the complex comprises: (a) a first polypeptide according to N-[IL-2]-[linker]-[V_H]-[C_H]-[hinge]-Fc-[scFv]-C; and (b) a second polypeptide according to N-[V_L]-[C_L]-C, wherein N- denotes a peptide N-terminus, C- denotes a peptide C-terminus, [linker] denotes said linker, V_H indicates a heavy chain variable domain of an anti-PD-1 monoselective antibody, C_H indicates a heavy chain constant domain of an immunoglobulin, V_L denotes a light chain variable domain of an anti-PD-1 monoselective antibody, [hinge] denotes a hinge region of an immunoglobulin, Fc denotes an Fc region of an immunoglobulin, C_L denotes a light chain constant domain of an immunoglobulin, and [scFv] denotes an scFv comprising V_H and V_L domains of said DBA. In some embodiments, said scFv is oriented according to N-[V_H]-[linker2]-[V_L]-C. In some embodiments, said scFv comprising V_H and V_L domains of said DBA comprises: (a) a V_H domain comprising a sequence having at least 80% identity to a V_H domain of any one of AB002022_2B07v1, AB002328_2B07v4, AB002360_7A04v1, AB002413_2A11v3, AB002342_2B07v5, AB002345_2B07v6, or AB002365_7A04v2; or (b) a V_L domain comprising a sequence having at least 80% identity to a V_L domain of any one of AB002022_2B07v1, AB002328_2B07v4, AB002360_7A04v1, AB002413_2A11v3, AB002342_2B07v5, AB002345_2B07v6, or AB002365_7A04v2. In some embodiments, said scFv comprising V_H and V_L domains of said DBA comprises: (a) heavy chain CDRs of any one of AB002022_2B07v1, AB002328_2B07v4, AB002360_7A04v1, AB002413_2A11v3, AB002342_2B07v5, AB002345_2B07v6, or AB002365_7A04v2; or (b) light chain CDRs of any one of AB002022_2B07v1, AB002328_2B07v4, AB002360_7A04v1, AB002413_2A11v3, AB002342_2B07v5, AB002345_2B07v6, or AB002365_7A04v2. In some embodiments, the complex comprises any one of AF003864, AF003871, AF003872, AF003913, AF003918, AF003923, AF003927, AF004502, AF004503, AF004504, AF004505, AF004892, or AF004893. In some embodiments, the complex comprises: (a) a first polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc[knob]-[linker]-[IL-2]-C; a second polypeptide according to N-[V_L]-

[C_L]-C; and a third polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc[hole]-[linker]-[scFv]-C, or (b) a first polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc[hole]-[linker]-[IL-2]-C; a second polypeptide according to N-[V_L]-[C_L]-C; and a third polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc[knob]-[linker]-[scFv]-C; wherein N- denotes a peptide N-terminus, C- denotes a peptide C-terminus, [linker] denotes said linker, V_H indicates a heavy chain variable domain of said DBA, C_H indicates a heavy chain constant domain of an immunoglobulin, V_L denotes a light chain variable domain of said DBA, [hinge] denotes a hinge region of an immunoglobulin, Fc[knob] denotes an Fc of an immunoglobulin comprising a knob mutation, Fc[hole] denotes an Fc region of an immunoglobulin comprising a hole mutation, C_L denotes a light chain constant domain of an immunoglobulin, and [scFv] denotes an scFv of said DBA. In some embodiments, the complex comprises any one of AF004693, AF004695, AF004696, AF005416, AF005418, or AF005419. In some embodiments, the complex comprises: (a) a first polypeptide according to N-[V_H]-[C_H]-[het-hinge]-Fc[knob]-[linker]-[IL-2]-C; a second polypeptide according to N-[V_L]-[C_L]-C; and a third polypeptide according to N-[V_H]-[C_H]-[het-hinge]-Fc[hole]-C, or (b) a first polypeptide according to N-[V_H]-[C_H]-[het-hinge]-Fc[hole]-[linker]-[IL-2]-C; a second polypeptide according to N-[V_L]-[C_L]-C; and a third polypeptide according to N-[V_H]-[C_H]-[het-hinge]-Fc[knob]-C, wherein N- denotes a peptide N-terminus, C- denotes a peptide C-terminus, [linker] denotes said linker, V_H indicates a heavy chain variable domain of said DBA, C_H indicates a heavy chain constant domain of an immunoglobulin, V_L denotes a light chain variable domain of said DBA, [het hinge] denotes a hinge region heterologous to said Fc region, Fc[knob] denotes an Fc of an immunoglobulin comprising a knob mutation, Fc[hole] denotes an Fc region of an immunoglobulin comprising a hole mutation, and C_L denotes a light chain constant domain of an immunoglobulin. In some embodiments, the hinge region heterologous to said Fc region is: (a) a hinge region derived from an IgG3 antibody, or (b) a G4S-based linker. In some embodiments, the complex comprises AF003632 or AF003634. In some embodiments, the IL-2 peptide comprises a wild-type human IL-2 peptide. In some embodiments, the IL-2 peptide comprises a sequence having at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or substantially 100% sequence identity to human IL-2. In some embodiments, the IL-2 peptide comprises a mutation at least one of R38, K43, E61, F42, Y45, L72, T3, or C125 of human IL-2. In some embodiments, the complex comprises any one of AF003232, AF003243, AF003246, AF003247, AF003341, AF003345, AF003632,

AF003634, AF003644, AF003651, AF003652, AF003653, AF003657, AF003740, AF003744, AF003747, AF003749, AF003753, AF003864, AF003873, AF003876, AF003877, AF003913, AF003918, AF003923, AF003927, AF003930, AF003931, AF003933, AF003934, AF003935, AF003941, AF003945, AF003946, AF003947, AF003948, AF003951, AF003952, AF003953, AF003955, AF003956, AF004262, AF004265, AF004273, AF004276, AF004284, AF004287, AF004295, AF004298, AF004385, AF004386, AF004387, AF004388, AF004389, AF004404, AF004405, AF004413, AF004414, AF004415, AF004416, AF004504, AF004505, AF004693, AF004695, AF004696, AF004771, AF004773, AF004892, or AF004893.

[0004] In some aspects, the present disclosure provides for a method of enhancing T-cell reactivity to heterologous cells, comprising administering any of the complexes described herein to a subject in need thereof. In some embodiments, the heterologous cells are cancer cells.

[0005] In some aspects, the present disclosure provides for a method of treating a subject in need thereof comprising administering the complex of any one of claims [0162]1-[0162]32 to the subject in need thereof. In some embodiments, the administering comprises intravenous, intramuscular, or subcutaneous administration. In some embodiments, the subject in need thereof has cancer. In some embodiments, the therapeutic domain treats the subject in need thereof. In some embodiments, the subject in need thereof is a mammal. In some embodiments, the subject in need thereof is a human.

[0006] In some aspects, the present disclosure provides for a composition comprising a recombinant nucleic acid encoding any of the complexes described herein. In some aspects, the present disclosure provides for a host cell comprising any of the recombinant nucleic acids encoding any of the complexes described herein.

In some aspects, the present disclosure provides for a pharmaceutical composition comprising any of the complexes described herein and a pharmaceutically acceptable excipient.

[0007] In various aspects, the present disclosure provides a complex comprising: a) a therapeutic domain; b) a linker; and c) a sensor domain, wherein the therapeutic domain is an IL-2 agonist, the therapeutic domain is linked to the sensor domain by the linker, and wherein the sensor domain is dual-binding antibody (DBA) that is capable of binding the therapeutic domain (the IL-2 agonist domain) and a marker, wherein the marker is PD-1.

[0008] In some aspects, the sensor domain is bound to the therapeutic domain in an absence of the marker. In some aspects, the therapeutic domain is blocked from binding the sensor domain upon binding of the sensor domain to the marker. In some aspects, the activity of the therapeutic domain is reduced upon binding of the therapeutic domain to the sensor domain. In some aspects, the therapeutic domain is capable of exhibiting therapeutic activity upon binding of the

sensor domain to the marker. In some aspects, the therapeutic domain is therapeutically active upon binding of the sensor domain to the marker.

[0009] In some aspects, the sensor domain comprises an antibody. In some aspects, the antibody is an antibody fragment or antibody derivative. In some aspects, the complex comprises an Fc domain. In some aspects, the complex comprises a domain that improves kinetic properties. In some aspects, the complex includes two heavy chains and two light chains.

[0010] In some aspects, the complex comprises two therapeutic domains. In some aspects, the complex comprises two sensor domains. In some aspects, the complex is a regulated therapeutic protein. In some aspects, the antibody or the antibody fragment comprises an IgG, a single domain antibody fragment, a nanobody, or a single chain variable fragment (scFv).

[0011] In some aspects, the therapeutic domain is an IL-2 receptor agonist. In some aspects, the IL-2 receptor agonist is IL-2, IL-15, or variants or fusions thereof. In some aspects, the therapeutic domain binds to the sensor domain.

[0012] In some aspects, the linker is a polypeptide linker. In some aspects, the linker comprises from 2 to 200 amino acids in length. In some aspects, the linker is: attached to a heavy chain of the sensor domain, attached to a light chain of the sensor domain, is a fusion with an N-terminus of the sensor domain, or is a fusion with a C-terminus of the sensor domain. In some aspects, the linker is: attached to a heavy chain of the therapeutic domain, attached to a light chain of the therapeutic domain, is a fusion with an N-terminus of the therapeutic domain, or is a fusion with a C-terminus of the therapeutic domain.

[0013] In some aspects, the activity of the therapeutic domain is reduced when bound to the sensor domain. In some aspects, the therapeutic domain is inactive when bound to the sensor domain. In some aspects, the sensor domain blocks the activity of the therapeutic domain when bound to the therapeutic domain. In some aspects, the therapeutic domain is active when the sensor domain is bound to the marker. In some aspects, an affinity of the sensor domain for the marker is equal to or greater than an affinity of the sensor domain for the therapeutic domain.

[0014] In some aspects, an affinity of the sensor domain for the marker is at least 2 times, 5 times, 10 times, 100 times, 1000 times, 10000, or 100000 times greater than an affinity of the sensor domain for the therapeutic domain.

[0015] In some aspects, the sensor domain is an antibody or a fragment thereof. In some aspects, the sensor domain comprises one or both antigen binding domains of a bispecific antibody. In some aspects, the bispecific antibody comprises a first antigen binding domain that is capable of binding to the therapeutic domain and is capable of binding to the marker, and a second antigen

binding domain that is capable of binding to the marker. In some aspects, the bispecific antibody comprises a single therapeutic domain.

[0016] In some aspects, the sensor antibody binds to an IL-2 receptor agonist and to PD-1. In some aspects, the IL-2 receptor agonist is IL-2, IL-15, or variants or fusions thereof.

[0017] In some aspects, the sensor domain comprises a complementarity determining region (CDR) selected from Table 3, Table 7, Table 8, or Table 19. In some aspects, the sensor domain is selected from **TABLE 8 or TABLE 18**. In some aspects, the complex is selected from **TABLE 15 or TABLE 2A**. In some aspects, the sensor domain comprises a complementarity determining region having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any of the complementary determining regions selected from Table 3, Table 7, Table 8, or Table 19. In some aspects, the sensor domain comprises a V_H or V_L domain having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any of the V_H or V_L domains recited in **TABLE 8 OR TABLE 18**. In some aspects, the sensor domain comprises a complementarity determining region having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 20 or SEQ ID NO: 142 – SEQ ID NO: 173, or SEQ ID NO: 238-252. In some aspects, the sensor domain has at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to any one of SEQ ID NO: 21 – SEQ ID NO: 27, SEQ ID NO: 31 – SEQ ID NO: 39, or SEQ ID NO: 127 – SEQ ID NO: 141. In some aspects, the protein complex has at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to any one of SEQ ID NO: 41, SEQ ID NO: 44, SEQ ID NO: 80 – SEQ ID NO: 112, SEQ ID NO: 174-175, SEQ ID NO: 181-182, SEQ ID NO: 195-196, SEQ ID NO: 205-206, SEQ ID NO: 210-212, SEQ ID NO: 220-223, SEQ ID NO: 226-231, SEQ ID NO: 259-261, SEQ ID NO: 266-282, SEQ ID NO: 289-293, or a fragment thereof.

[0018] In various aspects, the present disclosure provides a method comprising administering any of the above complexes to a subject in need thereof. In various aspects, the present disclosure provides a method of treating a subject in need thereof comprising administering any

of the above complexes to the subject in need thereof. In some aspects, the administering comprises intravenous, intramuscular, or subcutaneous administration. In some aspects, the subject in need thereof has cancer. In some aspects, the therapeutic domain treats the subject in need thereof. In some aspects, the subject in need thereof is a mammal. In some aspects, the subject in need thereof is a human.

[0019] In some aspects, the present disclosure provides IL-2 conjugates that can be delivered systemically but may exhibit diminished systemic toxicity.

INCORPORATION BY REFERENCE

[0020] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0022] **FIG. 1A and 1B** shows a schematic of the protein complexes of the present disclosure. **FIG. 1A** shows an exemplary dual binding protein complex in an inactive state. The protein complex has a sensor domain and a therapeutic domain. The sensor domain and therapeutic domain are linked by a linker. The sensor domain is shown bound to the therapeutic domain, rendering the therapeutic domain inactive. **FIG. 1B** shows an exemplary dual binding protein complex in an active state. The protein complex has a sensor domain and a therapeutic domain. The sensor domain and therapeutic domain are linked by a linker. The sensor domain is shown bound to the marker, rendering the therapeutic domain active.

[0023] **FIG. 2** shows schematics of protein complexes of the present disclosure comprising one or more sensor domains and one or more therapeutic domain. **FIGs. 2A, 2B, 2C, 2D, 2E, 2F, 2G, 2H, and 2I** show example schematics of arrangements of protein complexes detailed in the Examples.

[0024] **FIG. 3** shows that IL-2 signaling by five exemplary PD-1/IL-2 DBA-cytokine protein complexes (2_A08, 2_A11, 2_B05, 2_B07, and 7_A04, SEQ ID NO: 67 – SEQ ID NO: 68, SEQ ID NO: 69 – SEQ ID NO: 70, SEQ ID NO: 71 – SEQ ID NO: 72, SEQ ID NO: 73 – SEQ ID

NO: 74 and SEQ ID NO: 75 – SEQ ID NO: 76 respectively) is reduced as compared to a control IL-2-Anti-HER2 protein complex (SEQ ID NO: 65 – SEQ ID NO: 66).

[0025] FIGs. 4A, 4B, 4C, 4D, 4E, and 4F provide IL-2 activity of protein complexes comprising the structure depicted in **FIG. 2B** in wells coated with PD-1-Fc or an IgG1 control protein. Activity was measured as growth of a 630 nm signal from HEK-Blue™ IL-2 reporter cells. **FIGs. 4A-C** provide the IL-2 activities of three different PD-1/IL-2 DBA-IL-2 complexes. **FIG. 4D** provides the activity of an anti-PD-1 antibody-IL-2 complex. **FIG. 4E** provides the activity of an anti-Her-2 antibody-IL-2 complex. **FIG. 4F** provides the activity of an anti-IL-2 antibody-IL-2 complex.

[0026] FIGs. 5A, 5B, 5C, 5D, 5E, 5F, 5G, and 5H provide IL-2 activity of protein complexes comprising the structure depicted in **FIG. 2H** in wells coated with PD-1-Fc or an IgG1 control protein. Activity was measured as growth of a 630 nm signal from HEK-Blue™ IL-2 reporter cells. **FIG. 5B -5D** provide results for two PD-1/IL-2 DBA complexes comprising anti-PD-1 domains in the Fab arms and a PD-1/IL-2 DBA scFv on the Fc arm. **FIG. 5A, 5C, and 5E-H** provide results for control protein complexes

[0027] FIG. 6 provides rates of serum concentration decreases in the blood of wild-type mice of a PD-1/IL-2 DBA-cytokine complex ('2B07 IL-2 mut') and two control complexes.

[0028] FIGs. 7A, 7B, 7C, and 7D provide CD8⁺ T cell and NK cell counts in blood and spleen tissue collected from wild-type mice 5 days following treatment with a PD-1/IL-2 DBA-cytokine complex ('2B07 IL-2 mut') and two control complexes.

[0029] FIG. 8 provides tumor volume measurements as a function of the number of days post tumor cell implant in mice. Mice received various intravenous doses of a PD-1/IL-2 DBA-IL-2 complex, a PD-1/IL-2 DBA complex lacking IL-2, or an isotype control.

[0030] FIG. 9 provides the IL-2RBG binding for symmetric complexes comprising the structure depicted in **FIG. 2E**.

[0031] FIG. 10 provides the IL-2RBG binding for asymmetric complexes comprising the structure depicted in **FIG. 2B**.

[0032] FIG. 11 shows a plot depicting *in vitro* IL-2RBG binding by PD-1/IL-2 DBA-cytokine complexes and non-regulated control complexes and compares complexes made with two different forms of IL-2 including WT IL-2 and IL-2 3x. **FIG. 11A** shows IL-2RBG binding for complexes with wild-type IL-2 and **FIG. 11B** shows IL-2RBG binding for complexes with the IL-2 3x mutant.

[0033] FIGs. 12A, 12B, 13A, 13B, 14A, 14B, 15, and 16 show plots depicting IL-2 activity of IL-2-linked protein complexes comprising the structures depicted in **FIGs. 2E, 2B, 2H, 2G and**

2I, respectively in cells treated with PD-1-Fc or an IgG1 control protein. Activity was measured as growth of a 630 nm signal from HEK-Blue™ IL-2 reporter cells (an engineered human kidney cell line which generates a detectable color change in upon activation of its IL-2 receptor).

[0034] FIG. 17 shows plots depicting IL-2 activity of IL-2-linked protein complexes comprising the structures depicted in **FIG. 2H** with a human Fc domain in cells treated with PD-1-Fc or an IgG1 control protein. Activity was measured as growth of a 630 nm signal from HEK-Blue™ IL-2 reporter cells (an engineered human kidney cell line which generates a detectable color change in upon activation of its IL-2 receptor).

[0035] FIG. 18 shows plots depicting IL-2 activity in protein complexes comprising the structure depicted in **FIG. 2B** with the IL-2 3x variant.

[0036] FIGs. 19A, 19B, and 19C show plots depicting IL-2 activity of IL-2-3x-linked protein complexes comprising the structure depicted in **FIG. 2H with three different anti-PD-1 Fab arms (Nivolumab, 4C10 and Knd respectively)** in cells plated in wells coated with PD-1-Fc or an IgG1 control protein. Activity was measured as growth of a 630 nm signal from HEK-Blue™ IL-2 reporter cells (an engineered human kidney cell line which generates a detectable color change in upon activation of its IL-2 receptor).

[0037] FIG. 20 shows plots depicting IL-2 activity of IL-2-linked protein complexes comprising the structure depicted in **FIG. 2B** with linkers of varying lengths. Complexes were tested in wells coated with PD-1-Fc or an IgG1 control protein. Activity was measured as growth of a 630 nm signal from HEK-Blue™ IL-2 reporter cells (an engineered human kidney cell line which generates a detectable color change in upon activation of its IL-2 receptor).

[0038] FIGs. 21A and 21B show plots depicting PD-1 dependent induction of STAT5 phosphorylation by PD-1/IL-2 DBA-Cytokine Complexes in Human Primary CD8⁺ T Cells as measured by flow cytometry.

[0039] FIGs. 22A and 22B show plots depicting PD-1/IL-2 DBA-cytokine complex modulation of human T cell activation in a mixed lymphocyte reaction as assessed by Granzyme B release.

[0040] FIGs. 23A and 23B show plots depicting PD-1/IL-2 DBA-Cytokine Complex Modulation of Anti-Tumor Immunity in a Syngeneic Tumor Model. 500,000 MC38 tumor cells were implanted subcutaneously in human PD-1 knock-in mice, mice were treated intravenously with complexes, and tumor volume was assessed.

DETAILED DESCRIPTION

[0041] The present disclosure provides compositions of protein complexes and methods of use thereof. Interleukin 2 therapeutics are often unable to be realized due to systemic on-target toxicity. Provided herein are protein complexes, which specifically exhibit therapeutic efficacy on PD-1 positive cells, specifically antigen-experienced T cells. Moreover, protein complexes of the present disclosure are self-regulated, remaining inactive in the absence of a PD-1 and activating when bound to PD-1. The protein complexes disclosed herein may include a sensor domain (e.g., an antibody, Fab or scFv) that is linked to an IL-2 receptor agonist (the therapeutic domain) via a linker. The sensor domain may be a dual binding antibody that has affinity for the therapeutic domain and for PD-1, such that the PD-1 and the therapeutic domain compete for binding to the sensor domain. In the absence of PD-1, the sensor domain binds the therapeutic domain, rendering the therapeutic domain unable to exert activity on IL-2 receptors. When the sensor domain is bound to PD-1, the therapeutic domain is unbound and may exert activity. In some embodiments, regulation of IL-2 receptor agonist activity by the complex may be reversible, that is, when the sensor domain disassociates from PD-1, the sensor domain may bind the therapeutic domain, rendering the therapeutic domain once again unable to exert activity. Thus, the protein complexes of the present disclosure comprise sensor domains that regulate IL-2 receptor agonist domains in the presence of PD-1, bind the PD-1, and render the IL-2 receptor agonist domain active. Various structures and compositions of protein complexes are disclosed herein, including pharmaceutical formulations. Also provided herein are methods for treating a subject in need thereof by administering the protein complex to the subject.

[0042] As used herein, a “sensor domain” generally refers to a dual-binding antibody capable of binding PD-1 and of binding an IL-2 receptor agonist.

[0043] As used herein, a “therapeutic domain” generally refers to an IL-2 receptor agonist. Non-limiting examples of a therapeutic domain include IL-2, IL-15 or any other molecule that acts on an IL-2 receptor in a manner similar to IL-2.

[0044] As used herein, a “marker” generally refers to PD-1 protein.

[0045] As used herein, an “antibody” generally refers to an antibody, an antibody derivative, or fragment(s) thereof that contains part or all of an antibody variable domain.

[0046] The term “recombinant nucleic acid” generally refers to synthetic nucleic acid having a nucleotide sequence that is not naturally occurring. A recombinant nucleic acid may be synthesized in the laboratory. A recombinant nucleic acid is prepared by using recombinant DNA technology by using enzymatic modification of DNA, such as enzymatic restriction digestion, ligation, and DNA cloning. A recombinant nucleic acid as used herein can be DNA, or

RNA. A recombinant DNA may be transcribed in vitro, to generate a messenger RNA (mRNA), the recombinant mRNA may be isolated, purified and used to transfect a cell. A recombinant nucleic acid may encode a protein or a polypeptide. A recombinant nucleic acid, under suitable conditions, can be incorporated into a living cell, and can be expressed inside the living cell. As used herein, “expression” of a nucleic acid usually refers to transcription and/or translation of the nucleic acid. The product of a nucleic acid expression is usually a protein but can also be an mRNA. Detection of an mRNA encoded by a recombinant nucleic acid in a cell that has incorporated the recombinant nucleic acid, is considered positive proof that the nucleic acid is “expressed” in the cell.

[0047] As used herein, the term “therapeutic domain” generally refers to a protein domain having the minimum sequence features to activate a given therapeutic activity in a cell or organism. In the case where a therapeutic domain is a ligand of a ligand-receptor pair, the ligand has the minimum sequence and/or structural features to allow for binding to or activation of the receptor.

[0048] The process of inserting or incorporating a nucleic acid into a cell can be via transformation, transfection or transduction. Transformation is the process of uptake of foreign nucleic acid by a bacterial cell. This process is adapted for propagation of plasmid DNA, protein production, and other applications. Transformation introduces recombinant plasmid DNA into competent bacterial cells that take up extracellular DNA from the environment. Some bacterial species are naturally competent under certain environmental conditions, but competence is artificially induced in a laboratory setting. Transfection is the forced introduction of small molecules such as DNA, RNA, or antibodies into eukaryotic cells. Just to make life confusing, ‘transfection’ also refers to the introduction of bacteriophage into bacterial cells. ‘Transduction’ is mostly used to describe the introduction of recombinant viral vector particles into target cells, while ‘infection’ refers to natural infections of humans or animals with wild-type viruses.

Protein Complexes

[0049] The present disclosure provides complexes that may self-regulate IL-2 receptor agonist activity. Protein complexes of the present disclosure may include a dual-binding antibody with affinity for PD-1 and affinity for an IL-2 receptor agonist (the “sensor domain”) and an IL-2 receptor agonist (the “therapeutic domain”). The sensor domain and therapeutic domain may be linked by a linker. The sensor domain may regulate the activity of the therapeutic domain. Regulation of the activity of the therapeutic domain may include binding of the sensor domain to the therapeutic domain, rendering the therapeutic domain unable to exert activity on the IL-2

receptor. Regulation of the activity of the therapeutic domain may further include unbinding, or release, of the therapeutic domain by the sensor domain upon binding of the sensor domain to PD-1. Thus, the protein complexes of the present disclosure are superior drug candidates as the sensor domain-dependent activity of the IL-2 receptor agonist allows for cell-specific activity, even upon systemic administration of the protein complex. Compared to IL-2 receptor agonists administered on their own, the protein complexes of the present disclosure exhibit regulated therapeutic activity. As a result, compared to free IL-2 receptor agonists administered on their own, the protein complexes of the present disclosure exhibit reduced systemic on-target toxicity.

[0050] The protein complexes of the present disclosure can have an Fc region. The protein complexes of the present disclosure can have a domain that improves kinetic properties. For example, the protein complexes of the present disclosure may be further coupled to a half-life extender, such as an Fc region, albumin, PEG, or another zwitterionic polymer. The protein complexes of the present disclosure may have two heavy chains and two light chains. The protein complexes of the present disclosure may have two heavy chains and one light chain. The protein complexes of the present disclosure may include multiple sensor domains and multiple therapeutic domains. For example, a protein complex of the present disclosure may include two sensor domains and two therapeutic domains, all of which are linked and in which the two therapeutic domains are bound to the two sensor domains. In some embodiments, a protein complex of the present disclosure may include two sensor domains and one therapeutic domain, all of which are linked and in which the therapeutic domain may bind to both sensor domains or only one of the two sensor domains.

[0051] In some embodiments, the PD-1 may be a surface protein, such as a cell surface protein. In some embodiments, the PD-1 may be expressed on antigen-experienced T cells.

[0052] In some embodiments the IL-2 receptor agonist may be IL-2, a variant of IL-2 or a truncated version of IL-2. In some embodiments the IL-2 receptor agonist may be IL-15, IL-15-sushi, a variant of IL-15 or a variant of IL-15-sushi. In some embodiments the IL-2 receptor agonist may be an engineered or designed peptide that binds IL2 receptor beta and IL-2 receptor gamma. In some embodiments the IL-2 receptor agonist may be an antibody that binds IL2 receptor beta and IL-2 receptor gamma.

[0053] In some embodiments, binding of the sensor domain to the therapeutic domain versus binding of the sensor domain to PD-1 is regulated by the relative affinity of the sensor domain for the therapeutic domain. In some embodiments, the sensor domain may have a dissociation constant (K_d) for PD-1 that is lower than the dissociation constant of the sensor domain for the therapeutic domain. Thus, the sensor may have a higher affinity (lower K_d) for PD-1 than for the

therapeutic domain. The sensor domains of the present disclosure may be engineered, for example by affinity maturation, to have a higher affinity (lower dissociation constant) for PD-1 than the therapeutic domain. In the absence of the marker, the sensor domain of the present disclosure may have a sufficiently high affinity for the therapeutic domain such that the therapeutic domain is bound by the sensor domain. In the presence of the marker, the affinity of the sensor domain for PD-1 is sufficiently high (low dissociation constant), such that PD-1 outcompetes the therapeutic domain for binding to the sensor domain. As a result, the equilibrium binding shifts from a state in which the sensor domain is bound to the IL-2 receptor agonist domain to a state in which the IL-2 receptor agonist domain is unbound, and the sensor domain binds PD-1.

[0054] The sensor domain may have an affinity for PD-1 that is at least 2-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 5-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 10-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 15-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 20-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 25-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 30-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 35-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 40-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 45-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 50-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 60-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 70-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 80-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 90-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 100-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 150-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is at least 200-fold higher than an affinity for the therapeutic domain. The sensor

[illegible]

from 30 to 70-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is from 40 to 60-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is from 20 to 50-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is from 10 to 1000-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is from 70 to 500-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is from 100 to 500-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is from 500 to 750-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is from 250 to 750-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is from 1000 to 100000-fold higher than an affinity for the therapeutic domain. The sensor domain may have an affinity for PD-1 that is from 2 to 100000-fold higher than an affinity for the therapeutic domain.

[0055] A protein complex of the present disclosure, or a fragment thereof, may comprise one or more complementary determining regions (CDRs) having have at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or substantially 100% sequence identity to any one of the CDRs disclosed herein. A protein complex of the present disclosure, or a fragment thereof, may comprise one or more heavy chain or light chain variable regions having have at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or substantially 100% sequence identity to any one of the heavy chain or light chain variable regions described herein. For example, a protein complex of the present disclosure, or a fragment thereof, may comprise one or more CDRs having at least 80% sequence identity to any one of SEQ ID NOs: 1 –20, SEQ ID NOs: 142-173, or SEQ ID NOs: 238-252. A protein complex of the present disclosure, or a fragment thereof, may comprise one or more CDRs having at least 85% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 20, SEQ ID NO: 142-173, or SEQ ID NO: 238-252. A protein complex of the present disclosure, or a fragment thereof, may comprise one or more CDRs having at least 90% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 20, SEQ ID NO: 142-173, or

SEQ ID NO: 238-252. A protein complex of the present disclosure, or a fragment thereof, may comprise one or more CDRs having at least 92% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 20, SEQ ID NO: 142-173, or SEQ ID NO: 238-252. A protein complex of the present disclosure, or a fragment thereof, may comprise one or more CDRs having at least 95% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 20, SEQ ID NO: 142-173, or SEQ ID NO: 238-252. A protein complex of the present disclosure, or a fragment thereof, may comprise one or more CDRs having at least 97% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 20, SEQ ID NO: 142-173, or SEQ ID NO: 238-252. A protein complex of the present disclosure, or a fragment thereof, may comprise one or more CDRs having at least 99% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 20, SEQ ID NO: 142-173, or SEQ ID NO: 238-252. A protein complex of the present disclosure, or a fragment thereof, may comprise one or more CDRs having any one of SEQ ID NO: 1 – SEQ ID NO: 20, SEQ ID NO: 142-173, or SEQ ID NO: 238-252.

[0056] A protein complex, or a fragment thereof, can have at least 80% sequence identity to any one of SEQ ID NO: 41, SEQ ID NO: 44, SEQ ID NO: 80 – SEQ ID NO: 112, SEQ ID NO: 174-175, SEQ ID NO: 181-182, SEQ ID NO: 195-196, SEQ ID NO: 205-206, SEQ ID NO: 210-212, SEQ ID NO: 220-223, SEQ ID NO: 226-231, SEQ ID NO: 259-261, SEQ ID NO: 266-282, or SEQ ID NO: 289- 293, or a fragment thereof. A protein complex can have at least 85% sequence identity to any one of SEQ ID NO: 41, SEQ ID NO: 44, SEQ ID NO: 80 – SEQ ID NO: 112, SEQ ID NO: 174-175, SEQ ID NO: 181-182, SEQ ID NO: 195-196, SEQ ID NO: 205-206, SEQ ID NO: 210-212, SEQ ID NO: 220-223, SEQ ID NO: 226-231, SEQ ID NO: 259-261, SEQ ID NO: 266-282, or SEQ ID NO: 289- 293, or a fragment thereof. A protein complex can have at least 90% sequence identity to any one of SEQ ID NO: 41, SEQ ID NO: 44, SEQ ID NO: 80 – SEQ ID NO: 112, SEQ ID NO: 174-175, SEQ ID NO: 181-182, SEQ ID NO: 195-196, SEQ ID NO: 205-206, SEQ ID NO: 210-212, SEQ ID NO: 220-223, SEQ ID NO: 226-231, SEQ ID NO: 259-261, SEQ ID NO: 266-282, or SEQ ID NO: 289- 293, or a fragment thereof. A protein complex can have at least 92% sequence identity to any one of SEQ ID NO: 41, SEQ ID NO: 44, SEQ ID NO: 80 – SEQ ID NO: 112, SEQ ID NO: 174-175, SEQ ID NO: 181-182, SEQ ID NO: 195-196, SEQ ID NO: 205-206, SEQ ID NO: 210-212, SEQ ID NO: 220-223, SEQ ID NO: 226-231, SEQ ID NO: 259-261, SEQ ID NO: 266-282, or SEQ ID NO: 289- 293, or a fragment thereof. A protein complex can have at least 95% sequence identity to any one of SEQ ID NO: 41, SEQ ID NO: 44, SEQ ID NO: 80 – SEQ ID NO: 112, SEQ ID NO: 174-175, SEQ ID NO: 181-182, SEQ ID NO: 195-196, SEQ ID NO: 205-206, SEQ ID NO: 210-212, SEQ ID NO: 220-223, SEQ ID NO: 226-231, SEQ ID NO: 259-261, SEQ ID NO: 266-282, or SEQ ID NO: 289-

293, or a fragment thereof. A protein complex can have at least 97% sequence identity to any one of SEQ ID NO: 41, SEQ ID NO: 44, SEQ ID NO: 80 – SEQ ID NO: 112, SEQ ID NO: 174-175, SEQ ID NO: 181-182, SEQ ID NO: 195-196, SEQ ID NO: 205-206, SEQ ID NO: 210-212, SEQ ID NO: 220-223, SEQ ID NO: 226-231, SEQ ID NO: 259-261, SEQ ID NO: 266-282, or SEQ ID NO: 289- 293, or a fragment thereof. A protein complex can have at least 99% sequence identity to any one of SEQ ID NO: 41, SEQ ID NO: 44, SEQ ID NO: 80 – SEQ ID NO: 112, SEQ ID NO: 174-175, SEQ ID NO: 181-182, SEQ ID NO: 195-196, SEQ ID NO: 205-206, SEQ ID NO: 210-212, SEQ ID NO: 220-223, SEQ ID NO: 226-231, SEQ ID NO: 259-261, SEQ ID NO: 266-282, or SEQ ID NO: 289- 293, or a fragment thereof. A protein complex is any one of SEQ ID NO: 41, SEQ ID NO: 44, SEQ ID NO: 80 – SEQ ID NO: 112, SEQ ID NO: 174-175, SEQ ID NO: 181-182, SEQ ID NO: 195-196, SEQ ID NO: 205-206, SEQ ID NO: 210-212, SEQ ID NO: 220-223, SEQ ID NO: 226-231, SEQ ID NO: 259-261, SEQ ID NO: 266-282, or SEQ ID NO: 289- 293, or a fragment thereof.

[0057] A protein complex of the present disclosure may have at least 95% sequence identity to any one of SEQ ID NO: 41, SEQ ID NO: 44, SEQ ID NO: 80 – SEQ ID NO: 112, SEQ ID NO: 174-175, SEQ ID NO: 181-182, SEQ ID NO: 195-196, SEQ ID NO: 205-206, SEQ ID NO: 210-212, SEQ ID NO: 220-223, SEQ ID NO: 226-231, SEQ ID NO: 259-261, SEQ ID NO: 266-282, or SEQ ID NO: 289- 293, or a fragment thereof and have one or more CDRs with at least 80% sequence identity to any one SEQ ID NO: 1 – SEQ ID NO: 20, SEQ ID NO: 142-173, or SEQ ID NO: 238-252. The protein complexes of the present disclosure can have CDRs selected from SEQ ID NO: 1 – SEQ ID NO: 20, SEQ ID NO: 142-173, or SEQ ID NO: 238-252 arranged in any combination or order.

[0058] A fragment of any of the above may retain the functional binding domains of the sensor or any functional therapeutic domains of the therapeutic. For example, a dual binding antibody protein complex can include the entire antibody or a fragment having regions of the antibody that are capable of binding to a marker and the therapeutic domain. In the latter case, the fragment may be an scFv that can bind to a marker and the therapeutic domain. Exemplary sequence of protein complexes of the present disclosure is shown below in **TABLE 1**.

TABLE 1 – Exemplary Protein Complexes

SEQ ID NO	Sequence	Description
SEQ ID NO: 769	QVQLVESGGGVVQPGRSLRLDCKASGITFSN SGMHWVRQAPGKGLEWVAVIWYDGSKRYY ADSVKGRFTISRDN SKNTLFLQMNSLRAEDT AVYYCATNDDYWGQGLVTVSSAKTTAPSV YPLAPVCGDTTGSSVTLGCLVKGYFPEPVTL	PD1- IL2_3x_Cterm_Nivo _2B07_H_H37Y_L_ A107Y_S109R;

SEQ ID NO	Sequence	Description
	TWNSGSLSSGVHTFPAVLQSDLYTLSSSVTV TSSTWPSQSITCNVAHPASSTKVDKKIEPRGP TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVL ISLSPIVTCVVVDVSEDDPDVQISWVNNVEV HTAQTQTHREDYNSTLRVVSALPIQHQDWM SGKEFKCKVNNKDLGAPIERTISKPKGSVRAP QVYVLPPEKEMTKKQVSLTCLVKDFMPEDI YVEWTNNGKTELNYKNTEPVLKSDGSYFMY SKLTVEKKNWVERNYSYSCSVVHEGLHNHHT TKSFSRTPGGGGGSGGGGSGGGGSGGGGSQ VQLVQSGAEVKKPGASVKVSCASGDTFTR YYVHWVRQAPGQGLEWMGIINPSGGYASYA QKFQGRVTMTRDTSTSTVYMESSLRSEDTA VYYCAAGLFIWGQGTLLTVSSASGGGGSGG GGSGGGGSHASDIQMTQSPSSLSASVGDRVTI TCRASQSIGRWLAWYQQKPGKAPKLLIYSAS NLETGVPSRFSGSGSGTDFTLTISLQPEDFAT YYCQQYNRFPVTFGPGTKVDIK	AF4695_pep2
SEQ ID NO: 759	QVQLVESGGGVVQPGRSLRLDCKASGITFSN SGMHWVRQAPGKGLEWVAWIWYDGSKRYY ADSVKGRFTISRDN SKNTLFLQMNSLRAEDT AVYYCATNDDYWGGQGLTVTVSSAKTTAPSV YPLAPVCGD TTGSSVTLGCLVKGYFPEPVTL TWNSGSLSSGVHTFPAVLQSDLYTLSSSVTV TSSTWPSQSITCNVAHPASSTKVDKKIEPRGP TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVL ISLSPIVTCVVVDVSEDDPDVQISWVNNVEV HTAQTQTHREDYNSTLRVVSALPIQHQDWM SGKEFKCKVNNKDLGAPIERTISKPKGSVRAP QVYVLPPEEEMTKKQVTLTCMVTDMPEDI YVEWTNNGKTELNYKNTEPVLDSDGSYFMY SDLRVEKKNWVERNYSYSCSVVHEGLHNHHT TESFSRTPGGGGGSGGGGSGGGGSAPTSSST KKTQLQLEHLLLDLQMLNGINNYKNPKLTD MLTFEFYMPKKATELKHLQCLERELKPLEEV LNLAQSKNFHLRPRDLISNINVIVLELKGSETT FMCEYADETATIVEFLNRWITFCQSIISTLT	PD1- IL2_3x_Cterm_Nivo _2B07_H_H37Y_L_ A107Y_S109R; AF4695_pep1 AND AF4696_pep1
SEQ ID NO: 745	EIVLTQSPATLSLSPGERATLSCRASQSVSSYL AWYQQKPGQAPRLLIYDASNRATGIPARFSG SGSGTDFTLTISLLEPEDFAVYYCQQSSNWPR TFGQGTKVEIKRADAAPTVSIFPPSSEQLTSG GASVVCFLNFPKDIVKWKIDGSERQNG VLNSWTDQDSKDSTYSMSSTLTTLTKDEYERH NSYTCEATHKTSTSPIVKSFNRECE	PD1- IL2_3x_Cterm_Nivo _2B07_H_H37Y_L_ A107Y_S109R; AF4695_pep3 AND AF4695_pep3
SEQ ID NO: 770	QVQLVESGGGVVQPGRSLRLDCKASGITFSN SGMHWVRQAPGKGLEWVAWIWYDGSKRYY ADSVKGRFTISRDN SKNTLFLQMNSLRAEDT	PD1- IL2_3x_Cterm_Niv o 704var

SEQ ID NO	Sequence	Description
	AVYYCATNDDYWGQGTLLTVSSAKTTAPSV YPLAPVCGDTTGSSVTLGCLVKGYFPEPVTL TWNSGSLSSGVHTFPAVLQSDLYTLSSSVTV TSSTWPSQSITCNVAHPASSTKVDKKIEPRGP TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVL MISLSPIVTCVVVDVSEDDPDVQISWVFN NVEVHTAQTQTHREDYNSTLRVVSALPIQH QDWMMSGKEFKCKVNNKDLGAPIERTISK PKGSVRAPQVYVLPPEKEMTKKQVSLTCL VKDFMPEDIYVEWTNNGKTELNYKNTEP VLKSDGSYFMYSKLTVEKKNWVERN SYSCSVVHEGLHNHHTTKSFSRTPGGG GGSGGGGSGGGGSGGGGSGGGGSGQ VQLVQSGAEVKKPGASVKVSCKASGYTF TRYVYVHWVRQAPGQGLEWMGIINPRAGY TSYALKFQGRVTMTRDTSTSTVYMELSS LRSEDTAVYYCTSGWDVWGQGTLLTVSSA SGGGSGGGGSGGGGSHASDIQMTQSPSS LSASVGDRVITITCRASQSISTWLAWYQ QKPGKAPKLLIYAASSLD SGVPSRFSGS SGTDFTLTISSLQPEDFATYYCQQSY SFPVTFGQGTKVEIK	AF4696_pep2
SEQ ID NO: 181	APTSSSTKKTQLQLEHLLLDLQMLNGIN NYKNPKLTDMLTFEFYMPKKATELKHLQ CLERELKPLEEVLNLAQSKNFHLRPRDL ISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFCQSII STLTGGGGSG GGGSGGGGSGGGGSGGGGSGQVQLVQ SGAEVKKPGASVKVSCKASGDTFTRYV YVHWVRQAPGQGLEWMGIINPSGGYAS YAQKFQGRVTMTRDTSTSTVYMELSS LRSEDTAVYYCAAGLFIWGQGTLLTVSS AKTTAPSVYPLAPVCGDTTGSSVTLGCL VKGYFPEPVTLTWNSGSLSSGVHTFPA VLQSDLYTLSSSVTVTSSTWPSQSITCN VAHPASSTKVDKKIEPRGPTIKPCPPCK CPAPNAAGGPSVFIFPPKIKDVLMI SLSPIVTCVVVDVSEDDPDVQISWVFN NVEVHTAQTQTHREDYNSTLRVVSALPI QHQDWMMSGKEFKCKVNNKDLGAPIER TISKPKGSVRAPQVYVLPPEEEMTKKQ VTLTCMVTDFMPEDIYVEWTNNGKTEL NYKNTEPVLDSYFMYSDLRVEKKNWVER NSYSCSVVHEGLHNHHTTESFSRTPGK	PD1-IL2_3x_Asym_PD1 - IL2_2B07_H_H37Y_L_W38Y_A107Y AF4386_pep1
SEQ ID NO: 182	QVQLVQSGAEVKKPGASVKVSCKASGDT FTRYVYVHWVRQAPGQGLEWMGIINPS GGYASYAQKFQGRVTMTRDTSTSTVYM ELSSLRSEDTAVYYCAAGLFIWGQGTLL TVSSAKTTAPSVYPLAPVCGDTTGSSV TLGCLVKGYFPEPVTLTWNSGSLSSGV HTFPAVLQSDLYTLSSSVTVTSSTWPS QSITCNVAHPASSTKVDKKIEPRGPTIK PCPPCKCPAPNAAGGPSVFIFPPKIKD VLMISLSPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNSTLRVVS ALPIQH	PD1-IL2_3x_Asym_PD1 - IL2_2B07_H_H37Y_L_W38Y_A107Y AF4386_pep2

SEQ ID NO	Sequence	Description
	QDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQ VYVLPPEKEMTKKQVSLTCLVKDFMPEDIYVEWTNN GKTELNYKNTEPVLKSDGSYFMYSKLTVEKKNWVERNS YSCSVVHEGLHNHHTTKSFSRTPGGGGSGGGSHHHHH H	
SEQ ID NO: 212	DIQMTQSPSSLSASVGDRVTITCRASQSIGRY LAWYQQKPGKAPKLLIYSASNLETGVPSRFS GSGSGTDFLTISSLQPEDFATYYCQQYNSFP VTFGPGTKVDIKRADAAPTVSIFPPSSEQLTS GGASVVCFLNNFYPKDINVKWKIDGSERQN GVLNSWTDQDSKSTYSMSSTLTTLTKDEYER HNSYTCEATHKTSTSPIVKSFNRNEC	PD1- IL2_3x_Asym_PD1 - IL2_2B07_H_H37Y _L_W38Y_A107Y AF4386_pep3
SEQ ID NO: 183	APTSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTDM LTFEFYMPKKATELKHLQCLERELKPLEEVLNLAQSKNF HLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLN RWITFCQSIISTLTGGGGSGGGGSGGGGSGGGGSGVQV LVQSGAEVKKPGASVKVSKASGYTFTDYYMHVVRQ APGQGLEWMGIINPRAGYTSYALKFQGRVTMTRDTST STVYMELSSLRSEDTAVYYCTSGWDVWVGQGLTVTVSS AKTTAPSVYPLAPVCGDGTGSSVTLGCLVKGYFPEPVTL TWNSSGLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPS QSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPN AAGGPSVFIFPPKIKDVLMSLSPIVTCVVVDVSEDDPDV QISWVFNNEVHTAQTQTHREDYNSTLRVVSALPIQH QDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQ VYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNN GKTELNYKNTEPVLDSGSYFMYSDLRVEKKNWVERN SYSCSVVHEGLHNHHTTESFSRTPGK	PD1- IL2_3x_Asym_PD1 - IL2_7A04_H_M115 W_L_Q68D AF4387_pep1
SEQ ID NO: 184	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYYMHV VRQAPGQGLEWMGIINPRAGYTSYALKFQGRVTMTR DTSTSTVYMELSSLRSEDTAVYYCTSGWDVWVGQGLTV TVSSAKTTAPSVYPLAPVCGDGTGSSVTLGCLVKGYFPE PVTLTWNSSGLSSGVHTFPAVLQSDLYTLSSSVTVTSST WPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCP APNAAGGPSVFIFPPKIKDVLMSLSPIVTCVVVDVSED DPDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSAL PIQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVR APQVYVLPPEKEMTKKQVSLTCLVKDFMPEDIYVEWT NNGKTELNYKNTEPVLKSDGSYFMYSKLTVEKKNWVE RNSYSCSVVHEGLHNHHTTKSFSRTPGGGGSGGGSHH HHHH	PD1- IL2_3x_Asym_PD1 - IL2_7A04_H_M115 W_L_Q68D AF4387_pep2
SEQ ID NO: 185	DIQMTQSPSSLSASVGDRVTITCRASQSISTW LAWYQQKPGKAPKLLIYAASSLDSGVPSRFS	PD1- IL2_3x_Asym_PD1

SEQ ID NO	Sequence	Description
	GSGSGTDFTLTISSLQPEDFATYYCQQSYSPF VTFGQGTKVEIKRADAAPTVSIFPPSSEQLTS GGASVVCFLNNFYPKDINVKWKIDGSERQN GVLNSWTDQDSKSTYSMSSTLTLTKDEYER HNSYTCEATHKTSTSPIVKSFNRNEC	- IL2_7A04_H_M115 W_L_Q68D AF4387_pep3

A. Sensor Domains

[0059] Protein complexes of the present disclosure include sensor domains comprised of a dual-binding antibody with affinity for PD-1 and affinity for an IL-2 receptor agonist. A sensor domain may be any protein that is capable of sensing the presence of a first moiety and regulating a second moiety, where the first moiety is PD-1 and the second moiety is an IL-2 receptor agonist. For example, the present disclosure provides a sensor domain that may be an antibody or antibody fragment capable of binding a first moiety and binding and blocking the activity of a second moiety, wherein the first moiety is PD-1 and the second moiety is IL-2 or another IL-2 receptor agonist. In the absence of the first moiety, the sensor domain binds the second moiety. If the first moiety is introduced into the system, the sensor domain binds the first moiety and unbinds the second moiety. Thus, the binding and unbinding of the second moiety is reversible. The sensor domain inactivates or blocks the activity of the IL-2 receptor agonist domain by binding the IL-2 receptor agonist domain and preventing it from binding to its target (the IL-2 receptor). The sensor domain regulates the IL-2 receptor agonist domain by releasing it to act on its target upon binding of PD-1.

[0060] In some embodiments, the sensor domain is a dual binding antibody. A dual binding antibody may be capable of binding PD-1 and the IL-2 receptor agonist domain. A dual binding antibody of the present disclosure may be selected or engineered to bind PD-1 and the therapeutic domain. The dual binding protein may have a higher affinity for PD-1 as compared to the IL-2 receptor agonist domain. The dual binding protein may be affinity matured to have a higher affinity for PD-1 as compared to the IL-2 receptor agonist domain.

[0061] In some embodiments, the sensor domain is an antibody. The sensor domain may also be a fragment of an antibody. A fragment of an antibody consistent with the sensor domains disclosed herein retains its ability to exhibit dual binding to both PD-1 and an IL-2 receptor agonist domain. One or both domains of a bispecific antibody may be sensor domains of the protein complexes of the present disclosure. In the instance that bispecific antibodies are used, the bispecific antibody may include a first antigen binding domain that may bind an IL-2 receptor agonist domain and PD-1 and may also include a second antigen binding domain capable of binding PD-1.

[0062] In some embodiments, the sensor domain is an anti-PD1 antibody or fragment thereof (e.g., an scFv that binds PD1 or PD-L1).

B. Therapeutic Domains

[0063] Protein complexes of the present disclosure include therapeutic domains comprised of an IL-2 receptor agonist. A therapeutic domain of the present disclosure is linked to a sensor domain via a linker to form a protein complex. The therapeutic domain may exert therapeutic activity by binding to an IL-2 receptor.

[0064] In some embodiments, the protein complexes of the present disclosure comprise a therapeutic domain comprising IL-2, or variants or fusions of this cytokine. The therapeutic domain may also be a fragment of the above-mentioned moiety. A fragment retains functional regions of the moiety needed for binding to its target (e.g., IL-2 receptor) and any functional regions needed for activity.

[0065] In some embodiments, the protein complexes of the present disclosure comprise a therapeutic domain comprising IL-15, or variants or fusions of this cytokine. The therapeutic domain may also be a fragment of the above-mentioned moiety. A fragment retains functional regions of the moiety needed for binding to its target (e.g., IL-2 receptor) and any functional regions needed for activity.

[0066] In some embodiments, the protein complexes of the present disclosure comprise a therapeutic domain comprising a peptide, and engineered protein or an antibody capable of binding IL-2 receptor beta and IL-2 receptor gamma. The therapeutic domain may also be a fragment of the above-mentioned moiety. A fragment retains functional regions of the moiety needed for binding to its target (e.g., IL-2 receptor) and any functional regions needed for activity.

C. Linkers

[0067] A protein complex disclosed herein may comprise a linker. The linker may connect two domains, such as a sensor domain and a therapeutic domain. Various linkers are consistent with the protein complexes of the present disclosure. In some embodiments, the linker may be an amino acid linker or a chemical linker.

[0068] The linker may be a stable linker. For example, a linker may maintain a connection between a therapeutic domain and a sensor domain even upon binding of the sensor domain to a marker and, thereby, unbinding of the therapeutic domain from the sensor domain. For example, although the sensor domain may unbind the therapeutic domain, the therapeutic domain may

remain linked to the sensor domain via the linker. Examples of linkers that are consistent with this activity may include non-cleavable linkers.

[0069] The linker may also be a flexible linker. A flexible linker is a linker that is long enough to allow for the therapeutic domain to bind to the IL-2 receptor, once it is unbound from the sensor domain. Flexibility of the linker may affect therapeutic efficacy. For example, upon binding of the sensor domain to PD-1 and unbinding of the therapeutic domain, the therapeutic domain needs to be able to encounter and bind its target, the IL-2 receptor. If the linker is not flexible enough to allow for the therapeutic domain to bind the IL-2 receptor, therapeutic efficacy may be reduced or not exerted. When the linker is flexible, therapeutic domains may be able to bind the IL-2 receptor and exert high therapeutic efficacy. Flexibility of a linker may arise from the length of the linker. For example, short linkers may sterically hinder the therapeutic domain from binding the IL-2 receptor. Longer linkers may allow for the protein complex to be more flexible and allow for therapeutic domains to bind the IL-2 receptor. In some embodiments, a linker that is too long may impact the ability of the sensor domain to bind the therapeutic domain and inhibit activity in the absence of PD-1. In some embodiments, a linker that is too long may impact the stability of a protein therapeutic domain or the half-life of the protein therapeutic domain *in vivo*.

[0070] In some embodiments, the linker may be attached to a heavy chain of the sensor domain or a light chain of the sensor domain. A linker may be fused to the N-terminus or C-terminus of the sensor domain. In some embodiments, the linker may be fused with the N-terminus or C-terminus of the IL-2 receptor agonist domain. For example, a linker may be attached to an N-terminus or C-terminus of an scFV or an ScFab.

[0071] Amino Acid Linkers. An amino acid linker may comprise any amino acid residues. In some embodiments, favored amino acid residues are amino acid residues that are entropically flexible. Favored amino acid residues in an amino acid linker of the present disclosure may include glycine and serine. Other preferred amino acid residues may include alanine, proline, threonine, and glutamic acid. In preferred embodiments, the amino acid linker may comprise from 3 to 60 amino acid residues in length. In some embodiments, the amino acid linker may comprise 20 amino acid residues. In some embodiments, the amino acid linker may comprise 40 amino acid residues. In some embodiments, the amino acid linker may comprise 60 amino acid residues. In some embodiments, the amino acid linker may comprise 80 amino acid residues. An amino acid linker may comprise at least 5 amino acid residues. An amino acid linker may comprise at least 10 amino acid residues. An amino acid linker may comprise at least 15 amino acid residues. An amino acid linker may comprise at least 20 amino acid residues. An amino acid

[illegible]

[illegible]

than 300 amino acid residues. An amino acid linker may comprise no more than 400 amino acid residues. An amino acid linker may comprise no more than 500 amino acid residues.

Non-Cleavable Linkers.

[0072] A non-cleavable linker may include a non-proteolytically cleavable peptide. A non-proteolytically cleavable peptide may be inert to proteases present in a given sample or organism. For example, a peptide may be inert to all human protease cleavage sequences, and thereby may comprise a high degree of stability within humans and human samples. Such a peptide may also comprise a secondary structure which renders a protease cleavage site inert or inaccessible to a protease. A non-cleavable linker of the present disclosure may comprise a half-life for cleavage of at least 1 hour, at least 2 hours, at least 4 hours, at least 8 hours, at least 12 hours, at least 16 hours, at least 1 day, at least 2 days, at least 3 days, at least 1 week, at least 2 weeks, or at least 1 month in the presence of human proteases at 25 °C in pH 7 buffer.

D. Protein Complex Structures

[0073] The present disclosure provides a wide variety of protein complexes spanning a range of structures. A protein complex of the present disclosure may comprise an IL-2 receptor agonist domain and a sensor domain expressed as a single unit. An IL-2 receptor agonist domain may be expressed as an N-terminal extension of a sensor domain, as a C-terminal extension of a sensor domain, or disposed within a sensor domain. For example, a protein complex may comprise a peptide which comprises, from N-terminus to C-terminus, an IL-2 receptor agonist domain, a peptide linker, an scFv domain, and optionally a tag, such as a purification tag (e.g., a V5 or myc tag) or a localization signal.

[0074] A protein complex may comprise a plurality of protein subunits. The plurality of protein subunits (e.g., an IL-2 receptor agonist domain and a sensor domain, two sensor domains, or two subunits of a sensor domain) may be chemically or physically coupled following expression. The plurality of protein subunits may comprise a plurality of sensor and/or therapeutic domains. A sensor and/or a therapeutic domain may be comprised of a single protein subunit, of multiple protein subunits, or by portions thereof. For example, a sensor domain may comprise an antibody Fab region comprising portions of an immunoglobulin light chain and an immunoglobulin heavy chain.

[0075] A plurality of protein subunits may comprise physical handles which facilitate their selective coupling. The physical handles may enable spontaneous, irreversible, and/or non-mediated (e.g., not requiring a chaperone protein or a catalytic complex) coupling between the protein subunits, thereby enabling complex and asymmetric protein complexes. For example,

two distinct protein complex subunits expressed in a single Chinese hamster ovary (CHO) cell, may comprise physical handles which spontaneously and irreversibly couple prior to cellular export. Such physical handles may comprise a 'knob-into-hole' (KIH) construct or a charge-swap construct, in which two protein subunits comprise physical structures with mutual binding affinities and specificities. Such physical handles may comprise a covalently binding pair, such as a plurality of thiols configured to form disulfide bonds. Physical handles may enable facile production of protein complexes comprising identical or distinct domains.

[0076] A protein complex may comprise two or more identical domains. An example of such a protein complex is provided in **FIG. 2E**, which illustrates an antibody (multi-sensor domain) coupled to two IL-2 therapeutic domains. In this example, the protein complex comprises two protein immunoglobulin light chain subunits and two immunoglobulin heavy chain subunits complexed to form a competent antibody. The two immunoglobulin heavy chain subunits comprise N-terminal linkers coupled to IL-2 therapeutic domains. Each immunoglobulin heavy chain is coupled to an immunoglobulin light chain, such that the protein complex comprises two Fab regions, each separately coupled to a therapeutic domain by a linker. A second example of such a protein complex is provided in **FIG. 2D**, which illustrates an antibody (multi-sensor domain) coupled to two IL-2 therapeutic domains. In this example, the protein complex comprises two protein immunoglobulin light chain subunits and two immunoglobulin heavy chain subunits complexed to form a competent antibody. The two immunoglobulin light chain subunits comprise N-terminal linkers coupled to IL-2 therapeutic domains. Each immunoglobulin heavy chain is coupled to an immunoglobulin light chain, such that the protein complex comprises two Fab regions, each separately coupled to a therapeutic domain by a linker. A third example of such a protein complex is provided in **FIG. 2G**, which illustrates an antibody (multi-sensor domain) coupled to two IL-2 therapeutic domains and four sensor domains. In this example, the protein complex comprises two protein immunoglobulin light chain subunits and two immunoglobulin heavy chain subunits complexed to form a competent antibody. The two immunoglobulin heavy chain subunits comprise N-terminal linkers coupled to IL-2 therapeutic domains and comprise C-terminal linkers coupled to a sensor domain which does not target a therapeutic domain (an anti-PD-1 scFv domain). Each immunoglobulin heavy chain is coupled to an immunoglobulin light chain, such that the protein complex comprises two Fab regions, each separately coupled to a therapeutic domain by a linker and two Fc domains, each separately coupled to a sensor by a linker.

[0077] While the above example provides a symmetric protein complex with two identical sensor domains and two identical therapeutic domains, a protein complex may also comprise a

plurality of distinct sensor and/or therapeutic domains. Such a protein complex may comprise an immunoglobulin unit with a first arm comprised of a heavy chain-light chain pair, and a second arm comprised of an antibody fragment such as an scFv, an scFab, a VH, or a fragment thereof. In such cases, the heavy chain, the antibody fragment, or the light chain may comprise an N-terminal extension with a linker and a therapeutic domain, as illustrated in **FIG. 2A, C, and F**, respectively. Alternatively, the heavy chain, the antibody fragment, or the light chain may comprise a C-terminal extension with a linker and a therapeutic domain. A protein complex may also comprise a symmetric immunoglobulin unit with a single therapeutic domain. For example, as shown in **FIG. 2B**, an immunoglobulin unit may comprise an N-terminal linker and therapeutic unit on a single heavy chain. Alternatively, an immunoglobulin unit may comprise an N-terminal linker and therapeutic unit on a single light chain. An immunoglobulin unit may also comprise a pair of antibody fragments coupled to a single Fc region. An immunoglobulin unit may comprise a nanobody. An immunoglobulin unit may comprise a diabody.

[0078] A protein complex may comprise flexible linker between the Fab arm and the Fc domain of a competent antibody, such that the Fab sensor domain is capable of binding a therapeutic domain linked to the C-term of the Fc domain, as shown in FIG. 5I. In this example, the protein complex comprises two protein immunoglobulin light chain subunits and two immunoglobulin heavy chain subunits complexed to form the sensor antibody domains.

[0079] A protein complex may comprise a sensor domain which does not target a therapeutic domain. Such a sensor domain may aid in target localization, or may enhance the binding of a separate sensor domain to PD-1. An example of a protein complex comprising a sensor domain which does not target a therapeutic domain is provided in **FIG. 2H**. This system comprises a monospecific anti-PD-1 antibody, wherein a first heavy chain comprises a C-terminal linker coupled to a therapeutic domain, and a second heavy chain comprises a C-terminal linker coupled to a sensor domain with dual specificity for the IL-2 receptor agonist domain and for PD-1.

[0080] A protein complex may comprise a range of sensor-to-therapeutic domain ratios. A protein complex may comprise equal numbers of sensor domains and therapeutic domains, examples of which are provided by **FIGS. 2D and 2E**, which illustrate protein complexes with 2 sensor domains and 2 therapeutic domains. A protein complex may comprise a greater number of sensor domains than therapeutic domains, such as the protein complexes of **FIGS. 2A, 2B, 2C, 2F and 2I**, which each comprise two sensor domains and one therapeutic domain, or such as **FIG. 2G**, which comprises four sensor domains and two therapeutic domains and **FIG. 2H**, which comprises three sensor domains and one therapeutic domain. In such cases, a therapeutic

domain may be capable of interacting with multiple sensor domains, or may be constrained from interacting with more than one sensor domain. The number of therapeutic domains with which a sensor domain may interact may depend on its linker. A linker may be sufficiently short so as to prevent a therapeutic domain from interacting with a sensor domain, or may be sufficiently long so as to allow a therapeutic domain to interact with multiple sensor domains.

[0081] In specific cases, a protein complex may comprise an antibody with Fc-coupled therapeutic and sensor domains. As illustrated in **FIG. 2H**, a protein complex may comprise an antibody with a first heavy chain C-terminal extension comprising a linker and a therapeutic domain, and a second heavy chain C-terminal extension comprising a linker and a sensor domain. An antibody of this design may comprise common targets across its Fab and C-terminal extension sensor domain. For example, the antibody Fab regions and C-terminal extension sensor domain may each target the same epitope on PD-1. Conversely, an antibody of this design may comprise separate targets across its Fab regions and C-terminal extension sensor domain. For example, the antibody Fab regions and C-terminal extension sensor domain may each target a different epitope on PD-1. As illustrated in **FIG. 2I**, a protein complex may comprise an antibody with a first heavy chain comprising a flexible linker the CH1 and CH2 domains (between the Fab arm and the Fc domain) and a heavy chain C-terminal extension comprising a linker and a therapeutic domain, and a second first heavy chain comprising a flexible linker the CH1 and CH2 domains (between the Fab arm and the Fc domain) with no C-terminal extension.

[0082] In some embodiments, an amino acid in the protein complex described herein may comprise a conservative substitution. A conservative substitution may comprise a substitution of one amino acid with a different amino acid with similar biochemical properties (e.g. charge, hydrophobicity, and size). Examples of conservative substitutions, as well as substitutions that may be, but are not necessarily, preferred, are provided in TABLE 2 below.

TABLE 2 – Example Conservative Substitutions

Original Residue	Example Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Lys; Arg	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn

Original Residue	Example Substitutions	Preferred Substitutions
Glu (E)	Asp	Asp
Gly (G)	Pro; Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Leu; Val; Ile; Ala; Tyr	Leu
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0083] In some embodiments, the present disclosure describes a recombinant nucleic acid that encodes the protein complex disclosed herein. In some embodiments, the recombinant nucleic acid comprises a plasmid or a vector that encodes the entire protein complex. In some embodiments, the recombinant nucleic acid comprises plasmids or vectors that encode the therapeutic domain, the sensor domain, and the linker respectively. In some embodiments, the recombinant nucleic acid comprises plasmids or vectors that encode any two of the therapeutic domain, the sensor domain, and the linker together.

Pharmaceutical Formulations

[0084] A protein complex or a recombinant nucleic acid encoding the protein complex of the present disclosure may be formulated as a pharmaceutical composition. A pharmaceutical composition may comprise a pharmaceutically acceptable carrier or excipient. As used herein “pharmaceutically acceptable” or “pharmacologically acceptable” includes molecular entities

and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a subject, as appropriate. "Pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients are often also incorporated into the compositions.

Applications

[0085] A protein complex of the present disclosure may be used for various therapeutic applications. A protein complex of the present disclosure may be used as a therapeutic to administer to a subject in need thereof. The subject may be a human or non-human mammal. The subject may have a disease. The disease may be cancer. The cancer may be acute lymphoblastic leukemia (ALL); acute myeloid leukemia (AML); cancer in adolescents; adrenocortical carcinoma; aids-related cancers; Kaposi sarcoma (soft tissue sarcoma); aids-related lymphoma (lymphoma); primary CNS lymphoma (lymphoma); anal cancer; appendix cancer - see gastrointestinal carcinoid tumors; astrocytomas, childhood (brain cancer); atypical teratoid/rhabdoid tumor, childhood, central nervous system (brain cancer); basal cell carcinoma of the skin - see skin cancer; bile duct cancer; bladder cancer; bone cancer (includes Ewing sarcoma and osteosarcoma and malignant fibrous histiocytoma); brain tumors; breast cancer; bronchial tumors (lung cancer); Burkitt lymphoma - see non-Hodgkin lymphoma; carcinoid tumor (gastrointestinal); carcinoma of unknown primary; cardiac (heart) tumors, childhood; central nervous system; atypical teratoid/rhabdoid tumor, childhood (brain cancer); medulloblastoma and other CNS embryonal tumors, childhood (brain cancer); germ cell tumor, childhood (brain cancer); primary CNS lymphoma; cervical cancer; childhood cancers; cancers of childhood, unusual; cholangiocarcinoma - see bile duct cancer; chordoma, childhood (bone cancer); chronic lymphocytic leukemia (CLL); chronic myelogenous leukemia (CML); chronic myeloproliferative neoplasms; colorectal cancer; craniopharyngioma, childhood (brain cancer); cutaneous t-cell lymphoma - see lymphoma (mycosis fungoides and Sezary syndrome); ductal carcinoma in situ (DCIS) - see breast cancer; embryonal tumors, medulloblastoma and other central nervous system, childhood (brain cancer); endometrial cancer (uterine cancer); ependymoma, childhood (brain cancer); esophageal cancer; esthesioneuroblastoma (head and neck cancer); Ewing sarcoma (bone cancer); extracranial germ cell tumor, childhood; extragonadal germ cell tumor; eye cancer; intraocular melanoma; retinoblastoma; fallopian tube

cancer; fibrous histiocytoma of bone, malignant, and osteosarcoma; gallbladder cancer; gastric (stomach) cancer; gastrointestinal carcinoid tumor; gastrointestinal stromal tumors (GIST) (soft tissue sarcoma); germ cell tumors; childhood central nervous system germ cell tumors (brain cancer); childhood extracranial germ cell tumors; extragonadal germ cell tumors; ovarian germ cell tumors; testicular cancer; gestational trophoblastic disease; hairy cell leukemia; head and neck cancer; heart tumors, childhood; hepatocellular (liver) cancer; histiocytosis, Langerhans cell; Hodgkin lymphoma; hypopharyngeal cancer (head and neck cancer); intraocular melanoma; islet cell tumors, pancreatic neuroendocrine tumors; Kaposi sarcoma (soft tissue sarcoma); kidney (renal cell) cancer; Langerhans cell histiocytosis; laryngeal cancer (head and neck cancer); leukemia; lip and oral cavity cancer (head and neck cancer); liver cancer; lung cancer (non-small cell, small cell, pleuropulmonary blastoma, and tracheobronchial tumor); lymphoma; male breast cancer; malignant fibrous histiocytoma of bone and osteosarcoma; melanoma; melanoma, intraocular (eye); merkel cell carcinoma (skin cancer); mesothelioma, malignant; metastatic cancer; metastatic squamous neck cancer with occult primary (head and neck cancer); midline tract carcinoma with nut gene changes; mouth cancer (head and neck cancer); multiple endocrine neoplasia syndromes; multiple myeloma/plasma cell neoplasms; mycosis fungoides (lymphoma); myelodysplastic syndromes, myelodysplastic/myeloproliferative neoplasms; myelogenous leukemia, chronic (CML); myeloid leukemia, acute (AML); myeloproliferative neoplasms, chronic; nasal cavity and paranasal sinus cancer (head and neck cancer); nasopharyngeal cancer (head and neck cancer); neuroblastoma; non-Hodgkin lymphoma; non-small cell lung cancer; oral cancer, lip and oral cavity cancer and oropharyngeal cancer (head and neck cancer); osteosarcoma and malignant fibrous histiocytoma of bone; ovarian cancer; pancreatic cancer; pancreatic neuroendocrine tumors (islet cell tumors); papillomatosis (childhood laryngeal); paraganglioma; paranasal sinus and nasal cavity cancer (head and neck cancer); parathyroid cancer; penile cancer; pharyngeal cancer (head and neck cancer); pheochromocytoma; pituitary tumor; plasma cell neoplasm/multiple myeloma; pleuropulmonary blastoma (lung cancer); pregnancy and breast cancer; primary central nervous system (CNS) lymphoma; primary peritoneal cancer; prostate cancer; rectal cancer; recurrent cancer; renal cell (kidney) cancer; retinoblastoma; rhabdomyosarcoma, childhood (soft tissue sarcoma); salivary gland cancer (head and neck cancer); sarcoma; childhood rhabdomyosarcoma (soft tissue sarcoma); childhood vascular tumors (soft tissue sarcoma); Ewing sarcoma (bone cancer); Kaposi sarcoma (soft tissue sarcoma); osteosarcoma (bone cancer); soft tissue sarcoma; uterine sarcoma; Sezary syndrome (lymphoma); skin cancer; small cell lung cancer; small intestine cancer; soft tissue sarcoma; squamous cell carcinoma of the skin - see skin cancer; squamous

neck cancer with occult primary, metastatic (head and neck cancer); stomach (gastric) cancer; t-cell lymphoma, cutaneous - see lymphoma (mycosis fungoides and Sezary syndrome); testicular cancer; throat cancer (head and neck cancer); nasopharyngeal cancer; oropharyngeal cancer; hypopharyngeal cancer; thymoma and thymic carcinoma; thyroid cancer; tracheobronchial tumors (lung cancer); transitional cell cancer of the renal pelvis and ureter (kidney (renal cell) cancer); unknown primary carcinoma; unusual cancers of childhood; ureter and renal pelvis, transitional cell cancer (kidney (renal cell) cancer; urethral cancer; uterine cancer, endometrial; uterine sarcoma; vaginal cancer; vascular tumors (soft tissue sarcoma); vulvar cancer; Wilms tumor and other childhood kidney tumors; or cancer in young adults or any cancer mentioned at <https://www.cancer.gov/types>.

[0086] A protein complex may be administered as a pharmaceutical composition. A pharmaceutical composition of the disclosure can be a combination of any protein complex described herein with other chemical components, such as carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, and/or excipients. The pharmaceutical composition facilitates administration of a protein complex described herein to an organism. Pharmaceutical compositions can be administered in therapeutically-effective amounts as pharmaceutical compositions by various forms and routes including, for example, intravenous, subcutaneous, intramuscular, rectal, aerosol, parenteral, ophthalmic, pulmonary, transdermal, vaginal, optic, nasal, oral, inhalation, dermal, intra-articular, intrathecal, intranasal, and topical administration. A pharmaceutical composition can be administered in a local or systemic manner, for example, via injection of the protein complex described herein directly into an organ, optionally in a depot.

[0087] Parenteral injections can be formulated for bolus injection or continuous infusion. The pharmaceutical compositions can be in a form suitable for parenteral injection as a sterile suspension, solution or emulsion in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Pharmaceutical formulations for parenteral administration include aqueous solutions of a protein complex described herein in water-soluble form. Suspensions of protein complexes described herein can be prepared as oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. The suspension can also contain suitable stabilizers or agents which increase the solubility and/or reduces the aggregation of such protein complexes described herein to allow for the preparation of highly concentrated solutions.

Alternatively, the protein complexes described herein can be lyophilized or in powder form for re-constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. In some embodiments, a purified protein complex is administered intravenously. A protein complex of the present disclosure may comprise a sufficiently long serum half-life (e.g., as demonstrated herein, e.g., in EXAMPLE 7) to enable dosing regimens comprising daily, alternating day, twice weekly, weekly, biweekly, or monthly dosing frequencies. A protein complex of the present disclosure may comprise a serum half-life of at least 12 hours, at least 24 hours, at least 36 hours, at least 48 hours, at least 72 hours, at least 96 hours, at least 120 hours, at least 168 hours, at least 250 hours, at least 320 hours, or at least 400 hours. The serum half-life may be a human serum half-life, a murine serum half-life, a porcine serum-half life, a bovine serum half-life, a canine serum half-life, a feline serum half-life, or a leporine serum half-life.

[0088] A protein complex of the disclosure can be applied directly to an organ, or an organ tissue or cells, during a surgical procedure, or via transdermal, subcutaneous, intramuscular, intratumoral, intrathecal, topical, or local delivery. In some embodiments, a protein complex may be applied directly to a cancerous tissue (e.g., a tumor). The protein complexes described herein can be administered topically and can be formulated into a variety of topically administrable compositions, such as solutions, suspensions, lotions, gels, pastes, medicated sticks, balms, creams, and ointments. Such pharmaceutical compositions can contain solubilizers, stabilizers, tonicity enhancing agents, buffers and preservatives. The protein complexes may be expressed in spirulina and delivered orally.

[0089] In practicing the methods of treatment or use provided herein, therapeutically-effective amounts of the protein complex described herein are administered in pharmaceutical compositions to a subject suffering from cancer. In some embodiments, the subject is a mammal such as a human. A therapeutically-effective amount can vary widely depending on the severity of the disease, the age and relative health of the subject, the potency of the compounds used, and other factors.

[0090] Pharmaceutical compositions can be formulated using one or more physiologically-acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active compounds into preparations that can be used pharmaceutically. Formulation can be modified depending upon the route of administration chosen. Pharmaceutical compositions comprising a protein complex described herein can be manufactured, for example, by expressing the protein complex in a recombinant system, purifying the protein complex, lyophilizing the protein complex, mixing, or dissolving. The pharmaceutical compositions can include at least

one pharmaceutically acceptable carrier, diluent, or excipient and compounds described herein as free-base or pharmaceutically-acceptable salt form.

[0091] Methods for the preparation of protein complexes described herein include formulating the protein complex described herein with one or more inert, pharmaceutically-acceptable excipients or carriers to form a solid, semi-solid, or liquid composition. Solid compositions include, for example, powders, tablets, dispersible granules, capsules, cachets, and suppositories. These compositions can also contain minor amounts of nontoxic, auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, and other pharmaceutically-acceptable additives.

[0092] Certain methods described herein comprise administering to the subject an intravenous pharmaceutical composition comprising a protein complex of the present disclosure, for example, as described herein. Intravenous pharmaceutical compositions of protein complexes include any formulation suitable for administration to a subject via any intravenous method, including a bolus, an infusion which occurs over time or any other intravenous method known in the art. In some aspects, the rate of infusion is such that the dose is administered over a period of less than five minutes, more than five minutes but less than 15 minutes or greater than 15 minutes. In other aspects, the rate of infusion is such that the dose is administered over a period of less than 5 minutes. In other aspects, the rate of infusion is such that the dose is administered over a period of greater than 5 minutes and less than 15 minutes. In some other aspects, the rate of infusion is such that the dose is administered over a period of greater than 15 minutes.

[0093] “Product” or “dosage form” as used herein refers to any solid, semi-solid, lyophilized, aqueous, liquid or frozen formulation or preparation used for administration. Upon administration, the rate of release of an active moiety from a product is often greatly influenced by the excipients and/or product characteristics which make up the product itself. For example, an enteric coat on a tablet is designed to separate that tablet's contents from the stomach contents to prevent, for example, degradation of the stomach which often induces gastrointestinal discomfort or injury. According to the currently accepted conventional understanding, systemic exposure of the active moiety will be relatively insensitive to the small formulation changes.

[0094] Non-limiting examples of pharmaceutically-acceptable excipients can be found, for example, in Remington: The Science and Practice of Pharmacy, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H. A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980; and Pharmaceutical

Dosage Forms and Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins 1999), each of which is incorporated by reference in its entirety.

[0095] A protein complex of the present disclosure may be administered to a patient in an effective amount. The term “effective amount,” as used herein, can refer to a sufficient amount of an agent or a compound being administered which will relieve to some extent one or more of the symptoms of the disease or condition being treated. The result can be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. Compositions containing such agents or compounds can be administered for prophylactic, enhancing, and/or therapeutic treatments. An appropriate “effective” amount in any individual case can be determined using techniques, such as a dose escalation study.

[0096] The methods, compositions, and kits of this disclosure can comprise a method to prevent, treat, arrest, reverse, or ameliorate the symptoms of a condition. The treatment can comprise treating a subject (e.g., an individual, a domestic animal, a wild animal or a lab animal afflicted with a disease or condition) with a protein complex of the disclosure. Protein complexes of the present disclosure may be administered to treat a disease in a subject. The subject can be a human. A subject can be a human; a non-human primate such as a chimpanzee, or other ape or monkey species; a farm animal such as a cattle, horse, sheep, goat, swine; a domestic animal such as a rabbit, dog, and cat; a laboratory animal including a rodent, such as a rat, mouse and guinea pig, or the like. A subject can be of any age. A subject can be, for example, an elderly adult, adult, adolescent, pre-adolescent, child, toddler, infant, or fetus in utero.

[0097] Treatment can be provided to the subject before clinical onset of disease. Treatment can be provided to the subject after clinical onset of disease. Treatment can be provided to the subject after 1 day, 1 week, 6 months, 12 months, or 2 years or more after clinical onset of the disease. Treatment may be provided to the subject for more than 1 day, 1 week, 1 month, 6 months, 12 months, 2 years or more after clinical onset of disease. Treatment may be provided to the subject for less than 1 day, 1 week, 1 month, 6 months, 12 months, or 2 years after clinical onset of the disease. Treatment can also include treating a human in a clinical trial. A treatment can comprise administering to a subject a pharmaceutical composition, such as one or more of the pharmaceutical compositions described throughout the disclosure. A treatment can comprise a once daily dosing. A treatment can comprise delivering a protein complex of the disclosure to a subject, either intravenously, subcutaneously, intramuscularly, by inhalation, dermally, intra-articular injection, orally, intrathecally, transdermally, intranasally, via a peritoneal route, or directly onto or into a diseased tissue, e.g., via topical, intra-articular injection route or injection route of application.

[0098] In some embodiments, the present disclosure provides a method for treating a cancer, the method comprising administering to a subject in need thereof an effective amount of a protein complex of the present disclosure.

[0099] In some embodiments, the present disclosure provides a method for treating a cancer, the method comprising administering to a patient in need thereof an effective amount of a pharmaceutical composition comprising a protein complex of the present disclosure and a pharmaceutically acceptable carrier.

Kits

[0100] A protein complex of the present disclosure may be provided in various kits. In some embodiments, pharmaceutical compositions comprising a protein complex of the present disclosure may be supplied as a kit. A kit may comprise a container that comprises a protein complex. Therapeutic protein complexes can be provided in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a therapeutic protein complexes. Such a kit may further comprise written information on indications and usage of the pharmaceutical composition.

[0101] Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. Any reference to “or” herein is intended to encompass “and/or” unless otherwise stated.

[0102] Whenever the term “at least,” “greater than,” or “greater than or equal to” precedes the first numerical value in a series of two or more numerical values, the term “at least,” “greater than” or “greater than or equal to” applies to each of the numerical values in that series of numerical values. For example, greater than or equal to 1, 2, or 3 is equivalent to greater than or equal to 1, greater than or equal to 2, or greater than or equal to 3.

[0103] Whenever the term “no more than,” “less than,” “less than or equal to,” or “at most” precedes the first numerical value in a series of two or more numerical values, the term “no more than,” “less than” or “less than or equal to,” or “at most” applies to each of the numerical values in that series of numerical values. For example, less than or equal to 3, 2, or 1 is equivalent to less than or equal to 3, less than or equal to 2, or less than or equal to 1.

[0104] Where values are described as ranges, it will be understood that such disclosure includes the disclosure of all possible sub-ranges within such ranges, as well as specific numerical values

that fall within such ranges irrespective of whether a specific numerical value or specific sub-range is expressly stated.

[0105] TABLE 2A—Example Peptide Components of Multipeptide Immunocytokine Designs Described herein

peptideID	Sequence	Comments	SEQ ID NO:
PEP000113	DIQMTQSPSSLSASVGDRVTITCRASQDVNT AVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSRSGTDFTLTISSLQPEDFATYYCQQHY TTPPTFGQGTKVEIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSE RQNGVLNSWTDQDSKDYSTYSMSSTLTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRECE		671
PEP000169	DIQMTQSPSSLSASVGDRVTITCRASQSISS WLAWYQQKPGKAPKLLIYAASLTQSGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCQQS YSTPLTFGGGTKLEIKRADAAPTVSIFPPSSE QLTSGGASVVCFLNNFYPKDINVKWKIDGS ERQNGVLNSWTDQDSKDYSTYSMSSTLTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRECE		672
PEP000243	DIVMTQSPDSLAVSLGERATINCKASESVDT SDNSFIHWYQQKPGQSPKLLIYRSSTLES GV PDRFSGSGSGTDFTLTISSLQAEDVAVYYCQ QNYDVPWTFGQGTKVEIKRADAAPTVSIFP PSSEQLTSGGASVVCFLNNFYPKDINVKWKI DGSERQNGVLNSWTDQDSKDYSTYSMSSTL TLTKDEYERHNSYTCEATHKTSTSPIVKSFN RNECE		673
PEP000244	DIVMTQSPDSLAVSLGERATINCKASESVDT SDNSFIHWYQQKPGQSPKLLIYRSSTLES GV PDRFSGSGSGTDFTLTISSLQAEDVAVYYCQ QNYDVPWTFGQGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCFLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDYSTYSLSSTL TLTKADYEEKHKVYACEVTHQGLSSPVTKSF NRGEC		674
PEP000245	DIVMTQSPDSLAVSLGERATINCKASQSLH SSSNKNYLAWYQQKPGQPPKLLIYWASTRE SGVPDRFSGSGSGTDFTLTISSLQAEDVAVY YCQQYYSTPITFGPGTKVDIKRADAAPT VSI FPPSSEQLTSGGASVVCFLNNFYPKDINV KWKIDGSE RQNGVLNSWTDQDSKDYSTYSMS STLTLTKDEYERHNSYTCEATHKTSTSPIV KSFNRECE		675
PEP000288	EVQLLESGGGLVQPGGSLRLSCAASGFSFSS YTMSWVRQAPGKGLEWVATISGGGRDIYY PDSVKGRFTISRDNKNTLYLQMNSLRAED TAVYYCVLLTGRVYFALDSWGQGT LTVTS SASTKGPSVFPLAPSSKSTSGGTAALGCLVK		676

peptideID	Sequence	Comments	SEQ ID NO:
	DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEAAGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNK ALGAPIEKTISKAKGQPREPQVYTLPPCRDE LTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG GGGGSGGGGSGGGGSAPASSSTKKTQLQLE HLLDLQMILNGINNYKNPKLTRMLTAKFA MPKKATELKHLQCLEEELKPLEEVLNGAQS KNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFAQSIISTLT		
PEP001315	MSTSTEQKLISEEDLQVQLVQSGAEVKKPG ASVKVSCKASGYTFTTYMHWRQAPGQ GLEWMGIINPSGGGTLYAQKFQGRVTMTR DTSTSTVYMELSSLRSEDVAVYYCAAGLFI WGQGTITVTVSSASGGGGSGGGGSGGGGSH ASDIVMTQSPDSLAVSLGERATINCKASQSL LHSSSNKNYLAWYQQKPGQPPKLLIYWAST RESGVPDRFSGSGSGTDFTLTISSLQAEDVA VYYCQQYYSTPITFGPGTKVDIKGSGLNDIF EAQKIEWHEGKPIPNPLLGLDST	PD1_R04_C1 0 - Internal anti-PD1 mAb	677
PEP003213	MSTSTEQKLISEEDLQVQLVQSGAEVKKPG ASVKVSCKASGDTFTRHYVHWVRQAPGQG LEWMGIINPSGGYASYAQKFQGRVTMTRD TSTSTVYMELSSLRSEDVAVYYCAAGLFIW GQGTITVTVSSASGGGGSGGGGSGGGGSHA SDIQMTQSPSSLSASVGDRVTITCRASQSIGR WLAWYQQKPGKAPKLLIYSASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYE SFPVTFGPGTKVDIKGKPIPNPLLGLDST	AB002022_2 B07v1	678
PEP003626	EVQLLESGLLVQPGGSLRLSCAASGFSFSS YTMSWVRQAPGKGLEWVATISGGGRDIYY PDSVKGRFTISRDN SKNTLYLQMNSLRAED TAVYYCVLLTGRVYFALDSWGQGTITVTVS SAKTTAPSVYPLAPVCGDTTGSSVTLGCLV KGYFPEPVTLTWNSGSLSSGVHTFPAVLQS DLYTLSSSVTVTSSTWPSQSITCNVAHPASS TKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP SVFIFPPKIKDVLMI SLSPIVTCVVVDVSEDD PDVQISWVFNNEVHTAQTQTHREDYNSTL RVVSALPIQHQDWMSGKEFKCKVNNKDLG APIERTISKPKGSVRAPQVYVLPPEEEMTK KQVTLTCMVTD FMPEDIYVEWTNNGKTEL NYKNTEPVLDSDGSYFMYSDLRVEKKNWV ERNYSYSCSVVHEGLHNHHTTESFSRTPGGG		679

peptideID	Sequence	Comments	SEQ ID NO:
	GGSGGGGSGGGGSAPASSSTKKTQLQLEHL LLDLQMILNGINNYKNPKLTRMLTAKFAMP KKATELKHLQCLEEELKPLEEVLNGAQSKN FHLRPRDLISNINVIVLELKGSETTFMCEYA DETATIVEFLNRWITFAQSIISTLT		
PEP003639	APASSSTKKTQLQLEHLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGDTF TRHYVHWVRQAPGQGLEWMGIINPSGGYA SYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGTLLTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMSLSPIVTCVVVDVSEDDPDVQIS WVFVNNVEVHTAQTQTHREDYNSTLRVVS LPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDSGYSYFMYSDLRVEKKNWVERNSY SCSVVHEGLHNHHTTESFSRTPGK		680
PEP003641	DIQMTQSPSSLSASVGDRTITCRASQSIGR WLAWEYQQKPGKAPKALIIYSASFYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYFCQQYY TYPYTFGGGTKLEIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSE RQNGVLNSWTDQDSKDYSTYSMSSTLTTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRENEC		681
PEP003642	DIQMTQSPSSLSASVGDRTITCRASQSIGR WLAWEYQQKPGKAPKALIIYSASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYE SFPVTFGPGTKVDIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSE RQNGVLNSWTDQDSKDYSTYSMSSTLTTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRENEC		682
PEP003648	QVQLVQSGAEVKKPGASVKVSCKASGDTF TRHYVHWVRQAPGQGLEWMGIINPSGGYA SYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGTLLTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMSLSPIVTCVVVDVSEDDPDVQIS WVFVNNVEVHTAQTQTHREDYNSTLRVVS		683

peptideID	Sequence	Comments	SEQ ID NO:
	LPIQHQDWMMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKNT EPVLKSDGSYFMYSKLTVEKKNWVERNSY SCSVVHEGLHNHHTTKSFSRTPGGGGSGGG SHHHHHH		
PEP003654	APASSSTKKTKLQLEHLLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGSE VQLVESGGGLVKPGGSLRLSCAASGFTFSS YTLAWVRQAPGKGLEWVAIDSSSYTSP DTVRGRFTISRDNANKSLYLQMNSLRAEDT AVYYCARDSDNWDALDYWGQGTLLTVSSA KTTAPSVYPLAPVCGDTTGSSVTLGCLVKG YFPEPVTLTWNSGSLSSGVHTFPAVLQSDL YTLSSSVTVTSSTWPSQSITCNVAHPASSTK VDKKIEPRGPTIKPCPPCKCPAPNAAGGPSV FIFPPKIKDVLMISSLPIVTCVVVDVSEDDPD VQISWVFNNEVHTAQTQTHREDYNSTLR VVSALPIQHQDWMMSGKEFKCKVNNKDLGA PIERTISKPKGSVRAPQVYVLPPEEEMTKK QVTLTCTMVTDFMPEDIYVEWTNNGKTELN YKNTPEVLDSYFMYSKLRVEKKNWVE RNSYSCSVVHEGLHNHHTTKSFSRTPGK		684
PEP003655	APASSSTKKTKLQLEHLLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGSE VQLVESGGGLVQPGGSLRLSCAASGFNIKD TYIHWVRQAPGKGLEWVARIYPTNGYTRY ADSVKGRFTISADTSKNTAYLQMNSLRAED TAVYYCSRWGGDGFYAMDYWGQGTLLTV SSAKTTAPSVYPLAPVCGDTTGSSVTLGCL VKGYFPEPVTLTWNSGSLSSGVHTFPAVLQ SDLYTLSSSVTVTSSTWPSQSITCNVAHPAS STKVDKKIEPRGPTIKPCPPCKCPAPNAAGG PSVFIFPPKIKDVLMISSLPIVTCVVVDVSED DPDVQISWVFNNEVHTAQTQTHREDYNS TLRVVSALPIQHQDWMMSGKEFKCKVNNKD LGAPIERTISKPKGSVRAPQVYVLPPEEEM TKKQVTLTCTMVTDFMPEDIYVEWTNNGKT ELNYKNTPEVLDSYFMYSKLRVEKKN WVERNSYSCSVVHEGLHNHHTTKSFSRTPG K		685
PEP003657	APASSSTKKTKLQLEHLLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE		686

peptideID	Sequence	Comments	SEQ ID NO:
	EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGDTF TRHYVHWVRQAPGQGLEWMGIINPSGGYA SYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGTLLTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMSLSPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNSTLRVVS LPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDSGSGYFMYSKLRVEKKNWVERNSY SCSVVHEGLHNHHTTKSFSRTPGK		
PEP003780	APASSSTKKTQLQLEHLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGDTF TRHYVHWVRQAPGQGLEWMGIINPSGGYA SYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGTLLTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPEAAGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK		687
PEP003782	DIQMTQSPSSLSASVGDRVTITCRASQSIGR WLAWYQQKPGKAPKLLIYSASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYE SFPVTFGPGTKVDIKRTVAAPSVFIFPPSDEQ LKSGTASVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDYSLSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC		688
PEP003786	APASSSTKKTQLQLEHLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT		689

peptideID	Sequence	Comments	SEQ ID NO:
	FAQSIISTLTGGGGSGGGGSGGGGSGGGGS DIQMTQSPSSLASVGDRVITITCRASQSIGR WLAWYQQKPGKAPKLLIYSASNLETGVPSR FSGSGSGTDFTLTISLQPEDFATYYCQQYE SFPVTFGPGTKVDIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDYSLSTLTLKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC		
PEP003791	QVQLVQSGAEVKKPGASVKVSCKASGDTF TRHYVHWVRQAPGQGLEWMGIINPSGGYA SYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPEAAGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFIYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK		690
PEP004129	EVQLLES GGGLVQP GGSRLRLSCAASGFSFSS YTMSWVRQAPGKGLEWVATISGGGRDIYY PDSVKGRFTISRDN SKNTLYLQMNSLRAED TAVYYCVLLTGRVYFALDSWGQGLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLS SVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTSPPSPEPKSSDTPP PSPRSPEPKSSDTPPPSPRSPEPKSCDTPPPCP RAPEAAGGPSVFLFPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALGAPIEKTISKAKGQPREPQ VCTLPPSRDELTKNQVSLSCAVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSP		691
PEP004130	EVQLLES GGGLVQP GGSRLRLSCAASGFSFSS YTMSWVRQAPGKGLEWVATISGGGRDIYY PDSVKGRFTISRDN SKNTLYLQMNSLRAED TAVYYCVLLTGRVYFALDSWGQGLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLS SVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTSPPSPEPKSSDTPP PSPRSPEPKSSDTPPPSPRSPEPKSCDTPPPCP		692

peptideID	Sequence	Comments	SEQ ID NO:
	RAPEAAGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALGAPIEKTISKAKGQPREPQ VYTLPPCRDELTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGGGGGSGGGGSGGGGSAPASS STKKTQLQLEHLLDLQMILNGINNYKNPK LTRMLTAKFAMPKKATELKHLQCLEEELKP LEEVLNGAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQS IISTLT		
PEP004133	QVQLVQSGAEVKKPGASVKVSCKASGDTF TRHYVHWVRQAPGQGLEWMGIINPSGGYA SYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGTLLTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTSPPSPEPKSSDTPPPSPRSP EPKSSDTPPPSPRSPEPKSCDTPPPCPRAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALGAPIEKTISKAKGQPREPQVCTLPP SRDELTKNQVSLSCAVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLVSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLS LSP		693
PEP004134	QVQLVQSGAEVKKPGASVKVSCKASGDTF TRHYVHWVRQAPGQGLEWMGIINPSGGYA SYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGTLLTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTSPPSPEPKSSDTPPPSPRSP EPKSSDTPPPSPRSPEPKSCDTPPPCPRAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALGAPIEKTISKAKGQPREPQVYTLPP CRDELTKNQVSLWCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGGGGGSGGGGSGGGGSAPASSSTKKTQ LQLEHLLDLQMILNGINNYKNPKLTRMLT AKFAMPKKATELKHLQCLEEELKPLEEVLN		694

peptideID	Sequence	Comments	SEQ ID NO:
	GAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEFLNRWITFAQSIISTLT		
PEP004153	APASSSTKKTQLQLEHLLLDLQMLNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGDTF TRYVYVHWVRQAPGQGLEWMGIINPSGGYA SYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGLTVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMSLSPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNSTLRVVS LPIQHQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDSGYSYFMYSKLRVEKKNWVERN SCSVVHEGLHNHHTTKSFSRTPGK		695
PEP004158	DIQMTQSPSSLSASVGDRVTITCRASQSIGR WLAHYQQKPGKAPKLLIYSASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYN RFPVTFGPGTKVDIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVWKIDGSE RQNGVLNSWTDQDSKDYMSSTLTTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRECE		696
PEP004159	DIQMTQSPSSLSASVGDRVTITCRASQSIGR WLAHYQQKPGKAPKLLIYSASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYN SFPVTFGPGTKVDIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVWKIDGSE RQNGVLNSWTDQDSKDYMSSTLTTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRECE		697
PEP004161	DIQMTQSPSSLSASVGDRVTITCRASQSIGR YLAHYQQKPGKAPKLLIYSASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYN RFPVTFGPGTKVDIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVWKIDGSE RQNGVLNSWTDQDSKDYMSSTLTTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRECE		698
PEP004162	DIQMTQSPSSLSASVGDRVTITCRASQSIGR YLAHYQQKPGKAPKLLIYSASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYN SFPVTFGPGTKVDIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVWKIDGSE		699

peptideID	Sequence	Comments	SEQ ID NO:
	RQNGVLNSWTDQDSKDYMSSTLTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRECE		
PEP004163	DIQMTQSPSSLSASVGDRVTITCRASQSIGR YLAWEYQQKPGKAPKLLIYYASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYN SFPVTFGPGTKVDIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSE RQNGVLNSWTDQDSKDYMSSTLTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRECE		700
PEP004191	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGDTFTIRYYVHWVRQAPGQGLEWMGIINP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGTLLTVS SASGGGGSGGGGSGGGGSHASDIQMTQSPS SLASVGDRVTITCRASQSIGRWLAWEYQQK PGKAPKLLIYSASNLETGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYERFVTFGPG TKVDIKAAAGSGSEQKLISEEDLGKPIPNPL LGLDST	AB002342_2 B07v5	701
PEP004192	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGDTFTIRYYVHWVRQAPGQGLEWMGIINP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGTLLTVS SASGGGGSGGGGSGGGGSHASDIQMTQSPS SLASVGDRVTITCRASQSIGRWLAWEYQQK PGKAPKLLIYSASNLETGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYESFVTFGPGT KVDIKAAAGSGSEQKLISEEDLGKPIPNPLL GLDST	AB002353_2 B07v9	702
PEP004195	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGDTFTIRYYVHWVRQAPGQGLEWMGIINP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGTLLTVS SASGGGGSGGGGSGGGGSHASDIQMTQSPS SLASVGDRVTITCRASQSIGRWLAWEYQQK PGKAPKLLIYSASNLETGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYNRFVTFGPG TKVDIKAAAGSGSEQKLISEEDLGKPIPNPL LGLDST	AB002328_2 B07v4	703
PEP004198	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGDTFTIRYYVHWVRQAPGQGLEWMGIINP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGTLLTVS SASGGGGSGGGGSGGGGSHASDIQMTQSPS SLASVGDRVTITCRASQSIGRWLAWEYQQK PGKAPKLLIYSASNLETGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYNSFVTFGPG TKVDIKAAAGSGSEQKLISEEDLGKPIPNPL LGLDST	AB002293_2 B07v2	704

peptideID	Sequence	Comments	SEQ ID NO:
PEP004207	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGDTFTRYVHWVRQAPGQGLEWMGIINP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGTLLTVS SASGGGGSGGGGSGGGGSHASDIQMTQSPS SLSASVGDRVTITCRASQSIGRYLAWYQQK PGKAPKLLIYSASNLETGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQYNRFPVTFGPG TKVDIKAAAGSGSEQKLISEEDLGKPIPNPL LGLDST	AB002347_2 B07v7	705
PEP004208	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGDTFTRYVHWVRQAPGQGLEWMGIINP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGTLLTVS SASGGGGSGGGGSGGGGSHASDIQMTQSPS SLSASVGDRVTITCRASQSIGRYLAWYQQK PGKAPKLLIYSASNLETGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQYNSFPVTFGPG TKVDIKAAAGSGSEQKLISEEDLGKPIPNPL LGLDST	AB002345_2 B07v6	706
PEP004210	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGDTFTRYVHWVRQAPGQGLEWMGIINP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGTLLTVS SASGGGGSGGGGSGGGGSHASDIQMTQSPS SLSASVGDRVTITCRASQSIGRYLAWYQQK PGKAPKLLIYYASNLETGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQYNSFPVTFGPG TKVDIKAAAGSGSEQKLISEEDLGKPIPNPL LGLDST	AB002348_2 B07v8	707
PEP004211	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGDTFTRYVHWVRQAPGQGLEWMGIINP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGTLLTVS SASGGGGSGGGGSGGGGSHASDIQMTQSPS SLSASVGDRVTITCRASQSIGSWLAWYQQK PGKAPKLLIYSASNLETGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQYNSFPVTFGPG TKVDIKAAAGSGSEQKLISEEDLGKPIPNPL LGLDST	AB002326_2 B07v3	708
PEP004243	APASSSTKKKTQLQLEHLLLDLQMLNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGDTF TRYVHWVRQAPGQGLEWMGIINPSGGYA SYAQKFQGRVTMTRDTSTSTVYMESSLRS EDTAVYYCAAGLFIWGQGTLLTVSSAKTT		709

peptideID	Sequence	Comments	SEQ ID NO:
	APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMISSLPIVTCVVVDVSEDDPDVQIS WVFVNNVEVHTAQTQTHREDYNSTLRVVS LPIQHQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDSGYSYFMYSDLRVEKKNWVERN SYSCSVVHEGLHNHHTTESFSRTPGK		
PEP004247	QVQLVQSGAEVKKPGASVKVSCKASGDTF TRYVYVHWVRQAPGQGLEWMGIINPSGGYA SYAQKFQGRVTMTRDTSTSTVYMESSLRS EDTAVYYCAAGLFIWGQGLTVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMISSLPIVTCVVVDVSEDDPDVQIS WVFVNNVEVHTAQTQTHREDYNSTLRVVS LPIQHQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKNT EPVLKSDGYSYFMYSKLTVEKKNWVERN SYSCSVVHEGLHNHHTTKSFSRTPGK		710
PEP004251	APASSSTKKTKLQLEHLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGYTF TKNYMHWVRQAPGQGLEWLGWVSPDSGY TGYYAQKFQGRVTMTRDTSTSTVYMESSLR SEDTAVYYCTDLLSLELDDAFDIWGQGT MTVTVSSAKTTAPSVYPLAPVCGDTTGSSVTL GCLVKGYFPEPVTLTWNSGSLSSGVHTFPA VLQSDLYTLSSSVTVTSSTWPSQSITCNVAH PASSTKVDKKIEPRGPTIKPCPPCKCPAPNA AGGPSVFIFPPKIKDVLMISSLPIVTCVVVDV SEDDPDVQISWVFVNNVEVHTAQTQTHRED YNSTLRVVSALPIQHQDWMSGKEFKCKVN NKDLGAPIERTISKPKGSVRAPQVYVLPPE EEMTKKQVTLTCMVTDFMPEDIYVEWTNN GKTELNYKNTPEVLDSGYSYFMYSDLRVE KKNWVERN SYSCSVVHEGLHNHHTTESFS RTPGK		711
PEP004261	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGDTFTDYMHVVRQAPGQGLEWMGIINP	AB002381_7 A04v4	712

peptideID	Sequence	Comments	SEQ ID NO:
	RAGYTSYALKFQGRVTMTRDTSTSTVYME LSSLRSEDNAVYYCTSGWDVWGQGLVTV SSASGGGGSGGGGSGGGGSHASDIQMTQSP SSLSASVGDRVTITCRASQSISTWLAWYQQ KPGKAPKLLIYAASSLDGVPSPRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSPVTFGQ GTKVEIKAAAGSGSEQKLISEEDLGKPIPNP LLGLDST		
PEP004271	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGHTFTRYMHWRQAPGQGLEWMGIINP SGGYATYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDNAVYYCASGLFIWGQGLVTVS SASGGGGSGGGGSGGGGSHASDIQMTQSPS SLSASVGDRVTITCRASQSINSWLAWYQQK PGKAPKLLIYATSTLESGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQSYRFPVTFGQG TKVEIKAAAGSGSEQKLISEEDLGKPIPNPLL GLDST	AB002427_2 A11v5	713
PEP004272	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGHTFTRYMHWRQAPGQGLEWMGIINP SGGYATYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDNAVYYCASGLFIWGQGLVTVS SASGGGGSGGGGSGGGGSHASDIQMTQSPS SLSASVGDRVTITCRASQSINSWLAWYQQK PGKAPKLLIYATSTLESGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQYYRFPVTFGQG TKVEIKAAAGSGSEQKLISEEDLGKPIPNPLL GLDST	AB002413_2 A11v3	714
PEP004273	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGHTFTRYMHWRQAPGQGLEWMGIINP SGGYATYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDNAVYYCASGLFIWGQGLVTVS SASGGGGSGGGGSGGGGSHASDIQMTQSPS SLSASVGDRVTITCRASQSINSWLAWYQQK PGKAPKLLIYATSTLESGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQYYSPVTFGQG TKVEIKAAAGSGSEQKLISEEDLGKPIPNPLL GLDST	AB002417_2 A11v4	715
PEP004281	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGHTFTRYMHWRQAPGQGLEWMGIINP SGGYATYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDNAVYYCASGWFVWGQGLVTV SSASGGGGSGGGGSGGGGSHASDIQMTQSP SSLSASVGDRVTITCRASQSINSWLAWYQQ KPGKAPKLLIYDTSTLESGVPSRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSPVTFGQ GTKVEIKAAAGSGSEQKLISEEDLGKPIPNP LLGLDST	AB002410_2 A11v2	716

peptideID	Sequence	Comments	SEQ ID NO:
PEP004299	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGYTFTDYYMHWVRQAPGQGLEWMGIINP RAGYTSYALKFQGRVTMTRDTSTSTVYME LSSLRSEDNAVYYCTSGWDVWGQGTLVTV SSASGGGGSGGGGSGGGGSHASDIQMTQSP SSLSASVGDRVTITCRASQSISTWLAWYQQ KPGKAPKLLIYAASSLD SGVPSRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSPVTFGQ GTKVEIKAAAGSGSEQKLISEEDLGKPIPNP LLGLDST	AB002360_7 A04v1	717
PEP004340	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGYTFTTRYMHWVRQAPGQGLEWMGIINP RAGYTSYALKFQGRVTMTRDTSTSTVYME LSSLRSEDNAVYYCTSGWDVWGQGTLVTV SSASGGGGSGGGGSGGGGSHASDIQMTQSP SSLSASVGDRVTITCRASQSISTWLAWYQQ KPGKAPKLLIYAASSLD SGVPSRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSPVTFGQ GTKVEIKAAAGSGSEQKLISEEDLGKPIPNP LLGLDST	AB002365_7 A04v2	718
PEP004349	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGYTFTTYYMHWVRQAPGQGLEWMGIINP RAGYTSYALKFQGRVTMTRDTSTSTVYME LSSLRSEDNAVYYCTSGWDVWGQGTLVTV SSASGGGGSGGGGSGGGGSHASDIQMTQSP SSLSASVGDRVTITCRASQSISTWLAWYQQ KPGKAPKLLIYAASSLD SGVPSRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSPVTFGQ GTKVEIKAAAGSGSEQKLISEEDLGKPIPNP LLGLDST	AB002370_7 A04v3	719
PEP004356	EVQLLES GGLVQPGGSLRLSCAASGFSFSS YTMSWVRQAPGKGLEWVATISGGGRDIYY PDSVKGRFTISRDN SKNTLYLQMNSLRAED TAVYYCVLLTGRVYFALDSWGQGTLVTVS SAKTTAPSVYPLAPVCGDTTGSSVTLGCLV KGYFPEPVTLTWNSGSLSSGVHTFPAVLQS DLYTLSSSVTVTSSTWPSQSITCNVAHPASS TKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP SVFIFPPKIKDVL MISLSPIVTCVVVDVSEDD PDVQISWVFNNEVHTAQTQTHREDYNSTL RVVSALPIQH QDWMSGKEFKCKVNNKDLG APIERTISKPKGSVRAPQVYVLPPEKEMTK KQVSLTCLVKDFMPEDIYVEWTNNGKTEL NYKNTEPVLKSDGSYFMYSKLTVEKKNWV ERNYSYSCSVVHEGLHNHHTTKSFSRTPGGG GGSGGGGSGGGGSGGGGSEVQLVESGGGL VKPGGSLRLSCAASGFTFSSYTLAWVRQAP GKGLEWVAIDSSSYTYSPD TVRGRFTISR NAKNSLYLQMNSLRAEDTAVYYCARD SN		720

peptideID	Sequence	Comments	SEQ ID NO:
	WDALDYWGQGTLLTVSSASGGGGSGGGG SGGGGSHASDIQMTQSPSSLASVGDRVSIT CKASQNVGTNVGWYQQKPGKAPKALIYSA SFRYSGVPSRFSGSGSGTDFTLTISLQPEDF ATYFCQQYYTYPYTFGGGKLEIKGGGSGG GSHHHHHH		
PEP004361	APASSSTKKTKLQLEHLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGDTF TRHYVHWVRQAPGQGLEWMGIINPSGGYA SYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGTLLTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMISSLPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNSTLRVVS LPIQHQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDSGYSYFMYSKLRVEKKNWVERNSY SCSVVHEGLHNHHTTKSFSRTPGGGGSGG GGSGGGGSGGGGSQVQLVQSGAEVKKPGA SVKVSCKASGYTFTTYMHVVRQAPGQGL EWMGIINPSGGGTLYAQKFQGRVTMTRDTS TSTVYMELSSLRSEDVAVYYCAAGLFIWGQ GTTVTVSSASGGGGSGGGGSGGGGSHASDI VMTQSPDSLAVSLGERATINCKASQSLHSS SNKNYLAWYQQKPGQPPKLLIYWASTRES GVPDRFSGSGSGTDFTLTISLQAEDVAVYY CQQYYSTPITFGPGTKVDIK		721
PEP004363	APASSSTKKTKLQLEHLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGDTF TRYVHWVRQAPGQGLEWMGIINPSGGYA SYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGTLLTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMISSLPIVTCVVVDVSEDDPDVQIS		722

peptideID	Sequence	Comments	SEQ ID NO:
	W F V N N V E V H T A Q T Q T H R E D Y N S T L R V V S A L P I Q H Q D W M S G K E F K C K V N N K D L G A P I E R T I S K P K G S V R A P Q V Y V L P P P E E M T K K Q V T L T C M V T D F M P E D I Y V E W T N N G K T E L N Y K N T E P V L D S D G S Y F M Y S K L R V E K K N W V E R N S Y S C S V V H E G L H N H H T T K S F S R T P G G G G G S G G G G S G G G G S G G G G S Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T T Y Y M H W V R Q A P G Q G L E W M G I I N P S G G G T L Y A Q K F Q G R V T M T R D T S T S T V Y M E L S S L R S E D T A V Y Y C A A G L F I W G Q G T T V T V S S A S G G G G S G G G G S G G G G S H A S D I V M T Q S P D S L A V S L G E R A T I N C K A S Q S L L H S S S N K N Y L A W Y Q Q K P G Q P P K L L I Y W A S T R E S G V P D R F S G S G S G T D F T L T I S S L Q A E D V A V Y Y C Q Q Y Y S T P I T F G P G T K V D I K		
PEP004395	E V Q L L E S G G G L V Q P G G S L R L S C A A S G F S F S S Y T M S W V R Q A P G K G L E W V A T I S G G G R D I Y Y P D S V K G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C V L L T G R V Y F A L D S W G Q G T L V T V S S A K T T A P S V Y P L A P V C G D T T G S S V T L G C L V K G Y F P E P V T L T W N S G S L S S G V H T F P A V L Q S D L Y T L S S S V T V T S S T W P S Q S I T C N V A H P A S S T K V D K K I E P R G P T I K P C P P C K C P A P N A A G G P S V F I F P P K I K D V L M I S L S P I V T C V V V D V S E D D P D V Q I S W F V N N V E V H T A Q T Q T H R E D Y N S T L R V V S A L P I Q H Q D W M S G K E F K C K V N N K D L G A P I E R T I S K P K G S V R A P Q V Y V L P P P E K E M T K K Q V S L T C L V K D F M P E D I Y V E W T N N G K T E L N Y K N T E P V L K S D G S Y F M Y S K L T V E K K N W V E R N S Y S C S V V H E G L H N H H T T K S F S R T P G G G G G S G G G G S G G G G S G G G G S Q V Q L V Q S G A E V K K P G A S V K V S C K A S G D T F T R H Y V H W V R Q A P G Q G L E W M G I I N P S G G Y A S Y A Q K F Q G R V T M T R D T S T S T V Y M E L S S L R S E D T A V Y Y C A A G L F I W G Q G T L V T V S S A S G G G G S G G G G S G G G G S H A S D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q S I G R W L A W Y Q Q K P G K A P K L L I Y S A S N L E T G V P S R F S G S G S G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y E S F P V T F G P G T K V D I K G G G S G G G S H H H H H H		723
PEP004398	E V Q L L E S G G G L V Q P G G S L R L S C A A S G F S F S S Y T M S W V R Q A P G K G L E W V A T I S G G G R D I Y Y P D S V K G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C V L L T G R V Y F A L D S W G Q G T L V T V S S A K T T A P S V Y P L A P V C G D T T G S S V T L G C L V K G Y F P E P V T L T W N S G S L S S G V H T F P A V L Q S D L Y T L S S S V T V T S S T W P S Q S I T C N V A H P A S S T K V D K K I E P R G P T I K P C P P C K C P A P N A A G G P S V F I F P P K I K D V L M I S L S P I V T C V V V D V S E D D		724

peptideID	Sequence	Comments	SEQ ID NO:
	PDVQISWVFNNEVHTAQTQTHREDYNSTL RVVSALPIQHQDWMSGKEFKCKVNNKDLG APIERTISKPKGSVRAPQVYVLPPPEKEMTK KQVSLTCLVKDFMPEDIYVEWTNNGKTEL NYKNTEPVLKSDGSYFMYSKLTVEKKNWV ERNYSYSCSVVHEGLHNHHTTKSFSRTPGGG GGSGGGGSGGGGSGGGGSQVQLVQSGAEV KKPGASVKVSCKASGDTFTRYVHWVRQA PGQGLEWMGIINPSGGYASYAQKFQGRVT MTRDTSTSTVYMELSSLRSEDNAVYYCAAG LFIWGQGTLLTVSSASGGGGSGGGGSGGG GSHASDIQMTQSPSSLSASVGDRVTITCRAS QSIGRWLAWYQQKPGKAPKLLIYSASNLET GVPSRFSGSGSGTDFTLTISLQPEDFATYYC QQYNRFPVTFGPGTKVDIKGGGSGGGSHHH HHH		
PEP004404	EVQLLESQGGLVQPGGSLRLSCAASGFSFSS YTMSWVRQAPGKGLEWVATISGGGRDIYY PDSVKGRFTISRDNKNTLYLQMNSLRAED NAVYYCVLLTGRVYFALDSWGQGTLLTVS SAKTTAPSVYPLAPVCGDITGSSVTLGCLV KGYFPEPVTLLTWNSSGLSSGVHTFPAVLQS DLYTLSSSVTVTSSTWPSQSITCNVAHPASS TKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP SVFIFPPKIKDVLMISSLPIVTCVVVDVSEDD PDVQISWVFNNEVHTAQTQTHREDYNSTL RVVSALPIQHQDWMSGKEFKCKVNNKDLG APIERTISKPKGSVRAPQVYVLPPPEKEMTK KQVSLTCLVKDFMPEDIYVEWTNNGKTEL NYKNTEPVLKSDGSYFMYSKLTVEKKNWV ERNYSYSCSVVHEGLHNHHTTKSFSRTPGGG GGSGGGGSGGGGSGGGGSQVQLVQSGAEV KKPGASVKVSCKASGHTFTRYVMHWVRQ APGQGLEWMGIINPSGGYATYAQKFQGRV TMTRDTSTSTVYMELSSLRSEDNAVYYCAS GLFIWGQGTLLTVSSASGGGGSGGGGSGGG GSHASDIQMTQSPSSLSASVGDRVTITCRA SQSINSLAWYQQKPGKAPKLLIYATSTLE SGVPSRFSGSGSGTDFTLTISLQPEDFATYY CQQYYRFPVTFGQGTKEIKGGGSGGGSHH HHHH		725
PEP004406	EVQLLESQGGLVQPGGSLRLSCAASGFSFSS YTMSWVRQAPGKGLEWVATISGGGRDIYY PDSVKGRFTISRDNKNTLYLQMNSLRAED NAVYYCVLLTGRVYFALDSWGQGTLLTVS SAKTTAPSVYPLAPVCGDITGSSVTLGCLV KGYFPEPVTLLTWNSSGLSSGVHTFPAVLQS DLYTLSSSVTVTSSTWPSQSITCNVAHPASS TKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP		726

peptideID	Sequence	Comments	SEQ ID NO:
	SVFIFPPKIKDVLMSLSPIVTCVVVDVSEDD PDVQISWVFNNEVHTAQTQTHREDYNSTL RVVSALPIQHQDWMSGKEFKCKVNNKDLG APIERTISKPKGSVRAPQVYVLPPPEKEMTK KQVSLTCLVKDFMPEDIYVEWTNNGKTEL NYKNTEPVLKSDGSYFMYSKLTVEKKNWV ERNYSYSCSVVHEGLHNHHTTKSFSRTPGGG GGSGGGGSGGGGSGGGGSGVQLVQSGAEV KKPGASVKVSCKASGYTFTDYYMHWVRQ APGQGLEWMGIINPRAGYTSYALKFQGRVT MTRDTSTSTVYMESSLRSEDVAVYYCTSG WDVWGQGTLVTVSSASGGGGSGGGGSGG GGSHASDIQMTQSPSSLSASVGDRVTITCRA SQSISTWLAWYQQKPKAPKLLIYAASSLD SGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYSPVTFGQGTKVEIKGGGSGGGSHH HHHH		
PEP004416	APASSSTKKTQLQLEHLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGYTF TDYYMHWVRQAPGQGLEWMGIINPRAGY TSYALKFQGRVTMTRDTSTSTVYMESSLR SEDVAVYYCTSGWDVWGQGTLVTVSSAKT TAPSVYPLAPVCGDTTGSSVTLGCLVKGYF PEPVTLTWNSGSLSSGVHTFPAVLQSDLYTL SSSVTVTSSTWPSQSITCNVAHPASSTKVDK KIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFP PKIKDVLMSLSPIVTCVVVDVSEDDPDVQI SWFVFNNEVHTAQTQTHREDYNSTLRVVS ALPIQHQDWMSGKEFKCKVNNKDLGAPIE RTISKPKGSVRAPQVYVLPPPEEEMTKKQV TLTCMVTDFMPEDIYVEWTNNGKTELNYK NTEPVLDSGSYFMYSKLRVEKKNWVERN SYSCSVVHEGLHNHHTTKSFSRTPGK		727
PEP004418	APASSSTKKTQLQLEHLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGYTF TRYMHWVRQAPGQGLEWMGIINPRAGYT SYALKFQGRVTMTRDTSTSTVYMESSLRS EDVAVYYCTSGWDVWGQGTLVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSSVTVTSSTWPSQSITCNVAHPASSTKVDK		728

peptideID	Sequence	Comments	SEQ ID NO:
	IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVL MISLSPIVTCVVVDVSEDDPDVQIS W FVNNVEVHTAQTQTHREDYNSTLRVVS LPIQH QDWMSGKEFKCKVNNKDLGAPIERT ISKPKG SVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDS DGSYFMYSKLRVEKKNWVERN SCSVVHEGLHNHHTTKSFSRTPGK		
PEP004419	APASSSTKKTQLQLEHLLDLQMLNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGYTF TTYMHWRQAPGQGLEWMGIINPRAGYT SYALKFQGRVTMTRDTSTSTVYMESSLRS EDTAVYYCTSGWDVWGQGTLVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVL MISLSPIVTCVVVDVSEDDPDVQIS W FVNNVEVHTAQTQTHREDYNSTLRVVS LPIQH QDWMSGKEFKCKVNNKDLGAPIERT ISKPKG SVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDS DGSYFMYSKLRVEKKNWVERN SCSVVHEGLHNHHTTKSFSRTPGK		729
PEP004420	DIQMTQSPSSLSASVGDRVTITCRASQSIGR WLAWYQQKPGKAPKLLIYSASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYE RFPVTFGPGTKVDIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSE RQNGVLNSWTDQDSKDYSTYSMSSTLTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRECE		730
PEP004421	DIQMTQSPSSLSASVGDRVTITCRASQSIGS WLAWYQQKPGKAPKLLIYSASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYN SFPVTFGPGTKVDIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSE RQNGVLNSWTDQDSKDYSTYSMSSTLTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRECE		731
PEP004423	DIQMTQSPSSLSASVGDRVTITCRASQSINS WLAWYQQKPGKAPKLLIYATSTLES FSGSGSGTDFTLTISSLQPEDFATYYCQQYY RFPVTFGQGTKVEIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSE RQNGVLNSWTDQDSKDYSTYSMSSTLTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRECE		732

peptideID	Sequence	Comments	SEQ ID NO:
PEP004426	DIQMTQSPSSLSASVGDRVTITCRASQSIST WLAWYQQKPGKAPKLLIYAASSLDSGVPS RFSGSGSGTDFTLTISLQPEDFATYYCQQS YRFPVTFGQGTKVEIKRADAAPTVSIFPPSSE QLTSGGASVVCFLNFPKDIVKWKIDGS ERQNGVLNSWTDQDSKDSSTYSMSSTLTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRECE		733
PEP004427	DIQMTQSPSSLSASVGDRVTITCRASQSIST WLAWYQQKPGKAPKLLIYAASSLDSGVPS RFSGSGSGTDFTLTISLQPEDFATYYCQQS YSFPVTFGQGTKVEIKRADAAPTVSIFPPSSE QLTSGGASVVCFLNFPKDIVKWKIDGS ERQNGVLNSWTDQDSKDSSTYSMSSTLTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRECE		734
PEP004431	APASSSTKKKTQLQLEHLLLDLQMLNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGHTF TRYMHWRQAPGQGLEWMGIINPSGGYA TYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCASGLFIWGQGTLLTVSSAKTTA PSVYPLAPVCGDTTGSSVTLGCLVKGYFPEP VTLTWNSGSLSSGVHTFPAVLQSDLYTLSSS VTVTSSSTWPSQSITCNVAHPASSTKVDKKIE PRGPTIKPCPPCKCPAPNAAGGPSVFIFPKI KDVLMISLSPIVTCVVVDVSEDDPDVQISWF VNNVEVHTAQTQTHREDYNSTLRVVSALPI QHQQDWMSGKEFKCKVNNKDLGAPIERTIS KPKGSVRAPQVYVLPPEEEMTKKQVTLTC MVTDFMPEDIYVEWTNNGKTELNYKNTEP VLDSDGSYFMYSDLRVEKKNWVERNSYSC SVVHEGLHNHHTTESFSRTPGK		735
PEP004433	APASSSTKKKTQLQLEHLLLDLQMLNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGYTF TDYYMHWRQAPGQGLEWMGIINPRAGY TSYALKFQGRVTMTRDTSTSTVYMELSSLR SEDTAVYYCTSGWDVWGQGTLLTVSSAKT TAPSVYPLAPVCGDTTGSSVTLGCLVKGYF PEPVTLTWNSGSLSSGVHTFPAVLQSDLYTL SSSVTVTSSTWPSQSITCNVAHPASSTKVDK KIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFP PKIKDVLMISLSPIVTCVVVDVSEDDPDVQI SWFVNNVEVHTAQTQTHREDYNSTLRVVS		736

peptideID	Sequence	Comments	SEQ ID NO:
	ALPIQHQQDWMSGKEFKCKVNNKDLGAPIE RTISKPKGSVRAPQVYVLPPEEEMTKKQV TLTCMVTDFMPEDIYVEWTNNGKTELNYK NTEPVLDSGYSYFMYSDLRVEKKNWVERN SYSCSVVHEGLHNHHTTESFSRTPGK		
PEP004435	APASSSTKKTQLQLEHLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGYTF TRYMHVWRQAPGQGLEWMGIINPRAGYT SYALKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCTSGWDVWGQGLTVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMSLSPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNSTLRVVS LPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDSGYSYFMYSDLRVEKKNWVERN SYSCSVVHEGLHNHHTTESFSRTPGK		737
PEP004436	APASSSTKKTQLQLEHLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGYTF TTYMHVWRQAPGQGLEWMGIINPRAGYT SYALKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCTSGWDVWGQGLTVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMSLSPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNSTLRVVS LPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDSGYSYFMYSDLRVEKKNWVERN SYSCSVVHEGLHNHHTTESFSRTPGK		738
PEP004438	QVQLVQSGAEVKKPGASVKVSCKASGDTF TRYVHVWRQAPGQGLEWMGIINPSGGYA SYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGLTVTVSSAKTT		739

peptideID	Sequence	Comments	SEQ ID NO:
	APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMISSLPIVTCVVVDVSEDDPDVQIS WVFVNNVEVHTAQTQTHREDYNSTLRVVS LPIQHQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKNT EPVLKSDGSYFMYSKLTVEKKNWVERNSY SCSVVHEGLHNHHTTKSFSRTPGGGGSGGG SHHHHHH		
PEP004440	QVQLVQSGAEVKKPGASVKVSCKASGHTF TRYMHWRQAPGQGLEWMGIINPSGGYA TYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCASGLFIWGQGTLLTVSSAKTTA PSVYPLAPVCGDTTGSSVTLGCLVKGYFPEP VTLTWNSGSLSSGVHTFPAVLQSDLYTLSS VTVTSSTWPSQSITCNVAHPASSTKVDKKIE PRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKI KDVLMISSLPIVTCVVVDVSEDDPDVQISWF VNNVEVHTAQTQTHREDYNSTLRVVSALPI QHQDWMSGKEFKCKVNNKDLGAPIERTIS KPKGSVRAPQVYVLPPEKEMTKKQVSLTC LVKDFMPEDIYVEWTNNGKTELNYKNTEP VLKSDGSYFMYSKLTVEKKNWVERNSYSC SVVHEGLHNHHTTKSFSRTPGGGGSGGGSH HHHHH		740
PEP004442	QVQLVQSGAEVKKPGASVKVSCKASGYTF TDYYMHWRQAPGQGLEWMGIINPRAGY TSYALKFQGRVTMTRDTSTSTVYMELSSLR SEDTAVYYCTSGWDVWGQGTLLTVSSAKT TAPSVYPLAPVCGDTTGSSVTLGCLVKGYF PEPVTLTWNSGSLSSGVHTFPAVLQSDLYTL SSSVTVTSSTWPSQSITCNVAHPASSTKVDK KIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFP PKIKDVLMISSLPIVTCVVVDVSEDDPDVQI SWFVNNVEVHTAQTQTHREDYNSTLRVVS ALPIQHQDWMSGKEFKCKVNNKDLGAPIE RTISKPKGSVRAPQVYVLPPEKEMTKKQV SLTCLVKDFMPEDIYVEWTNNGKTELNYK NTEPVLKSDGSYFMYSKLTVEKKNWVERN SYSCSVVHEGLHNHHTTKSFSRTPGGGGSG GGSHHHHHH		741
PEP004443	QVQLVQSGAEVKKPGASVKVSCKASGYTF TKNYMHWRQAPGQGLEWLGVSPDSGY TGIAQKFQGRVTMTRDTSTSTVYMELSSLR SEDTAVYYCTDLLSLELDDAFDIWGQGT MTVTVSSAKTTAPSVYPLAPVCGDTTGSSVTL		742

peptideID	Sequence	Comments	SEQ ID NO:
	GCLVKGYFPEPVTLTWNSGSLSSGVHTFPA VLQSDLYTLSSSVTVTSSTWPSQSITCNVAH PASSTKVDKKIEPRGPTIKPCPPCKCPAPNA AGGPSVFIFPPKIKDVLMSLSPIVTCVVVDV SEDDPDVQISWVFNNEVHTAQTQTHRED YNSTLRVVSALPIQHQQDWMSGKEFKCKVN NKDLGAPIERTISKPKGSVRAPQVYVLPPE KEMTKKQVSLTCLVKDFMPEDIYVEWTNN GKTELNYKNTEPVLKSDGSYFMYSKLTVEK KNWVERNNSYSCSVVHEGLHNHHTTKSFSR TPGGGGSGGGSHHHHHH		
PEP004445	QVQLVQSGAEVKKPGASVKVSCKASGYTF TRYMHWRQAPGQGLEWMGIINPRAGYT SYALKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCTSGWDVWGQGTLLTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTL SSSVTVTSSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMSLSPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNSTLRVVS ALPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKN TEPVLKSDGSYFMYSKLTVEKKNWVERN SYSCSVVHEGLHNHHTTKSFSRTPGGGGSGGG SHHHHHH		743
PEP004446	QVQLVQSGAEVKKPGASVKVSCKASGYTF TTYMHWRQAPGQGLEWMGIINPRAGYT SYALKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCTSGWDVWGQGTLLTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTL SSSVTVTSSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMSLSPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNSTLRVVS ALPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKN TEPVLKSDGSYFMYSKLTVEKKNWVERN SYSCSVVHEGLHNHHTTKSFSRTPGGGGSGGG SHHHHHH		744
PEP004729	EIVLTQSPATLSLSPGERATLSCRASQSVSSY LAWYQQKPGQAPRLLIYDASNRATGIPARF SGSGSGTDFLTISLEPEDFAVYYCQQSSN WPRFTFGQGTKVEIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSE		745

peptideID	Sequence	Comments	SEQ ID NO:
	RQNGVLNSWTDQDSKDYMSSTLTTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRECE		
PEP004810	APASSSTKKTQLQLEHLLDLQMLNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGGSQVQLVQSGAEVKKPG ASVKVSCKASGDTFTRYVHVWRQAPGQG LEWMGIINPSGGYASYAQKFQGRVTMTRD TSTSTVYMESSLRSEDVAVYYCAAGLFIW GQGTLVTVSSAKTTAPSVYPLAPVCGDTTG SSVTLGCLVKGYFPEPVTLTWNSGSLSSGV HTFPAVLQSDLYTLSSSVTVTSSTWPSQSI CNVAHPASSTKVDKKIEPRGPTIKPCPPCKC PAPNAAGGPSVFIFPPKIKDVLMSLSPIVTC VVVDVSEDDPDVQISWVNNVEVHTAQQT THREDYNSTLRVVSALPIQHQQDWMSGKEF KCKVNNKDLGAPIERTISKPKGSVRAPQVY VLPPEEEMTKKQVTLCMVTDMPEDIYV EWTNNGKTELNYKNTEPVLDSDGSYFMYS DLRVEKKNWVERNSYSCSVVHEGLHNHHT TESFSRTPGK		746
PEP004813	APASSSTKKTQLQLEHLLDLQMLNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGGSQVQLVQSGAEVKKPG ASVKVSCKASGYTFTRYMHVWRQAPGQG GLEWMGIINPRAGYTSYALKFQGRVTMTR DTSTSTVYMESSLRSEDVAVYYCTSGWDV WGQGTLVTVSSAKTTAPSVYPLAPVCGDTT GSSVTLGCLVKGYFPEPVTLTWNSGSLSSG VHTFPAVLQSDLYTLSSSVTVTSSTWPSQSI TCNVAHPASSTKVDKKIEPRGPTIKPCPPCK CPAPNAAGGPSVFIFPPKIKDVLMSLSPIVT CVVVDVSEDDPDVQISWVNNVEVHTAQQT QTHREDYNSTLRVVSALPIQHQQDWMSGKE FKCKVNNKDLGAPIERTISKPKGSVRAPQV YVLPPEEEMTKKQVTLCMVTDMPEDIY VEWTNNGKTELNYKNTEPVLDSDGSYFMY SDLRVEKKNWVERNSYSCSVVHEGLHNHH TTESFSRTPGK		747
PEP004818	QVQLVQSGAEVKKPGASVKVSCKASGYTF TRYMHVWRQAPGQGLEWMGIINPRAGYT SYALKFQGRVTMTRDTSTSTVYMESSLRS EDVAVYYCTSGWDVWGQGTLVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSTWPSQSITCNVAHPASSTKVDKK		748

peptideID	Sequence	Comments	SEQ ID NO:
	IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMISSLPIVTCVVVDVSEDDPDVQIS WVFVNNVEVHTAQQTQTHREDYNSTLRVVS ALPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKNT EPVLKSDGSYFMYSKLTVEKKNWVERN SYSCSVVHEGLHNHHTTKSFSRTPGK		
PEP004822	APASSSTKKTQLQLEHLLDLQMLNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSQVQLVQSGAE VKKPGASVKVSCCKASGDTFTRYVHWVRQ APGQGLEWMGIINPSGGYASYAQKFQGRV TMTRDTSTSTVYMELSSLRSEDNAVYYCAA GLFIWGQGTLLTVSSAKTTAPSVYPLAPVC GDTTGSSVTLGCLVKGYFPEPVTLTWNSGS LSSGVHTFPAVLQSDLYTLSSSVTVTSSTWP SQSITCNVAHPASSTKVDKKIEPRGPTIKPCP PCKCPAPNAAGGPSVFIFPPKIKDVLMISSL PIVTCVVVDVSEDDPDVQISWVFVNNVEVHTA QTQTHREDYNSTLRVVSALPIQHQQDWMSG KEFKCKVNNKDLGAPIERTISKPKGSVRAPQ VYVLPPEEEMTKKQVTLTCMVTDMPEDI YVEWTNNGKTELNYKNTPEVLDSGGSYFM YSDLRVEKKNWVERN SYSCSVVHEGLHNHHTTESFSRTPGK		749
PEP004825	APASSSTKKTQLQLEHLLDLQMLNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSQVQLVQSGAE VKKPGASVKVSCCKASGYTFTRYMHVWR QAPGQGLEWMGIINPRAGYTSYALKFQGR VTMTRDTSTSTVYMELSSLRSEDNAVYYCT SGWDVWGQGTLLTVSSAKTTAPSVYPLAP VCGDTTGSSVTLGCLVKGYFPEPVTLTWNS GSLSSGVHTFPAVLQSDLYTLSSSVTVTSST WPSQSITCNVAHPASSTKVDKKIEPRGPTIK PCPPCKCPAPNAAGGPSVFIFPPKIKDVLMI SSLPIVTCVVVDVSEDDPDVQISWVFVNNVEV HTAQQTQTHREDYNSTLRVVSALPIQHQQDW MSGKEFKCKVNNKDLGAPIERTISKPKGSV RAPQVYVLPPEEEMTKKQVTLTCMVTDMP EDIYVEWTNNGKTELNYKNTPEVLDSGGSY FMYSDLRVEKKNWVERN SYSCSVVHEGLHNHHTTESFSRTPGK		750

peptideID	Sequence	Comments	SEQ ID NO:
PEP004829	APASSSTKKKTQLQLEHLLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGVQLV QSGAEVKKPGASVKVSCASGDTFTRYVYV HWVRQAPGQGLEWMGIINPSGGYASYAQK FQGRVTMTRDTSTSTVYMESSLRSEDTAV YYCAAGLFIWGQGLTVTVSSAKTTAPSVYP LAPVCGD TTGSSVTLGCLVKGYFPEPVTLT WNSGSLSSGVHTFPAVLQSDLYTLSSSVTV TSSTWPSQSITCNVAHPASSTKVDKKIEPRG PTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDV LMISLSPIVTCVVVDVSEDDPDVQISWVFN VEVHTAQTQTHREDYNSTLRVVSALPIQH DWMSGKEFKCKVNNKDLGAPIERTISKPKG SVRAPQVYVLPPEEEMTKKQVTLTCMVT DFMPEDIYVEWTNNGKTELNYKNTEPVLDS DGSYFMYSDLRVEKKNWVERNSYSCSVVH EGLHNHHTTESFSRTPGK		751
PEP004832	APASSSTKKKTQLQLEHLLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGVQLV QSGAEVKKPGASVKVSCASGYTFTRYVM HWVRQAPGQGLEWMGIINPRAGYTSYALK FQGRVTMTRDTSTSTVYMESSLRSEDTAV YYCTSGWDVWGQGLTVTVSSAKTTAPSVY PLAPVCGD TTGSSVTLGCLVKGYFPEPVTLT WNSGSLSSGVHTFPAVLQSDLYTLSSSVTV TSSTWPSQSITCNVAHPASSTKVDKKIEPRG PTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDV LMISLSPIVTCVVVDVSEDDPDVQISWVFN VEVHTAQTQTHREDYNSTLRVVSALPIQH DWMSGKEFKCKVNNKDLGAPIERTISKPKG SVRAPQVYVLPPEEEMTKKQVTLTCMVT DFMPEDIYVEWTNNGKTELNYKNTEPVLDS DGSYFMYSDLRVEKKNWVERNSYSCSVVH EGLHNHHTTESFSRTPGK		752
PEP004836	APASSSTKKKTQLQLEHLLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS GGGGSGVQLVQSGAEVKKPGASVKVSCA SGDTFTRYVYVHWVRQAPGQGLEWMGIINP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGLTVTVS		753

peptideID	Sequence	Comments	SEQ ID NO:
	SAKTTAPSVYPLAPVCGDTTGSSVTLGCLV KGYFPEPVTLTWNSGSLSSGVHTFPAVLQS DLYTLSSSVTVTSSTWPSQSITCNVAHPASS TKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP SVFIFPPKIKDVL MISLSPIVTCVVVDVSEDD PDVQISWFVNNVEVHTAQTQTHREDYNSTL RVVSALPIQHQDWMSGKEFKCKVNNKDLG APIERTISKPKGSVRAPQVYVLPPEEEMTK KQVTLTCMVTDFMPEDIYVEWTNNGKTEL NYKNTEPVLDSDGSYFMYSDLRVEKKNWV ERNYSYSCSVVHEGLHNHHTTESFSRTPGK		
PEP004839	APASSSTKKTQLQLEHLLDLQMILNGINNY KNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNGAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWIT FAQSIISTLTGGGGSGGGGSGGGGSGGGGS GGGGSQVQLVQSGAEVKKPGASVKVSCKA SGYTFTRYMHWRQAPGQGLEWMGIINP RAGYTSYALKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCTSGWDVWGQGTLVTV SSAKTTAPSVYPLAPVCGDTTGSSVTLGCL VKGYFPEPVTLTWNSGSLSSGVHTFPAVLQ SDLYTLSSSVTVTSSTWPSQSITCNVAHPAS STKVDKKIEPRGPTIKPCPPCKCPAPNAAGG PSVFIFPPKIKDVL MISLSPIVTCVVVDVSED DPDVQISWFVNNVEVHTAQTQTHREDYNS TLRVVSALPIQHQDWMSGKEFKCKVNNKD LGAPIERTISKPKGSVRAPQVYVLPPEEEM TKKQVTLTCMVTDFMPEDIYVEWTNNGKT ELNYKNTEPVLDSDGSYFMYSDLRVEKKN WVERNSYSCSVVHEGLHNHHTTESFSRTPG K		754
PEP004957	APTSSSTKKTQLQLEHLLDLQMILNGINNY KNPKLTDMLTFEFYMPKKATELKHLQCLER ELKPLEEVLNLAQSKNFHLRPRDLISNINVIV LELKGSETTFMCEYADETATIVEFLNRWITF CQSIISTLTGGGGSGGGGSGGGGSGGGGSQ VQLVQSGAEVKKPGASVKVSCKASGDTFT RYYVHWVRQAPGQGLEWMGIINPSGGYAS YAQKFQGRVTMTRDTSTSTVYMESSLRSE DTAVYYCAAGLFIWGQGTLVTVSSAKTTAP SVYPLAPVCGDTTGSSVTLGCLVKGYFPEP VTLTWNSGSLSSGVHTFPAVLQSDLYTLSS VTVTSSTWPSQSITCNVAHPASSTKVDKKIE PRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKI KDVL MISLSPIVTCVVVDVSEDDPDVQISWF VNNVEVHTAQTQTHREDYNSTLRVVSALPI QHQDWMSGKEFKCKVNNKDLGAPIERTIS KPKGSVRAPQVYVLPPEEEMTKKQVTLTC		755

peptideID	Sequence	Comments	SEQ ID NO:
	MVTDFMPEDIYVEWTNNGKTELNYKNTEP VLDSGSGSYFMYSDLRVEKKNWVERNYSYSC SVVHEGLHNHHTTESFSRTPGK		
PEP004958	APTSSSTKKTQLQLEHLLLDLQMILNGINNY KNPKLTDMLTFEFYMPKKATELKHLQCLER ELKPLEEVLNLAQSKNFHLRPRDLISNINVIV LELKGETTFMCEYADETATIVEFLNRWITF CQSIISTLTGGGGSGGGGSGGGGSGGGGSQ VQLVQSGAEVKKPGASVKVSCKASGHTFT RYYMHWVRQAPGQGLEWMGIINPSGGYAT YAQKFQGRVTMTRDTSTSTVYMELSSLRSE DTAVYYCASGLFIWGQGTLLTVSSAKTTAP SVYPLAPVCGDTTGSSVTLGCLVKGYFPEP VTLTWNSGSLSSGVHTFPAVLQSDLYTLSSS VTVTSTWPSQSITCNVAHPASSTKVDKKIE PRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKI KDVLMISLSPIVTCVVVDVSEDDPDVQISWF VNNVEVHTAQTQTHREDYNSTLRVVSALPI QHGDWMSGKEFKCKVNNKDLGAPIERTIS KPKGSVRAPQVYVLPPEEEMTKKQVTLTC MVTDFMPEDIYVEWTNNGKTELNYKNTEP VLDSGSGSYFMYSDLRVEKKNWVERNYSYSC SVVHEGLHNHHTTESFSRTPGK		756
PEP004959	APTSSSTKKTQLQLEHLLLDLQMILNGINNY KNPKLTDMLTFEFYMPKKATELKHLQCLER ELKPLEEVLNLAQSKNFHLRPRDLISNINVIV LELKGETTFMCEYADETATIVEFLNRWITF CQSIISTLTGGGGSGGGGSGGGGSGGGGSQ VQLVQSGAEVKKPGASVKVSCKASGYTFT DYVMHWVRQAPGQGLEWMGIINPRAGYTS YALKFQGRVTMTRDTSTSTVYMELSSLRSE DTAVYYCTSGWDVWGQGTLLTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMISLSPIVTCVVVDVSEDDPDVQIS WVNNVEVHTAQTQTHREDYNSTLRVVSALPI QHGDWMSGKEFKCKVNNKDLGAPIERTIS KPKGSVRAPQVYVLPPEEEMTKKQVTLTC MVTDFMPEDIYVEWTNNGKTELNYKNTEP EPVLDSGSGSYFMYSDLRVEKKNWVERNYSY SCSVVHEGLHNHHTTESFSRTPGK		757
PEP004960	APTSSSTKKTQLQLEHLLLDLQMILNGINNY KNPKLTDMLTFEFYMPKKATELKHLQCLER ELKPLEEVLNLAQSKNFHLRPRDLISNINVIV LELKGETTFMCEYADETATIVEFLNRWITF CQSIISTLTGGGGSGGGGSGGGGSGGGGSQ VQLVQSGAEVKKPGASVKVSCKASGYTFT		758

peptideID	Sequence	Comments	SEQ ID NO:
	TTYMHWVRQAPGQGLEWMGIINPRAGYTS YALKFQGRVTMTRDTSTSTVYMELSSLRSE DTA VYYCTSGWDVWGQGLTVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVL MISLSPIVTCVVVDVSEDDPDVQIS W FVNNVEVHTAQTQTHREDYNSTLRV VSA LPIQH QDWMSGKEFKCKVNNKDLGAPIERT ISKPKG SVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDS DGSYFMYSDLRVEKKNWVERN SY SCSVVHEGLHNHHTTESFSRTPGK		
PEP004967	QVQLVESGGGVVQPGRSLRLDCKASGITFS NSGMHWVRQAPGKGLEWVA VIWYDGSKR YYADSVKGRFTISRDN SKNTLFLQMNSLRA EDTA VYYCATNDDYWGQGLTVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVL MISLSPIVTCVVVDVSEDDPDVQIS W FVNNVEVHTAQTQTHREDYNSTLRV VSA LPIQH QDWMSGKEFKCKVNNKDLGAPIERT ISKPKG SVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDS DGSYFMYSDLRVEKKNWVERN SY SCSVVHEGLHNHHTTESFSRTPGGGGGSGG GSGGGGSAPTSSSTKKTQLQLEHLLLDLQ MILNGINNYKNPKLTDMLTFEFYMPKKATE LKHLQCLERELKPLEEVLNLAQSKNFHLRP RDLISNINVIVLELKGSETTFMCEYADETATI VEFLNRWITFCQSIISTLT		759
PEP004973	QVQLVESGGGVVQPGRSLRLDCKASGITFS NSGMHWVRQAPGKGLEWVA VIWYDGSKR YYADSVKGRFTISRDN SKNTLFLQMNSLRA EDTA VYYCATNDDYWGQGLTVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVL MISLSPIVTCVVVDVSEDDPDVQIS W FVNNVEVHTAQTQTHREDYNSTLRV VSA LPIQH QDWMSGKEFKCKVNNKDLGAPIERT ISKPKG SVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKNT EPVLKSDGSYFMYSKLTVEKKNWVERN SY SCSVVHEGLHNHHTTKSFSRTPGGGGGSGG		760

peptideID	Sequence	Comments	SEQ ID NO:
	GGSGGGSGGGGSQVQLVQSGAEVKKPGA SVKVSCKASGHTFTRYMHVVRQAPGQGL EWMGIINPSGGYATYAQKFQGRVTMTRDT STSTVYMELSSLRSEDNAVYYCASGLFIWG QGTLVTVSSASGGGGSGGGSGGGGSHAS DIQMTQSPSSLSASVGDRVTITCRASQSINS WLAHYQQKPGKAPKLLIYATSTLESGVPSR FSGSGSGTDFTLTISLQPEDFATYYCQQYY RFPVTFGQGTKVEIKGGGSGGGSHHHHHH		
PEP004974	QVQLVESGGGVVQPGRSLRLDCKASGITFS NSGMHWVRQAPGKGLEWVAVIWDGSKR YYADSVKGRFTISRDNKNTLFLQMNSLRA EDNAVYYCATNDDYWGQGLVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTSL SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMISSLPIVTCVVVDVSEDDPDVQIS WLVNNEVHTAQTQTHREDYNSTLRVVSAL LPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKNT EPVLKSDGSYFMYSKLTVEKKNWVERNSY SCSVVHEGLHNHHTTKSFSRTPGGGGSGG GGSGGGSGGGGSQVQLVQSGAEVKKPGA SVKVSCKASGYTFDYYMHVVRQAPGQG LEWMGIINPRAGYTSYALKFQGRVTMTRDT STSTVYMELSSLRSEDNAVYYCTSGWDVW GQGLVTVSSASGGGGSGGGSGGGGSHA SDIQMTQSPSSLSASVGDRVTITCRASQSIST WLAHYQQKPGKAPKLLIYAASSLDGVP RFSGSGSGTDFTLTISLQPEDFATYYCQQS YSFPVTFGQGTKVEIKGGGSGGGSHHHHHH		761
PEP004978	QVQLVQSGAEVKKPGASVKVSCKASGYTF TTYMHVVRQAPGQGLEWMGIINPSGGGT LYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDNAVYYCAAGLFIWGQGTITVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTSL SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMISSLPIVTCVVVDVSEDDPDVQIS WLVNNEVHTAQTQTHREDYNSTLRVVSAL LPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEEEMTKKQVTL TCMVTDMPEDIYVEWTNNGKTELNYKNT EPVLDSGYSYFMYSDLRVEKKNWVERNSY SCSVVHEGLHNHHTTESFSRTPGGGGSGG GGSGGGGSAPTSSSTKKTQLQLEHLLDLQ		762

peptideID	Sequence	Comments	SEQ ID NO:
	MILNGINNYKNPKLTDMLTFEFYMPKKATE LKHLQCLERELKPLEEVLNLAQSKNFHLRP RDLISNINVIVLELKGSETTFMCEYADETATI VEFLNRWITFCQSIISTLT		
PEP004981	QVQLVQSGAEVKKPGASVKVSCKASGYTF TTYMHWRQAPGQGLEWMGIINPSGGGT LYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGTITVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMSLSPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNSTLRVVS LPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKNT EPVLKSDGSYFMYSKLTVEKKNWVERNSY SCSVVHEGLHNHHTTKSFSRTPGGGGGSGG GGSGGGGSGGGGSQVQLVQSGAEVKKPGA SVKVSCASGDTFTRYVHWVRQAPGQGL EWMGIINPSGGYASYAQKFQGRVTMTRDT STSTVYMELSSLRSEDVAVYYCAAGLFIWG QGTILTVSSASGGGGSGGGGSGGGGSHAS DIQMTQSPSSLSASVGDRVTITCRASQSIGR WLAWYQQKPGKAPKLLIYSASNLETGVPSR FSGSGSGTDFTLTISLQPEDFATYYCQQYE RFPVTFGPGTKVDIKGGGSGGGSHHHHHH		763
PEP004982	QVQLVQSGAEVKKPGASVKVSCKASGYTF TTYMHWRQAPGQGLEWMGIINPSGGGT LYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGTITVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMSLSPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNSTLRVVS LPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKNT EPVLKSDGSYFMYSKLTVEKKNWVERNSY SCSVVHEGLHNHHTTKSFSRTPGGGGGSGG GGSGGGGSGGGGSQVQLVQSGAEVKKPGA SVKVSCASGDTFTRYVHWVRQAPGQGL EWMGIINPSGGYASYAQKFQGRVTMTRDT STSTVYMELSSLRSEDVAVYYCAAGLFIWG QGTILTVSSASGGGGSGGGGSGGGGSHAS DIQMTQSPSSLSASVGDRVTITCRASQSIGR		764

peptideID	Sequence	Comments	SEQ ID NO:
	YLAWYQQKPGKAPKLLIYSASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYN SFPVTFGPGTKVDIKGGGSGGGSHHHHHH		
PEP004983	QVQLVQSGAEVKKPGASVKVSCKASGYTF TTYMHWRQAPGQGLEWMGIINPSGGGT LYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGTTVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMISSLPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNSTLRVVS LPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKNT EPVLKSDGSYFMYSKLTVEKKNWVERN SYSCSVVHEGLHNHHTTKSFSRTPGGGGGSGG GGSGGGGSGGGGSQVQLVQSGAEVKKPGA SVKVSCKASGHTFTRYMHWRQAPGQGL EWMGIINPSGGYATYLAQKFQGRVTMTRDT STSTVYMELSSLRSEDVAVYYCASGLFIWG QGTLTVSSASGGGGSGGGSGGGGSHAS DIQMTQSPSSLSASVGDRVTITCRASQSINS WLAWYQQKPGKAPKLLIYATSTLES GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYY RFPVTFGQGTKVEIKGGGSGGGSHHHHHH		765
PEP004984	QVQLVQSGAEVKKPGASVKVSCKASGYTF TTYMHWRQAPGQGLEWMGIINPSGGGT LYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCAAGLFIWGQGTTVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMISSLPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNSTLRVVS LPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKNT EPVLKSDGSYFMYSKLTVEKKNWVERN SYSCSVVHEGLHNHHTTKSFSRTPGGGGGSGG GGSGGGGSGGGGSQVQLVQSGAEVKKPGA SVKVSCKASGYTFDYYMHWRQAPGQG LEWMGIINPRAGYTSYALKFQGRVTMTRDT STSTVYMELSSLRSEDVAVYYCTSGWDVW GQGTTLTVSSASGGGGSGGGSGGGGSHA SDIQMTQSPSSLSASVGDRVTITCRASQSIST WLAWYQQKPGKAPKLLIYAASSLD SGVPS		766

peptideID	Sequence	Comments	SEQ ID NO:
	RFSGSGSGTDFTLTISSLQPEDFATYYCQQS YSFPVTFGQGTKVEIKGGGSGGGSHHHHHH		
PEP005089	QVQLVESGGGVVQPGRSLRLDCKASGITFS NSGMHWVRQAPGKGLEWVAWIYDGSKR YYADSVKGRFTISRDN SKNTLFLQMNSLRA EDTAVYYCATNDDYWGQGLVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMI SLSPIVTCVVVDVSEDDPDVQIS WVFNNVEVHTAQTQTHREDYNSTLRVVS LPIQH QDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDS DGSYFMYSDLRVEKKNWVERN SY SCSVVHEGLHNHHTTESFSRTPGGGGGSGG GGSGGGGSAPASSSTKKTQLQLEHLLDLQ MILNGINNYKNPKLTRMLTAKFAMPKKAT ELKHLQCLEELKPLEEVLNGAQS KNFHLR PRDLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFAQSIISTLT		767
PEP005090	QVQLVESGGGVVQPGRSLRLDCKASGITFS NSGMHWVRQAPGKGLEWVAWIYDGSKR YYADSVKGRFTISRDN SKNTLFLQMNSLRA EDTAVYYCATNDDYWGQGLVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMI SLSPIVTCVVVDVSEDDPDVQIS WVFNNVEVHTAQTQTHREDYNSTLRVVS LPIQH QDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKNT EPVLKSDGSYFMYSKLTVEKKNWVERN SY SCSVVHEGLHNHHTTKSFSRTPGGGGGSGG GGSGGGGSGGGGSEVQLVESGGGLVQPGG SLRLSCAASGFNIKDTYIHWVRQAPGKGLE WVARIYPTNGYTRYADSVKGRFTISADTSK NTAYLQMNSLRAEDTAVYYCSRWGGDGF YAMDYWGQGLVTVSSASGGGGSGGGGS GGGGSHASDIQMTQSPSSLSASVGDRVITITC RASQDVNTAVAWYQQKPKGAPKLLIYSAS FLYSGVPSRFSGSRSGTDFTLTISSLQPEDFA TYYCQQHYTTPPTFGQGTKVEIK	AB001203_tr astuzumab control	768
PEP005091	QVQLVESGGGVVQPGRSLRLDCKASGITFS NSGMHWVRQAPGKGLEWVAWIYDGSKR YYADSVKGRFTISRDN SKNTLFLQMNSLRA		769

peptideID	Sequence	Comments	SEQ ID NO:
	EDTAVYYCATNDDYWGQGLTVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMISSLPIVTCVVVDVSEDDPDVQIS WVFVNNVEVHTAQTQTHREDYNSTLRVVS LPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKNT EPVLKSDGSYFMYSKLTVEKKNWVERN SYSCSVVHEGLHNHHTTKSFSRTPGGGGGSGG GGSGGGGSGGGGSQVQLVQSGAEVKKPGA SVKVSCKASGDTFTRYVHWVRQAPGQGL EWMGIINPSGGYASYAQKFQGRVTMTRDT STSTVYMESSLRSEDTAVYYCAAGLFIWG QGTLTVTVSSASGGGGSGGGGSGGGGSHAS DIQMTQSPSSLSASVGDRVTITCRASQSIGR WLAWYQQKPGKAPKLLIYSASNLETGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYN RFPVTFGPGTKVDIK		
PEP005092	QVQLVESGGGVVQPGRSLRLDCKASGITFS NSGMHWVRQAPGKGLEWVAVIWYDGSKR YYADSVKGRFTISRDN SKNTLFLQMNSLRA EDTAVYYCATNDDYWGQGLTVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMISSLPIVTCVVVDVSEDDPDVQIS WVFVNNVEVHTAQTQTHREDYNSTLRVVS LPIQHQQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEKEMTKKQVSL TCLVKDFMPEDIYVEWTNNGKTELNYKNT EPVLKSDGSYFMYSKLTVEKKNWVERN SYSCSVVHEGLHNHHTTKSFSRTPGGGGGSGG GGSGGGGSGGGGSQVQLVQSGAEVKKPGA SVKVSCKASGYTFTRYMHVVRQAPGQGL EWMGIINPRAGYTSYALKFQGRVTMTRDTS TSTVYMESSLRSEDTAVYYCTSGWDVWG QGTLTVTVSSASGGGGSGGGGSGGGGSHAS DIQMTQSPSSLSASVGDRVTITCRASQSIST WLAWYQQKPGKAPKLLIYAASSLD SGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCQQS YSFPVTFGQG TKVEIK		770
PEP005469	DIQMTQSPSSLSASVGDRVTITCRASQTISRY LNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYS TPRTFGQGTKLEIKRADAAPTVSIFPPSSEQL		771

peptideID	Sequence	Comments	SEQ ID NO:
	TSGGASVVCFLNNFYPKDINVKWKIDGSER QNGVLNSWTDQDSKDSTYSMSSTLTLTKD EYERHNSYTCEATHKTSTSPIVKSFNRNEC		
PEP005470	QVQLVQSGAEVKKPGSSVKVSCASGYTFT AYYIHWVRQAPGQGLEFMGWIHPYSGGTN YAQKFQGRVTITADESTSTAYMELSSLRSE DTAVYYCAIGYYYGKFDYWGGQGLTVTVSS AKTTAPSVYPLAPVCGDTTGSSVTLGCLVK GYFPEPVTLTWNSGSLSSGVHTFPAVLQSD LYTLSSSVTVTSSTWPSQSITCNVAHPASST KVDKKIEPRGPTIKCPPCKCPAPNAAGGPS VFIFPPKIKDVLMISSLPIVTCVVVDVSEDDP DVQISWVFNNEVHTAQTQTHREDYNSTL RVVSALPIQHQQDWMSGKEFKCKVNNKDLG APIERTISKPKGSVRAPQVYVLPPEEEMTK KQVTLTCMVTDFMPEDIYVEWTNNGKTEL NYKNTEPVLDSGGSYFMYSDLRVEKKNWV ERNYSYSCSVVHEGLHNHHTTESFSRTPGGG GGSGGGGSGGGGSAPTSSSTKKTQLQLEHL LLDLQMILNGINNYKNPKLTDMLTFEFYMP KKATELKHLQCLERELKPLEEVLNLAQSKN FHLRPRDLISNINVIVLELKGSETTFMCEYA DETATIVEFLNRWITFCQSIISTLT		772
PEP005471	QVQLVQSGAEVKKPGSSVKVSCASGYTFT AYYIHWVRQAPGQGLEFMGWIHPYSGGTN YAQKFQGRVTITADESTSTAYMELSSLRSE DTAVYYCAIGYYYGKFDYWGGQGLTVTVSS AKTTAPSVYPLAPVCGDTTGSSVTLGCLVK GYFPEPVTLTWNSGSLSSGVHTFPAVLQSD LYTLSSSVTVTSSTWPSQSITCNVAHPASST KVDKKIEPRGPTIKCPPCKCPAPNAAGGPS VFIFPPKIKDVLMISSLPIVTCVVVDVSEDDP DVQISWVFNNEVHTAQTQTHREDYNSTL RVVSALPIQHQQDWMSGKEFKCKVNNKDLG APIERTISKPKGSVRAPQVYVLPPEKEMTK KQVSLTCLVKDFMPEDIYVEWTNNGKTEL NYKNTEPVLKSDGSYFMYSKLTVEKKNWV ERNYSYSCSVVHEGLHNHHTTKSFSRTPGGG GGSGGGGSGGGGSGGGGSQVQLVQSGAEV KKPGASVKVSCASGDTFTRYVHWVRQA PGQGLEWMGIINPSGGYASYAQKFQGRVT MTRDTSTSTVYMELSSLRSEDTAVYYCAAG LFIWGQGLTVTVSSASGGGGSGGGGSGGG GSHASDIQMTQSPSSLSASVGDRVTITCRAS QSIGRWLAWYQQKPGKAPKLLIYSASNLET GVPSRFSGSGSGTDFTLTISLQPEDFATYYC QQYNRFPVTFGPGTKVDIK		773
PEP005473	DIVMTQSPLSLPVTPGEPASISCRSSQSLLS NGYNYLDWYLLQKPGQSPQLLIYLGSRAS		774

peptideID	Sequence	Comments	SEQ ID NO:
	GVPDRFSGSGSGTDFTLKISRVEADVGVY YCMQGTHWPPTFGQGTKVEIKRADAAPTV SIFPPSSEQLTSGGASVVCFLNNFYPKDINVK WKIDGSERQNGVLNSWTDQDSKDSTYSMS STLTLTKDEYERHNSYTCEATHKTSTSPIVK SFNRNEC		
PEP005474	QVQLVQSGAEVKKPGASVKVSCKASGYIFN GYDIHWVRQAPGQGLEWMGWMNPDNGN TGLAQKFQGRVTITADESTSTAYMELSSLRS EDTAVYYCARGMATRFPYYYGMDVWGQ GTLVTVSSAKTTAPSVYPLAPVCGDTTGSS VTLGCLVKGYFPEPVTLTWNSGSLSSGVHT FPAVLQSDLYTLSSSVTVTSSTWPSQSITCN VAHPASSTKVDKKIEPRGPTIKPCPPCKCPA PNAAGGPSVFIFPPKIKDVLMSLSPIVTCVV VDVSEDDPDVQISWVNNVEVHTAQTQTH REDYNSTLRVVSALPIQHQQDWMSGKEFKC KVNNKDLGAPIERTISKPKGSVRAPQVYVL PPPEEEMTKKQVTLTCMVTDFMPEDIYVEW TNNGKTELNYKNTEPVLDSDGSYFMYSDL RVEKKNWVERNSYSCSVVHEGLHNHHTTE SFSRTPGGGGSGGGSGGGGSAPTSSSTK KTQLQLEHLLDLQMLNGINNYKNPKLTD MLTFEFYMPKKATELKHLQCLERELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGS ETTFMCEYADETATIVEFLNRWITFCQSIIST LT		775
PEP005475	QVQLVQSGAEVKKPGASVKVSCKASGYIFN GYDIHWVRQAPGQGLEWMGWMNPDNGN TGLAQKFQGRVTITADESTSTAYMELSSLRS EDTAVYYCARGMATRFPYYYGMDVWGQ GTLVTVSSAKTTAPSVYPLAPVCGDTTGSS VTLGCLVKGYFPEPVTLTWNSGSLSSGVHT FPAVLQSDLYTLSSSVTVTSSTWPSQSITCN VAHPASSTKVDKKIEPRGPTIKPCPPCKCPA PNAAGGPSVFIFPPKIKDVLMSLSPIVTCVV VDVSEDDPDVQISWVNNVEVHTAQTQTH REDYNSTLRVVSALPIQHQQDWMSGKEFKC KVNNKDLGAPIERTISKPKGSVRAPQVYVL PPPEKEMTKKQVSLTCLVKDFMPEDIYVEW TNNGKTELNYKNTEPVLSKSDGSYFMYSKLT VEKKNWVERNSYSCSVVHEGLHNHHTTKS FSRTPGGGGSGGGSGGGSGGGGSQVQ LVQSGAEVKKPGASVKVSCKASGDTFTRY YVHWVRQAPGQGLEWMGIINPSGGYASYA QKFQGRVTMTTRDTSTSTVYMESSLRSED TAVYYCAAGLFIWGQGTTLTVSSASGGGGS GGGGSGGGGSHASDIQMTQSPSSLSASVGD RVTITCRASQSIGRWLAWYQQKPGKAPKLL		776

peptideID	Sequence	Comments	SEQ ID NO:
	IYSASNLETGVPSRFSGSGSGTDFTLTISLQ PEDFATYYCQQYNRFPVTFGPGTKVDIK		
PEP005631	EVQLLES GGGLVQP GGSRLRLSCAASGFSFSS YTMSWVRQAPGKGLEWVATISGGGRDIYY PDSVKGRFTISRDN SKNTLYLQMNSLRAED TAVYYCVLLTGRVYFALDSWGQGLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEAAGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNK ALGAPIEKTISKAKGQPREPQVCTLPPSRDE LTKNQVSLSCAVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG GGGSGGGSGGGSGGGGSQVQLVQSGAE VKKPGASVKVSCKASGDTFTRYVHWVRQ APGQGLEWMGIINPSGGYASYAQKFQGRV TMTRDTSTSTVYMELSSLRSED TAVYYCAA GLFIWGQGLVTVSSASGGGGSGGGSGG GGSHASDIQMTQSPSSLSASVGDRVITCRA SQSIGRWLAWYQQKPGKAPKLLIYSASNLE TGVPSRFSGSGSGTDFTLTISLQPEDFATYY CQQYNRFPVTFGPGTKVDIK		777
PEP005634	EVQLLES GGGLVQP GGSRLRLSCAASGFSFSS YTMSWVRQAPGKGLEWVATISGGGRDIYY PDSVKGRFTISRDN SKNTLYLQMNSLRAED TAVYYCVLLTGRVYFALDSWGQGLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEAAGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNK ALGAPIEKTISKAKGQPREPQVCTLPPSRDE LTKNQVSLSCAVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG GGGSGGGSGGGSGGGGSQVQLVQSGAE VKKPGASVKVSCKASGYTFTRYMHWR QAPGQGLEWMGIINPRAGYTSYALKFQGR VTMTRDTSTSTVYMELSSLRSED TAVYYCT SGWDVWGQGLVTVSSASGGGGSGGGGS GGGGSHASDIQMTQSPSSLSASVGDRVITC RASQSISTWLAWYQQKPGKAPKLLIYAASS		778

peptideID	Sequence	Comments	SEQ ID NO:
	LDSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSPVTFGQGTKVEIK		
PEP005789	MSTSTQVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYD GSKRYYADSVKGRFTISRDN SKNTLFLQMN SLRAEDTAVYYCATNDDYW GQGTL VTVSS ASGGGGSGGGGSGGGGSHASEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASN RATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTFGQGT KVEIKEQKLISEEDLG SGLNDIFE AQKIEWH EGKPIPNPLLGLDSTNA	Nivolumab-scFV	779
PEP005790	MSTSTQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFKNEKFKNRVLT TDSSTTTAYME LKSLQFDDTAVYYCARRDYRFDMGFDYW GQGTTVTVSSASGGGGSGGGGSGGGGSHA SEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLES GVPARFSGSGSGTDFTLTISSLEPEDFAVYYC QHSRDLPLTFGGGTKVEIKEQKLISEEDLG SGLNDIFE AQKIEWHEGKPIPNPLLGLDSTNA	Pembrolizumab-scFv	780
PEP005794	MSTSTQVQLVQSGAEVKKPGASVKVSCKASGDEFTRYYYVHWVRQAPGQGLEWMGIINP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGT LTVS SGGGGSGGGGSGGGGSGGGGSDIQMTQSPSLSASVGDRVTITCRASQSIGRWLAWYQQKPGKAPKLLIYSASNLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNRFPVTFGPGTKVDIKEQKLISEEDLG SGLNDIFE AQKIEWHEGKPIPNPLLGLDST	AB003178_2 B07v12	781
PEP005797	MSTSTQVQLVQSGAEVKKPGASVKVSCKASGDEFTRYYYVHWVRQAPGQGLEWMGIQNP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGT LTVS SGGGGSGGGGSGGGGSGGGGSDIQMTQSPSLSASVGDRVTITCRASQSIGRELAWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNRFPVTFGPGTKVDIKEQKLISEEDLG SGLNDIFE AQKIEWHEGKPIPNPLLGLDST	AB003183_2 B07v14	782
PEP005798	MSTSTQVQLVQSGAEVKKPGASVKVSCKASGDEFTRYYYVHWVRQAPGQGLEWMGIQNP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGT LTVS SGGGGSGGGGSGGGGSGGGGSDIQMTQSPSLSASVGDRVTITCRASQSIGRWLAWYQQKPGKAPKLLIYSASNLETGVPSRFSGSGSGTD	AB003180_2 B07v13	783

peptideID	Sequence	Comments	SEQ ID NO:
	FTLTISSLQPEDFATYYCQQYNRFPVTFGPG TKVDIKEQKLISEEDLGSLNDIFEAKIEW HEGKPIPNPLLGLDST		
PEP005801	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGDTFTRYVHWVRQAPGQGLEWMGIINP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGTLLTVS SGGGGSGGGGSGGGGSGGGGSDIQMTQSPS SLSASVGDRVTITCRASQSIGRELAWYQQK PGKAPKLLIYSASNLETGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYNRFPVTFGPG TKVDIKEQKLISEEDLGSLNDIFEAKIEW HEGKPIPNPLLGLDST	AB003174_2 B07v11	784
PEP005803	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGDTFTRYVHWVRQAPGQGLEWMGIINP SGGYASYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCAAGLFIWGQGTLLTVS SGGGGSGGGGSGGGGSGGGGSDIQMTQSPS SLSASVGDRVTITCRASQSIGRWLAWYQQK PGKAPKLLIYSASNLETGVPSRFSGSGSGTD FTLTISSLQPEDFATYYCQQYNRFPVTFGPG TKVDIKEQKLISEEDLGSLNDIFEAKIEW HEGKPIPNPLLGLDST	AB002328_2 B07v4	785
PEP005817	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGETFTRYVHWVRQAPGQGLEWMGIINPS GGYASYAQKFQGRVTMTRDTSTSTVYMEL SSLRSEDTAVYYCAAGLFIWGQGTLLTVSS GGGGSGGGGSGGGGSGGGGSDIQMTQSPSS LSASVGDRVTITCRASQSIGRWLAWYQQKP GKAPKLLIYSASNLETGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQYNRFPVTFGPGT KVDIKEQKLISEEDLGSLNDIFEAKIEWH EGKPIPNPLLGLDST	AB003155_2 B07v10	786
PEP005840	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGYTFTRYMHWRQAPGQGLEWMGIINP RAGYTSYALKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCTSGWDVWGQGTLLTV SSGGGSGGGGSGGGGSGGGGSDIQMTQSP SSLSASVGDRVTITCRASQSISTWLAWYQQ KPGKAPKLLIYAASSLDEGVPSRFSGSGSGT DFTLTISSLQPEDFATYYCQQSYSPVTFGQ GTKVEIKEQKLISEEDLGSLNDIFEAKIE WHEGKPIPNPLLGLDST	AB003012_7 A04v7	787
PEP005842	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGYTFTRYMHWRQAPGQGLEWMGIINP RAGYTSYALKFQGRVTMTRDTSTSTVYME LSSLRSEDTAVYYCTSGWDVWGQGTLLTV SSGGGSGGGGSGGGGSGGGGSDIQMTQSP SSLSASVGDRVTITCRASQSISTWLAWYQQ	AB002365_7 A04v2	788

peptideID	Sequence	Comments	SEQ ID NO:
	KPGKAPKLLIYAASSLDGVSFRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSPVTFGQ GTKVEIKEQKLISEEDLGSLNDIFEAQKIE WHEGKPIPNPLLGLDST		
PEP005843	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGYTFTRYMHWRQAPGQGLEWMGIINP RAGYTSYALKFQGRVTMTRDTSTSTVYME LSSLRSEDVAVYYCTSGWEVWGQGLTVTV SSGGGGSGGGGSGGGGSGGGGSDIQMTQSP SSLSASVGDRVTITCRASQSISTWLAWYQQ KPGKAPKLLIYAASSLDEGVPSRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSPVTFGQ GTKVEIKEQKLISEEDLGSLNDIFEAQKIE WHEGKPIPNPLLGLDST	AB003007_7 A04v6	789
PEP005845	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGYTFTRYMHWRQAPGQGLEWMGIIP RAGYTSYALKFQGRVTMTRDTSTSTVYME LSSLRSEDVAVYYCTSGWDVWGQGLTVTV SSGGGGSGGGGSGGGGSGGGGSDIQMTQSP SSLSASVGDRVTITCRASQSISTWLAWYQQ KPGKAPKLLIYAASSLDEGVPSRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSPVTFGQ GTKVEIKEQKLISEEDLGSLNDIFEAQKIE WHEGKPIPNPLLGLDST	AB003005_7 A04v5	790
PEP005847	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGETFTNYYIHWVRQAPGQGLEWMGIIDPR AGYTSYALKFQGRVTMTRDTSTSTVYME LSSLRSEDVAVYYCAGGWEDWGQGLTVTVS SSGGGGSGGGGSGGGGSGGGGSDIQMTQSPS SLSASVGDRVTITCRISQSISFLAWYQQKP GKAPKLLIYSASSLQSGVPSRFSGSGSGTDF TLTISLQPEDFATYYCQQSFTSPITFGQGTR LEIKEQKLISEEDLGSLNDIFEAQKIEWHE GKPIPNPLLGLDST	AB003209_2 B05v4	791
PEP005853	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGETFTNYYIHWVRQAPGQGLEWMGVINP RAGYTSYALKFQGRVTMTRDTSTSTVYME LSSLRSEDVAVYYCAGGWEDWGQGLTVTV SSGGGGSGGGGSGGGGSGGGGSDIQMTQSP SSLSASVGDRVTITCRISQSISWLAWYQQK PGKAPKLLIYSASSLQSGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQSFTSPITFGQGT RLEIKEQKLISEEDLGSLNDIFEAQKIEWHE GKPIPNPLLGLDST	AB003200_2 B05v1	792
PEP005860	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGFTFTRYMHWRQAPGQGLEWMGVINP SGGYATYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDVAVYYCASGWDVWGQGLTVTV SSGGGGSGGGGSGGGGSGGGGSDIQMTQSP	AB003021_2 A11v7	793

peptideID	Sequence	Comments	SEQ ID NO:
	SSLSASVGDRVTITCRASQSISSWLAWYQQ KPGKAPKLLIYATSTLESGVPSRFSGSGSGT DFTLTISLQPEDFATYYCQQSYSSPVTFGQ GTKVEIKEQKLISEEDLGSLNDIFEAKIE WHEGKPIPNPLLGLDST		
PEP005863	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGGTFTNYYIHWVRQAPGQGLEWMGIIDPR AGYTSYALKFQGRVTMTRDTSTSTVYMEL SSLRSEDNAVYYCAGGWEDWGQGTLLTVS SGGGGSGGGGSGGGGSGGGGSDIQMTQSPS SLSASVGDRVTITCRISQSISSWLAWYQQK GKAPKLLIYSASSLQSGVPSRFSGSGSGTDF TLTISLQPEDFATYYCQQSFTSPITFGQGTR LEIKEQKLISEEDLGSLNDIFEAKIEWHE GKPIPNPLLGLDST	AB003201_2 B05v2	794
PEP005867	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGHTFTRYMHWRQAPGQGLEWMGIINP SGGYATYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDNAVYYCASGLFIWGQGTLLTVS SGGGGSGGGGSGGGGSGGGGSDIQMTQSPS SLSASVGDRVTITCRASQSINWLAWYQQK PGKAPKLLIYATSTLESGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQSYRFPVTFGQG TKVEIKEQKLISEEDLGSLNDIFEAKIEW HEGKPIPNPLLGLDST	AB002427_2 A11v5	795
PEP005868	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGHTFTRYMHWRQAPGQGLEWMGIINP SGGYATYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDNAVYYCASGLFIWGQGTLLTVS SGGGGSGGGGSGGGGSGGGGSDIQMTQSPS SLSASVGDRVTITCRASQSINWLAWYQQK PGKAPKLLIYATSTLESGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQYYRFPVTFGQG TKVEIKEQKLISEEDLGSLNDIFEAKIEW HEGKPIPNPLLGLDST	AB002413_2 A11v3	796
PEP005869	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGHTFTRYMHWRQAPGQGLEWMGIINP SGGYATYAQKFQGRVTMTRDTSTSTVYME LSSLRSEDNAVYYCASGLFIWGQGTLLTVS SGGGGSGGGGSGGGGSGGGGSDIQMTQSPS SLSASVGDRVTITCRASQSINWLAWYQQK PGKAPKLLIYATSTLESGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQYYSPVTFGQG TKVEIKEQKLISEEDLGSLNDIFEAKIEW HEGKPIPNPLLGLDST	AB002417_2 A11v4	797
PEP005880	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGHTFTRYMHWRQAPGQGLEWMGVIN PSGGYATYAQKFQGRVTMTRDTSTSTVYM ELSSLRSEDNAVYYCASGWDVWGQGTLLTV	AB002864_2 A11v6	798

peptideID	Sequence	Comments	SEQ ID NO:
	VSSGGGSGGGGSGGGGSGGGGSDIQMTQ SPSSLSASVGDRVTITCRASQSINSWLAWYQ QKPGKAPKLLIYATSTLESGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCQQSYSSPVTFG QGTKVEIKEQKLISEEDLGSLNDIFEAKKIE WHEGKPIPNPLLGLDST		
PEP005887	MSTSTQVQLVQSGAEVKKPGASVKVSCKA SGSTFTNYYIHWVRQAPGQGLEWMGIIDPR AGYTSYALKFQGRVTMTRDTSTSTVYMEL SSLRSEDTAVYYCAGGWEDWGQGLTVTVS SGGGGSGGGGSGGGGSGGGGSDIQMTQSPS SLSASVGDRVTITCRISQSISSFLAWYQQKP GKAPKLLIYSASSLQSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQSFTSPITFGQGTR LEIKEQKLISEEDLGSLNDIFEAKKIEWHE GKPIPNPLLGLDST	AB003202_2 B05v3	799
PEP006177	QVQLVESGGGVVQPGRSLRLDCKASGITFS NSGMHWVRQAPGKGLEWVAVIWYDGSKR YYADSVKGRFTISRDN SKNTLFLQMNSLRA EDTAVYYCATNDDYWGQGLTVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFP EPVTLTWNSGSLSSGVHTFPAVLQSDLYTLS SSVTVTSSWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP KIKDVLMI SLSPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNSTLRVVS LPIQH QDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDS DGSYFMYSDLRVEKKNWVERNSY SCSVVHEGLHNHHTTESFSRTPGGGGGSGG GGSGGGGSAPTSSSTKKTQLQLEHLLDLQ MILNGINNYKNPKLTRMLTFKFYMPKKATE LKHLQCLEEELKPLEEVLNLAQSKNFHLRP RDLISNINVIVLELKGSETTFMCEYADETATI VEFLNRWITFCQSIISTLT		800

EXAMPLES

[0106] The following examples are illustrative and non-limiting to the scope of the devices, methods, systems, and kits described herein.

EXAMPLE 1**Isolation of a set of dual-binding antibodies (DBAs) that bind human PD-1 and human IL-2**

[0107] This example describes the isolation of sensor domains of the present disclosure, specifically, a set of DBAs that bind human PD-1 and human IL-2. Anti-PD-1 and anti-IL-2 DBAs were isolated from a Tumbler antibody phage display library (Distributed Bio, Inc.). The antibody phage display library was constructed to incorporate the heavy chain CDR1, heavy chain CDR2, and light chain diversity of the Superhuman 2.0 antibody library combined with 10 heavy chain CDR3 sequences from PD-1 binding antibodies (SEQ ID NO: 11 – SEQ ID NO: 20).

TABLE 3 – HC-CDR3 of PD-1 binders

SEQ ID NO	Sequence	Description
SEQ ID NO: 11	CAAGLFIW	HC-CDR3 of PD-1 binder
SEQ ID NO: 12	CAGGWLDW	HC-CDR3 of PD-1 binder
SEQ ID NO: 13	CARDHLGGSYQPW	HC-CDR3 of PD-1 binder
SEQ ID NO: 14	CARDLVGVSPGINYVPRYYYY YYGMDVW	HC-CDR3 of PD-1 binder
SEQ ID NO: 15	CARDTGLGYYYGSGDFDYW	HC-CDR3 of PD-1 binder
SEQ ID NO: 16	CARSGYSYGYYFDYW	HC-CDR3 of PD-1 binder
SEQ ID NO: 17	CARTGGYPAIDSW	HC-CDR3 of PD-1 binder
SEQ ID NO: 18	CASGWDVW	HC-CDR3 of PD-1 binder
SEQ ID NO: 19	CASSPLQWVDVW	HC-CDR3 of PD-1 binder
SEQ ID NO: 20	CTSGMDVW	HC-CDR3 of PD-1 binder

[0108] This library was subjected to four rounds of selection with standard protocols. In brief, the phage library was incubated with the antigen, then captured on magnetic beads and washed on a Kingfisher magnetic particle processor, eluted from the magnetic beads and amplified by passaging in *E. coli*. Round 1 was incubated with 50 nM human PD-1-His fusion (R&D Systems, Prod. Num. 8986-PD) and captured with TRIS NTA Biotin (Sigma-Aldrich Prod. Num. 75543) and streptavidin magnetic beads. Round 2 was incubated with 100 nM biotinylated IL-2 (Creative Biomart, Prod. Num. IL2-501H, biotinylated using standard protocols) and captured on streptavidin magnetic beads. Round 3 was incubated with 50 nM cynomolgus PD-1-Fc fusion (R&D Systems, Prod. Num. 8578-PD) and captured on protein G magnetic beads. Round 4 was incubated with 50 nM biotinylated human IL-2 and captured on streptavidin magnetic beads. The final selection was plated as single colonies and 380 colonies picked for Sanger sequencing. One hundred and fifty-one unique clones were chosen for expression. The scFv sequence for each clone was codon-optimized for *E. coli* expression and the corresponding DNA sequences sent to Integrated DNA Technologies, Inc. (IDT) for synthesis as gBlocks with a T7 promoter, a

translation initiation site and a T7 terminator. Protein from each gBlock encoding an scFv was expressed using the PURExpress *In vitro* Protein Synthesis Kit (New England Biolabs, Inc., Prod. Num. E6800). The PURExpress scFv proteins were used directly in HTRF binding assays and cell-based functional assays. Each scFv was tested for binding to PD-1 and to human IL-2. Eighty-one of the antibodies showed dual-binding activity for both PD-1 and IL-2 and a summary of fluorescence signal values of binding curves is shown in **TABLE 5**. To examine the ability of DBA binding domains to block IL-2 receptor binding, V5-tagged DBA scFvs were serially diluted in a 384 well plate. Europium-labeled Streptavidin, biotin-labeled IL-2 (Acro Biosystems, Prod. Num. IL2-H82E4), IL-2 Receptor beta (Fc-IL2RB) (Acro Biosystems, Prod. Num. ILB-H5253), and APC-labeled anti-Fc antibody. Plates were incubated at room temperature for 2 hours, and the HTRF signal was read on an Envision (Perkin Elmer) as a measure of IL-2:IL2RB binding. Four scFvs (SEQ ID NO: 31 – SEQ ID NO: 34) bound PD-1, bound IL-2 and blocked binding of IL-2 to IL-2RB (**TABLE 4**).

TABLE 4 – DBAs and Controls

SEQ ID NO	Sequence	Description
SEQ ID NO: 31	QVQLVQSGAEVKKPGVSVKVSCKASGYTF PRSYIHWVRQAPGQGLEWMGWINPHSGDT YYAQNFGQGRVTMTRDTSTSTVYMELSSLR SEDTAVYYCARDTGLGYYYGSGDFDYWG QGTLVTVSSASGGGGSGGGGSGGGGSHAS DIQMTQSPSSLSASVGDRVTITCRASQSISR YLNWYQQKPGKAPKLLIYTASSLQSGVPSR FSGSGSGTDFTLTISLQPEDFATYYCQQAN RFPLTFGPGTKVDIK	DBA capable of binding a PD-1 marker and IL-2 therapeutic
SEQ ID NO: 32	QVQLVQSGAEVKKPGASVKVSCKASGYTF PRYHIHWVRQAPGQGLEWMGMINPSGGTT TYAQKFQGRVTMTRDTSTSTVYMELSSLR SEDTAVYYCARDTGLGYYYGSGDFDYWG QGTLVTVSSASGGGGSGGGGSGGGGSHAS DIQMTQSPSSLSASVGDRVTITCRASQSISS WLAWYQQKPGKAPKLLIYAASSLQSGVPS RFSGSGSGTDFTLTISLQPEDFATYYCQQS HSFPLTFGGGTKVEIK	DBA capable of binding a PD-1 marker and IL-2 therapeutic
SEQ ID NO: 33	QVQLVQSGAEVKKPGASVKVSCKASGYTF TRYIHWVRQAPGQGLEWMGWINAYNGD TNYAQKLQGRVTMTRDTSTSTVYMELSSL RSEDTAVYYCARDSYYYDSFDYWGQGTL VTVSSASGGGGSGGGGSGGGGSHASDIQM TQSPSSLSASVGDRVTITCRASQTITDWLA WYQQKPGKAPKLLIYGASNLOGGVPSRFS GSGSGTDFTLTISLQPEDFATYYCQQYYSS WTFGQGTKVEIK	DBA capable of binding a PD-1 marker and IL-2 therapeutic

SEQ ID NO	Sequence	Description
SEQ ID NO: 34	QVQLVQSGAEVKKPGASVKVSCKASGYTF TSYYMHWVRQAPGQGLEWMGIINPSDGST TYAQSFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYYCASGWDVWGQGLVTVSSASG GGGSGGGSGGGGSHASDIVMTQSPDSL VSLGERATINCKSSQSVFSSANNKNYLAW YQQKPGQPPKLLIWASTRESGVDPDRFSGS GSGTDFLTISLQAEDVAVYYCQQYFGTP VTFGGGTKVEIK	DBA capable of binding a PD-1 marker and IL-2 therapeutic

TABLE 5

Name	PD1 Binding	IL2 Binding	IL2RB Blocking
No DNA	5	6	1,490
PD1-IL2-R01-H08	576	-9	1,829
PD1-IL2-R01-H09	1,015	131	1,772
PD1-IL2-R02-A03	1,508	635	1,714
PD1-IL2-R02-A04	909	978	1,618
PD1-IL2-R02-A05	1,557	23	1,735
PD1-IL2-R02-A06	357	515	1,772
PD1-IL2-R02-A08	995	520	1,612
PD1-IL2-R02-A09	1,421	1,470	1,495
PD1-IL2-R02-A10	500	838	1,847
PD1-IL2-R02-A11	1,625	1,559	1,783
PD1-IL2-R02-A12	1,725	130	1,586
PD1-IL2-R02-B01	746	1,077	1,516
PD1-IL2-R02-B02	1,740	1,107	1,849
PD1-IL2-R02-B04	11	2,346	1,536
PD1-IL2-R02-B05	1,665	2,489	1,613
PD1-IL2-R02-B06	1,527	32	1,605
PD1-IL2-R02-B07	1,685	628	1,814
PD1-IL2-R02-B08	1,446	92	1,680
PD1-IL2-R02-B10	211	343	1,607
PD1-IL2-R02-B11	1,426	915	1,509
PD1-IL2-R02-B12	1,264	316	1,762
PD1-IL2-R02-C01	1,463	296	1,743
PD1-IL2-R02-C02 (SEQ ID NO: 31)	1,299	298	1,069
PD1-IL2-R02-C03 (SEQ ID NO: 32)	1,383	293	1,211
PD1-IL2-R02-C04	1,622	575	1,857
PD1-IL2-R02-C06	1,376	34	1,684
PD1-IL2-R02-C07	34	87	1,607
PD1-IL2-R02-C08	1,468	619	1,671

Name	PD1 Binding	IL2 Binding	IL2RB Blocking
PD1-IL2-R02-C10	174	256	1,757
PD1-IL2-R02-C12	1,367	340	1,723
PD1-IL2-R02-D01	1,421	68	1,614
PD1-IL2-R02-D02	1,473	539	1,726
PD1-IL2-R06-A10	1,269	9	1,796
PD1-IL2-R06-A11	1,376	34	1,762
PD1-IL2-R06-A12	1,305	7	1,681
PD1-IL2-R06-B01	10	2,109	1,307
PD1-IL2-R06-B02	1,666	15	1,799
PD1-IL2-R06-B03	923	4	1,661
PD1-IL2-R06-B04	1,782	28	1,666
PD1-IL2-R06-B06	1,223	17	1,648
PD1-IL2-R06-B08	1,777	1,160	1,738
PD1-IL2-R06-B10	13	31	1,847
PD1-IL2-R06-B11	1,534	24	1,699
PD1-IL2-R06-B12	822	1,125	1,604
PD1-IL2-R06-C02	1,667	26	1,671
PD1-IL2-R06-C04	1,491	7	1,759
PD1-IL2-R06-C08	1,448	8	1,693
PD1-IL2-R06-C09	1,158	1,525	1,602
PD1-IL2-R06-C11	1,879	-2	1,785
PD1-IL2-R06-C12	1,669	1,998	1,033
PD1-IL2-R06-D02	280	432	1,677
PD1-IL2-R06-D03	9	93	1,606
PD1-IL2-R06-D05	505	-3	1,786
PD1-IL2-R06-D07	1,577	24	1,820
PD1-IL2-R06-D10	1,751	49	1,719
PD1-IL2-R06-D11	405	593	1,576
PD1-IL2-R06-D12	1,024	1,423	1,649
PD1-IL2-R06-E01	1,628	3	1,724
PD1-IL2-R06-E02	1,554	16	1,598
PD1-IL2-R06-E04 (SEQ ID NO: 33)	50	247	1,108
PD1-IL2-R06-E05	1,364	14	1,734
PD1-IL2-R06-E06	1,627	15	1,735
PD1-IL2-R06-E07	1,801	12	1,698
PD1-IL2-R06-E09	1,467	11	1,511
PD1-IL2-R06-E11	1,805	294	1,767
PD1-IL2-R06-E12	4	-7	1,735
PD1-IL2-R06-F01	196	280	1,629
PD1-IL2-R06-F03	1,377	28	1,642
PD1-IL2-R06-F04	26	779	1,726

Name	PD1 Binding	IL2 Binding	IL2RB Blocking
PD1-IL2-R06-F05	1,493	18	1,625
PD1-IL2-R06-F06	1,577	46	1,595
PD1-IL2-R06-F07	1,544	335	1,682
PD1-IL2-R06-F08	1,570	9	1,780
PD1-IL2-R06-F09	30	41	1,776
PD1-IL2-R06-F10	1,745	24	1,607
PD1-IL2-R06-F11	1,586	12	1,574
PD1-IL2-R06-F12	623	8	1,645
PD1-IL2-R06-G01	130	184	1,640
PD1-IL2-R06-G02	1,754	20	1,623
PD1-IL2-R06-G04	1,348	13	1,596
PD1-IL2-R06-G05	1,382	10	1,846
PD1-IL2-R06-G06	1,383	4	1,744
PD1-IL2-R06-G08	1,708	124	1,533
PD1-IL2-R06-G09	557	756	1,527
PD1-IL2-R06-G10	1,595	35	1,703
PD1-IL2-R06-G11	1,469	17	1,709
PD1-IL2-R06-G12	1,281	1,479	1,713
PD1-IL2-R06-H01	381	4	1,647
PD1-IL2-R06-H02	1,501	20	1,748
PD1-IL2-R06-H03	1,132	1,449	1,617
PD1-IL2-R06-H04	355	1	1,677
PD1-IL2-R06-H05	1,409	21	1,561
PD1-IL2-R06-H06	1,491	23	1,650
PD1-IL2-R06-H07	12	13	1,701
PD1-IL2-R06-H08	847	1,118	1,746
PD1-IL2-R06-H09	1,732	22	1,662
PD1-IL2-R06-H10	830	1,151	1,569
PD1-IL2-R07-A03	1,786	28	1,511
PD1-IL2-R07-A04	730	973	1,613
PD1-IL2-R07-A05	477	663	1,327
PD1-IL2-R07-A08	1,628	841	1,618
PD1-IL2-R07-A09 (SEQ ID NO: 34)	1,235	2,040	910
PD1-IL2-R07-A10	1,716	63	1,518
PD1-IL2-R07-B01	1,397	32	1,565
PD1-IL2-R07-B02	192	321	1,634
PD1-IL2-R07-B03	65	202	1,604
PD1-IL2-R07-B04	1,862	410	1,527
PD1-IL2-R07-B05	965	351	1,389
PD1-IL2-R07-B06	1,882	44	1,497
PD1-IL2-R07-B07	6	2,549	1,517

Name	PD1 Binding	IL2 Binding	IL2RB Blocking
PD1-IL2-R07-B08	906	1,047	1,475
PD1-IL2-R07-B09	1,788	27	1,384
PD1-IL2-R07-B10	18	19	1,635
PD1-IL2-R07-B11	1,765	9	1,641
PD1-IL2-R07-C01	230	367	1,536
PD1-IL2-R07-C02	236	304	1,500
PD1-IL2-R07-C03	20	1,347	1,536
PD1-IL2-R07-C07	15	275	1,665
PD1-IL2-R07-C10	1,064	317	1,550
PD1-IL2-R07-C11	1,523	642	1,460
PD1-IL2-R07-C12	1,377	49	1,707
PD1-IL2-R07-D01	1,541	79	1,657
PD1-IL2-R07-D03	1,483	33	1,481
PD1-IL2-R07-D04	923	1,104	1,517
PD1-IL2-R07-D06	1,664	416	1,734
PD1-IL2-R07-D07	6	835	1,512
PD1-IL2-R07-D10	1,580	193	1,572
PD1-IL2-R07-D11	1,401	798	1,614
PD1-IL2-R07-E02	1,473	992	1,830
PD1-IL2-R07-E03	1,459	422	1,683
PD1-IL2-R07-E05	512	913	1,513
PD1-IL2-R07-E06	1,483	1,178	1,526
PD1-IL2-R07-E07	1,181	1,060	1,524
PD1-IL2-R07-E08	1,604	472	1,717
PD1-IL2-R07-E09	1,733	23	1,569
PD1-IL2-R07-E10	1,472	251	1,545
PD1-IL2-R07-E11	1,146	56	1,777
PD1-IL2-R07-E12	1,698	106	1,764
PD1-IL2-R07-F01	3	17	1,529
PD1-IL2-R07-F02	348	752	1,537
PD1-IL2-R07-F03	1,788	520	1,750
PD1-IL2-R07-F04	1,416	145	1,767
PD1-IL2-R07-F06	1,422	438	1,579
PD1-IL2-R07-F09	1,589	17	1,456
PD1-IL2-R07-F10	24	19	1,778
PD1-IL2-R07-F12	505	196	1,553
PD1-IL2-R07-G01	4	214	1,560
PD1-IL2-R07-G02	1,610	61	1,735
PD1-IL2-R07-G04	82	147	1,600
PD1-IL2-R07-G05	981	216	1,475
PD1-IL2-R07-G06	860	512	1,655
PD1-R04-C10	1,552	4	1,550

Name	PD1 Binding	IL2 Binding	IL2RB Blocking
PD1-R07-A05	653	19	1,730
PD1-R07-A10	484	25	2,290
PD1-R07-C09	1,911	20	2,080
PD1-R07-D03	1,733	22	2,208
PD1-R07-D05	1,760	16	1,578
PD1-R07-D06	1,997	22	1,749
PD1-R07-E05	633	24	2,246
PD1-R07-G12	907	11	1,577
PD1-R15-B02	1,671	28	1,797
PDL1-DB03-H02	18	11	1,725
Anti-Her2 (SEQ ID NO: 28)	4	20	1,636

[0109]

[0110]

EXAMPLE 2**Dual Binding Antibody (DBA)-Cytokine Protein Complexes**

[0111] This example describes dual binding antibody (DBA)-cytokine protein complexes of the present disclosure. Various DBA-cytokine protein complexes of the present disclosure were designed to include a cytokine, a linker, and one or more dual binding antibody domains.

Pictorial representations of exemplary constructs are shown in **FIG. 5**.

[0112] A series of DBA-cytokine protein complexes may be designed with two marker binding domains and one therapeutic domain. The DBAs used in this series, provided in **TABLE 6** with sequences provided in **TABLE 8**, exhibit a range of affinities for the marker and the therapeutic domain. Exemplary DBA complexes are provided in **TABLE 6**, **TABLE 9**, and **TABLE 10**.

TABLE 6 – Exemplary DBA Cytokine Protein Complexes

SEQ ID NO	Sequences	Description
SEQ ID NO: 51	QVQLVQSGAEVKKPGASVKVSCKASGYTFST YYIHWVRQAPGQGLEWMGIINPSGGGTVYA QKFQGRVTMTRDTSTSTVYMELSSLRSEDTA VYYCAAGLFIWGQGLTVTVSSAKTTAPSVYP LAPVCGDTTGSSVTLGCLVKGYFPEPVTLTW NSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSST WPSQSITCNVAHPASSTKVDKKIEPRGPTIKPC PPCKCPAPNAAGGPSVFIFPPKIKDVLMI SLSPIVTCVVVDVSEDDPDVQISWVNNVEVHTAQ TQTHREDYNSTLRVVSALPIQHQDWMSGKEF KCKVNNKDLGAPIERTISKPKGSVRAPQVYV LPPPEEEMTKKQVTLTCMVTDFMPEDIYVEW	PD1- IL2_6C12_N36T_S ym_L_Long_Pepl ; Symmetric DBA- Cytokine Complex IgG format

SEQ ID NO	Sequences	Description
	TNNGKTELNYKNTEPVLDSDGSYFMYSKLRV EKKNWVERNSYSCSVVHEGLHNHHTTKSFSR TPGK	
SEQ ID NO: 52	APTSSSTKKTQLQLEHLLLDLQMLNGINNYK NPKLTRMLTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFCQSII STLTVPGVGVPGAGVPGVGVPGGGVPGVGV PGGGVPGAGVPGGGVPGVGVPGAGVPGVGV PGGGDIQMTQSPSSLSASVGDRVTITCRASQY ISSGLAWYQQKPGKAPKLLIYKASSLDNGVPS RFSGSGSGTDFTLTISLQPEDFATYYCQQYE RLPLTFGGGTKVEIKRADAAPTVSIFPPSSEQL TSGGASVVCFLNFPKDVNVKWKIDGSERQ NGVLNSWTDQDSKDYSTYSMSSTLTLTCKDEYE RHNSYTCEATHKTSTSPIVKSFNRECE	PD1- IL2_6C12_N36T_S ym_L_Long_Pep2 ; Symmetric DBA- Cytokine Complex IgG format
SEQ ID NO: 53	APTSSSTKKTQLQLEHLLLDLQMLNGINNYK NPKLTRMLTFKFYMPKKATELKHLQCLEEEL KPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFCQSII STLTGGGGSGGGGSGGGGSGGGGSQVQLVQ SGAEVKKPGASVKVSKKASGYTFSTYYIHWV RQAPGQGLEWMGIINPSGGGTVAQAQKFGQR VTMTRDTSTSTVYMELSSLRSEDTAVYYCAA GLFIWGQGTLLTVSSAKTTAPSVYPLAPVCG DTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSS GVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSIT CNVAHPASSTKVDKKIEPRGPTIKPCPPCKCP APNAAGGPSVFIFPPKIKDVLMISSLPIVTCVV VDVSEDDPDVQISWVFNVEVHTAQTQTHRE DYNSTLRVVSALPIQHQQDWMMSGKEFKCKVN NKDLGAPIERTISKPKGSVRAPQVYVLPPEEE MTKKQVTLTCMVTDMPEDIVYEWNTNNGKT ELNYKNTEPVLDSDGSYFMYSKLRVEKKNW VERNSYSCSVVHEGLHNHHTTKSFSRTPGK	PD1- IL2_6C12_N36T_ D68E_Sym_H_Sho rt_Pep1 ; Symmetric DBA- Cytokine Complex IgG format
SEQ ID NO: 54	DIQMTQSPSSLSASVGDRVTITCRASQYISSGL AWYQQKPGKAPKLLIYKASSLENGVPSRFSG SGSGTDFTLTISLQPEDFATYYCQQYERLPLT FGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGA SVVCFLNFPKDVNVKWKIDGSERQNGVLN SWTDQDSKDYSTYSMSSTLTLTCKDEYERHNSY TCEATHKTSTSPIVKSFNRECE	PD1- IL2_6C12_N36T_ D68E_Sym_H_Sho rt_Pep2 ;Symmetric DBA-Cytokine Complex IgG format
SEQ ID NO: 77	QVQLVQSGAEVKKPGASVKVSKKASGYTFST YYVHWVRQAPGQGLEWMGIINPSGGSTSYA QNFQGRVTMTRDTSTSTVYMELSSLRSEDTA VYYCASGWDVWGQGTITVTVSSAKTTAPSVY PLAPVCGDTTGSSVTLGCLVKGYFPEPVTLT WNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTS STWPSQSITCNVAHPASSTKVDKKIEPRGPTIK	PD1- IL2_L_7A05scFv_ PD1-R07- A05_Pep1 ;Asymm etric DBA- Cytokine Complex IgG-scFv format

SEQ ID NO	Sequences	Description
	PCPPCKCPAPNAAGGPSVFIFPPKIKDVLMI SPIVTCVVVDVSEDDPDVQISWVNNVEVHT AQTQTHREDYNSTLRVVSALPIQHQDWMSG KEFKCKVNNKDLGAPIERTISKPKGSVRAPQV YVLPPEEEMTKKQVTLTCMVTDFMPEDIYV EWTNNGKTELNYKNTEPVLDSDGSYFMYSD LRVEKKNWVERNSYSCSVVHEGLHNHHTTE SFSRTPGK	
SEQ ID NO: 78	QVQLVQSGAEVKKPGASVKVSCKASGYTFTT YYVHWVRQAPGQGLEWMGIINPSGGSTSYA QNFQGRVTMTRDTSTSTVYMELSSLRSEDTA VYYCASGWDVWGQGTTVTVSSASGGGGSG GGGSGGGGSHASEIVMTQSPATLSVSPGERAT LSCRASQSVNTYLAWYQQKPGQAPRLLIYGA STRATGIPARFSGSGSGTEFTLTISLQSEDFAV YYCQQYGSSPVTFGQGTRLEIKPRGPTIKPCPP CKCPAPNAAGGPSVFIFPPKIKDVLMI SPIVTCVVVDVSEDDPDVQISWVNNVEVHTAQT QTHREDYNSTLRVVSALPIQHQDWMSGKEFK CKVNNKDLGAPIERTISKPKGSVRAPQVYVLP PPEKEMTKKQVSLTCLVKDFMPEDIYVEWTN NGKTELNYKNTEPVLKSDGSYFMYSKLTVEK KNWVERNSYSCSVVHEGLHNHHTTKSFSRTP GGGSGGGGSHHHHHH	PD1- IL2_L_7A05scFv_ PD1-R07- A05_Pep2 ;Asymm etric DBA- Cytokine Complex IgG-scFv format
SEQ ID NO: 79	APTSSSTKKTKQLQLEHLLLDLQMLNGINNYK NPKLTDMLTFKFYMPKKATELKHLCLEEL KPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFCQSII STLTGGGGSGGGGSGGGGSGGGGSEIVMTQS PATLSVSPGERATLSCRASQSVNTYLAWYQQ KPGQAPRLLIYGA STRATGIPARFSGSGSGTEF TLTISLQSEDFAVYYCQQYGSSPVTFGQGTR LEIKRADAAPTVSIFPPSSEQLTSGGASVVCFL NNFYPKDINVKWKIDGSRQNGVLNSWTDQ DSKDSTYSMSSTLTLTKEDEYERHNSYTCEAT HKTSTSPIVKSFNRENEC	PD1- IL2_L_7A05scFv_ PD1-R07- A05_Pep3 ;Asymm etric DBA- Cytokine Complex IgG-scFv format

TABLE 7 – Dual-Binding Antibodies (DBAs)

Dual-Binding Antibody	Marker	Therapeutic	HV* SEQ ID NO:	LV** SEQ ID NO:	HV_cdr1 SEQ ID NO:	HV_cdr2 SEQ ID NO:	HV_cdr3 SEQ ID NO:	LV_cdr1 SEQ ID NO:	LV_cdr2 SEQ ID NO:	LV_cdr3 SEQ ID NO:
AB001718	PD-1	IL-2	127	135	142	148	154	157	163	168
AB001744	PD-1	IL-2	128	136	143	148	154	158	163	169
AB002022	PD-1	IL-2	129	137	144	149	154	159	164	170

*HV refers to the heavy chain variable region of the respective antibodies

**LV refers to the light chain variable region of the respective antibodies

TABLE 8 – Sequences of DBA Protein Components

SEQ ID NO:	DBA Protein Component	Sequence
SEQ ID NO: 127	AB001718_HV	QVQLVQSGAEVKKPGASVKVSCKASG DTFSTYYVHWVRQAPGQGLEWMGIINP SGGGTVYAQKFQGRVTMTRDTSTSTVY MELSSLRSED TAVYYCAAGLFIWGQGT LVTVSS
SEQ ID NO: 128	AB001744_HV	QVQLVQSGAEVKKPGASVKVSCKASG YTFSNYYIHWVRQAPGQGLEWMGIINP SGGGTVYAQKFQGRVTMTRDTSTSTVY MELSSLRSED TAVYYCAAGLFIWGQGT LVTVSS
SEQ ID NO: 129	AB002022_HV	QVQLVQSGAEVKKPGASVKVSCKASG DTFTRHYVHWVRQAPGQGLEWMGIIN PSGGYASYAQKFQGRVTMTRDTSTSTV YMELSSLRSED TAVYYCAAGLFIWGQG TLVTVSS
SEQ ID NO: 135	AB001718_LV	DIQMTQSPSSLSASVGDRVTITCRASQYI SSGLAWYQQKPGKAPKLLIYKASSLDN GVPSRFSGSGSGTDFTLTISLQPEDFAT YYCQQYERLPLTFGGGTKVEIK
SEQ ID NO: 136	AB001744_LV	DIQMTQSPSSLSASVGDRVTITCRASQSI GTGLAWYQQKPGKAPKLLIYKASSLDN GVPSRFSGSGSGTDFTLTISLQPEDFAT YYCQQYNRAPLTFGGGTKVEIK
SEQ ID NO: 137	AB002022_LV	DIQMTQSPSSLSASVGDRVTITCRASQSI GRWLAWYQQKPGKAPKLLIYSASNLET GVPSRFSGSGSGTDFTLTISLQPEDFAT YYCQQYESFPVTFGPGTKVDIK
SEQ ID NO: 142	AB001718_HV_cdr1	GDTFSTYYVH
SEQ ID NO: 146	AB001843_HV_cdr1	GYTFSGYYIH
SEQ ID NO: 147	AB001866_HV_cdr1	GYTFSNYYVH
SEQ ID NO: 148	AB001718_HV_cdr2	IINPSGGGTVYAQKFQG
	AB001744_HV_cdr2	<u>IINPSGGGTVYAQKFQG</u>
SEQ ID NO: 149	AB002022_HV_cdr2	IINPSGGYASYAQKFQG
SEQ ID NO: 154	AB001718_HV_cdr3	AAGLFI
SEQ ID NO: 157	AB001718_LV_cdr1	RASQYISSGLA
SEQ ID NO: 158	AB001744_LV_cdr1	RASQSIGTGLA
SEQ ID NO: 159	AB002022_LV_cdr1	RASQSIGRWLA
SEQ ID NO: 160	AB001609_LV_cdr1	RASQSISNRLA
SEQ ID NO: 161	AB001638_LV_cdr1	QASQSISNYLA
SEQ ID NO: 162	AB001843_LV_cdr1	RASQSISSYLN
SEQ ID NO: 163	AB001718_LV_cdr2	KASSLDN
	AB001744_LV_cdr2	<u>KASSLDN</u>
SEQ ID NO: 164	AB002022_LV_cdr2	SASNLET
SEQ ID NO: 168	AB001718_LV_cdr3	QQYERLPL
SEQ ID NO: 169	AB001744_LV_cdr3	QQYNRAPL

SEQ ID NO:	DBA Protein Component	Sequence
SEQ ID NO: 170	AB002022_LV_cdr3	QQYESFPV

TABLE 9 – Exemplary DBA-Cytokine Protein Complexes

Name	DBA / Therapeutic	DBA	Type	DBA domains	Therapeutic domains	2nd Ab domain	Heavy Chain 1 SEQ ID NO:	Heavy Chain 2 SEQ ID NO:	Heavy Chain 3 SEQ ID NO:
AF003229	PD-1 / IL-2	AB001718	FIG. 2B	2	1	N/A	80	97	114
AF003230	PD-1 / IL-2	AB001744	FIG. 2B	2	1	N/A	81	98	115
AF003232	PD-1 / IL-2	AB002022	FIG. 2B	2	1	N/A	82	99	116
AF003250	PD-1 / IL-2	AB001718	FIG. 2A	1	1	anti-PD-1	83	100	117
AF003251	PD-1 / IL-2	AB001744	FIG. 2A	1	1	anti-PD-1	84	101	118
AF003253	PD-1 / IL-2	AB002022	FIG. 2A	1	1	anti-PD-1	85	102	119

TABLE 10 – Sequences of Peptides in TABLE 9

SEQ ID NO:	DBA	Sequence
SEQ ID NO: 80	AF003229_Pep1	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTDMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLTGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKP GASVKVSCASGDTFSTYYVHWVRQAPGQGLEWMGIINPS GGGTVYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDNAVY YCAAGLFIWGQGTLLTVSSAKTTAPSVYPLAPVCGDTTGSS VTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYT LSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKP CPPCKCPAPNAAGGPSVFIFPPKIKDVLMSLSPIVTCVVVDV SEDDPDVQISWVFVNNVEVHTAQTQTHREDYNSTLRVVSALP IQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQV YVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTE LNYKNTEPVLDSGGSYFMYSDLRVEKKNWVERNYSYSCSVV HEGLHNHHTTESFSRTPGK
SEQ ID NO: 81	AF003230_Pep1	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTDMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLTGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKP GASVKVSCASGYTFSNYYIHWVRQAPGQGLEWMGIINPS GGGTVYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDNAVY YCAAGLFIWGQGTLLTVSSAKTTAPSVYPLAPVCGDTTGSS VTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYT LSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKP CPPCKCPAPNAAGGPSVFIFPPKIKDVLMSLSPIVTCVVVDV SEDDPDVQISWVFVNNVEVHTAQTQTHREDYNSTLRVVSALP

SEQ ID NO:	DBA	Sequence
		IQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQV YVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTE LNYKNTEPVLDSDGSYFMYSDLRVEKKNWVERNSYSCSVV HEGLHNHHTTESFSRTPGK
SEQ ID NO: 82	AF003232_Pep1	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTDMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLTGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKP GASVKVSCKASGDTFTRHYVHWVRQAPGQGLEWMGIINPS GGYASYAQKFQGRVTMTRDTSTSTVYMESSLRSED TAVY YCAAGLFIWGQGT LVTVSSAKTTAPSVYPLAPVCGDTTGSS VTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYT LSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKP CPPCKCPAPNAAGGPSVFIFPPKIKDVL MISLSPIVTCVVVDV SEDDPDVQISW FVNNVEVHTAQTQTHREDYNSTLRVVSALP IQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQV YVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTE LNYKNTEPVLDSDGSYFMYSDLRVEKKNWVERNSYSCSVV HEGLHNHHTTESFSRTPGK
SEQ ID NO: 83	AF003250_Pep1	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTDMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLTGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKP GASVKVSCKASGDTFSTYYVHWVRQAPGQGLEWMGIINPS GGGTVY AQKFQGRVTMTRDTSTSTVYMESSLRSED TAVY YCAAGLFIWGQGT LVTVSSAKTTAPSVYPLAPVCGDTTGSS VTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYT LSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKP CPPCKCPAPNAAGGPSVFIFPPKIKDVL MISLSPIVTCVVVDV SEDDPDVQISW FVNNVEVHTAQTQTHREDYNSTLRVVSALP IQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQV YVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTE LNYKNTEPVLDSDGSYFMYSDLRVEKKNWVERNSYSCSVV HEGLHNHHTTESFSRTPGK
SEQ ID NO: 84	AF003251_Pep1	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTDMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLTGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKP GASVKVSCKASGYTFSNYIHWVRQAPGQGLEWMGIINPS GGGTVY AQKFQGRVTMTRDTSTSTVYMESSLRSED TAVY YCAAGLFIWGQGT LVTVSSAKTTAPSVYPLAPVCGDTTGSS VTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYT LSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKP CPPCKCPAPNAAGGPSVFIFPPKIKDVL MISLSPIVTCVVVDV SEDDPDVQISW FVNNVEVHTAQTQTHREDYNSTLRVVSALP IQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQV YVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTE LNYKNTEPVLDSDGSYFMYSDLRVEKKNWVERNSYSCSVV HEGLHNHHTTESFSRTPGK

SEQ ID NO:	DBA	Sequence
SEQ ID NO: 85	AF003253_Pep1	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTDMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLTGGGGSGGGGSGGGGSGGGGSGVQLVQSGAEVKKP GASVKVSCKASGDTFTRHYVHWVRQAPGQGLEWMGIINPS GGYASYAQKFQGRVTMTRDTSTSTVYMESSLRSEDNAVY YCAAGLFIWGQGTLVTVSSAKTTAPSVYPLAPVCGDTTGSS VTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYT LSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKP CPPCKCPAPNAAGGPSVFIFPPKIKDVLMSLSPIVTCVVVDV SEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSALP IQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQV YVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTE LNYKNTEPVLDSDGSYFMYSDLRVEKKNWVERNSYSCSVV HEGLHNHHTTESFSRTPGK

EXAMPLE 3

Reduced CD8⁺ T cell STAT5 phosphorylation by a PD-1/IL-2 Dual Binding Antibody (DBA) Cytokine Complexes

[0113] This example describes reduction of IL-2 mediated signaling by addition of PD-1/IL-2 DBA moieties to IL-2 molecules by fusion, as read out using CD8⁺ T-cell STAT5 phosphorylation. Genes for the PD-1/IL-2 DBAs shown in **TABLE 11** were synthesized and expressed in HEK293 as IgG proteins with IL-2 fused to the N-terminus of the heavy or light chain through a linker (Genscript). Although only two of the antibodies blocked IL-2 binding to IL-2RB as scFvs, over 30 of the antibodies were able to reduce IL-2 signaling by a linked IL-2 domain in formats as shown in **FIG. 2D** and **2E**. An exemplary set of these DBAs were chosen for analysis and compared to a control anti-HER2-IL-2 immunocytokine (**TABLE 11** and **FIG. 3**).

TABLE 11 – IgG PD-1/IL-2 DBA protein complexes

Name	Heavy Chain Sequence SEQ ID NO	Light Chain Sequence SEQ ID NO
Anti-HER2	(SEQ ID NO: 65) EVQLVESGGGLVQPGGSLRLSCAA SGFNIKDTYIHWVRQAPGKGLEWV ARIYPTNGYTRYADSVKGRFTISAD TSKNTAYLQMNSLRAEDTAVYYCS RWGGDGFYAMDYWGQGTLVTVSS AKTTAPSVYPLAPVCGDTTGSSVTL GCLVKGYFPEPVTLTWNSGSLSSG	(SEQ ID NO: 66) APTSSSTKKTQLQLEHLLLDLQ MILNGINNYKNPKLTRMLTFKF YMPKKATELKHLQCLEEELKPL EEVLNLAQSKNFHLRPRDLISNI NVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLTVP GVGVPGAGVPGVGVPGGGVPG

Name	Heavy Chain Sequence SEQ ID NO	Light Chain Sequence SEQ ID NO
	VHTFPAVLQSDLYTLSSSVTVTSST WPSQSITCNVAHPASSTKVDKKIEP RGPTIKPCPPCKCPAPNAAGGPSVFI FPPKIKDVLMSLSPIVTCVVVDVSE DDPDVQISWVFVNNVEVHTAQTQTH REDYNSTLRVVSALPIQHQQDWMSG KEFKCKVNNKDLGAPIERTISKPKG SVRAPQVYVLPPEEEMTKKQVTL TCMVTDFMPEDIYVEWTNNGKTEL NYKNTEPVLDSDGSYFMYSKLRVE KKNWVERNSYSCSVVHEGLHNHH TTKSFSRTPGK	VGVPGGGVPGAGVPGGGVPGV GVPAGVPGVGVPGGGDIQMT QSPSSLSASVGDRVTITCRASQD VNTAVAWYQQKPGKAPKLLIY SASFLYSGVPSRFSGSRSGTDFT LTISSLQPEDFATYYCQQHYTTP PTFGQGTKVEIKRADAAPTVSIF PPSSEQLTSGGASVVCFLNNFY KDINVKWKIDGSRQNGVLNS WTDQDSKDYSTYSMSSTLTLTKD EYERHNSYTCEATHKTSTSPIVK SFNRNEC
2-A08	(SEQ ID NO: 67) QVQLVQSGAEVKKPGASVKVSCK VSGYTFTSYDINWVRQAPGQGLEW MGWINPNSGDTGYAQKFQGRVTM TRDTSTSTVYMELSSLRSEDTAVYY CARDTGLGYYYGSGDFDYWGQGT LVTVSSAKTTAPSVYPLAPVCGD GSSVTLGCLVKGYFPEPVTLTWNS GSLSSGVHTFPAVLQSDLYTLSSSV TVTSSTWPSQSITCNVAHPASSTKV DKKIEPRGPTIKPCPPCKCPAPNAA GGPSVFIFFPKIKDVLMSLSPIVTCV VVDVSEDDPDVQISWVFVNNVEVHT AQTQTHREDYNSTLRVVSALPIQH QDWMSGKEFKCKVNNKDLGAPIE RTISKPKGSVRAPQVYVLPPEEEM TKKQVTLTCMVTDFMPEDIYVEWT NNGKTELNYKNTEPVLDSDGSYFM YSKLRVEKKNWVERNSYSCSVVHE GLHNHHTTKSFSRTPGK	(SEQ ID NO: 68) APTSSSTKKTQLQLEHLLLDLQ MILNGINNYKNPKLTRMLTFKF YMPKKATELKHLCLEELKPL EEVLNLAQSKNFHLRPRDLISNI NVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLTVP GVGVPGAGVPGVGVPGGGVPGV VGVPGGGVPGAGVPGGGVPGV GVPAGVPGVGVPGGGDIQMT QSPSSLSASVGDRVTITCQASQD IHNYLNWYQQKPGKAPKLLIYD VSNLETGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQAISFPLT FGGGTKVEIKRADAAPTVSIFPP SSEQLTSGGASVVCFLNNFY DINVKWKIDGSRQNGVLNSW TDQDSKDYSTYSMSSTLTLTKDE YERHNSYTCEATHKTSTSPIVKS FNRNEC
2-A11	(SEQ ID NO: 69) QVQLVQSGAEVKKPGASVKVSCK ASGHTFTRYMHWRQAPGQGLEW WMGIINPSGGYATYAQKFQGRVTM TRDTSTSTVYMELSSLRSEDTAVYY CASGWDVWGQGT LVTVSSAKTTA PSVYPLAPVCGD TTGSSVTLGCLVK GYFPEPVTLTWNSGSLSSGVHTFPA VLQSDLYTLSSSVTVTSSTWPSQSIT CNVAHPASSTKVDKKIEPRGPTIKP CPPCKCPAPNAAGGPSVFIFFPKIKD VLMISLSPIVTCVVVDVSEDDPDVQ ISWVFVNNVEVHTAQTQTHREDYNS TLRVVSALPIQHQQDWMSGKEFKCK VNNKDLGAPIERTISKPKGSVRAPQ	(SEQ ID NO: 70) APTSSSTKKTQLQLEHLLLDLQ MILNGINNYKNPKLTRMLTFKF YMPKKATELKHLCLEELKPL EEVLNLAQSKNFHLRPRDLISNI NVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLTVP GVGVPGAGVPGVGVPGGGVPGV VGVPGGGVPGAGVPGGGVPGV GVPAGVPGVGVPGGGDIQMT QSPSSLSASVGDRVTITCRASQSI NSWLAWYQQKPGKAPKLLIYA TSTLESVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQSYSFPP TFGQGTKVEIKRADAAPTVSIFP

Name	Heavy Chain Sequence SEQ ID NO	Light Chain Sequence SEQ ID NO
	VYVLPPPEEEMTKKQVTLTCMVTD FMPEDIYVEWTNNGKTELNYKNTE PVLDSGGSYFMYSKLRVEKKNWV ERNYSYSCSVVHEGLHNHHTTKSFSR TPGK	PSSEQLTSGGASVVCFLNMFYPK DINVKWKIDGSRQNGVLNSW TDQDSKDYSTYSMSSTLTLTKDE YERHNSYTCEATHKTSTSPIVKS FNRNEC
2-B05	(SEQ ID NO: 71) QVQLVQSGAEVKKPGASVKVSCK ASGYTFTNYYIHWVRQAPGQGLE WMGIINPRAGYTSYALKFQGRVTM TRDTSTSTVYMELSSLRSEDVAVYY CAGGWLWDWGQGTLLTVSSAKTTA PSVYPLAPVCGDITGSSVTLGCLVK GYFPEPVTLTWNSGSLSSGVHTFPA VLQSDLYTLSSSVTVTSSTWPSQSIT CNVAHPASSTKVDKKIEPRGPTIKP CPPCKCPAPNAAGGPSVFIFPPKIKD VLMISLSPIVTCVVVDVSEDDPDVQ ISWVFNNEVHTAQTQTHREDYNS TLRVVSALPIQHQQDWMSGKEFKCK VNNKDLGAPIERTISKPKGSVRAPQ VYVLPPPEEEMTKKQVTLTCMVTD FMPEDIYVEWTNNGKTELNYKNTE PVLDSGGSYFMYSKLRVEKKNWV ERNYSYSCSVVHEGLHNHHTTKSFSR TPGK	(SEQ ID NO: 72) APTSSSTKKTQLQLEHLLLDLQ MILNGINNYKNPKLTRMLTFKF YMPKKATELKHLQCLEEELKPL EEVLNLAQSKNFHLRPRDLISNI NVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLTVP GVGVPGAGVPGVGVPGGGVPG VGVPGGGVPGAGVPGGGVPGV GVPGAGVPGVGVPGGGDIQMT QSPSSLSASVGDRVTITCRASQSI SSWLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQSFTMPI TFGQGRLEIKRADAAPTVSIFP PSSEQLTSGGASVVCFLNMFYPK DINVKWKIDGSRQNGVLNSW TDQDSKDYSTYSMSSTLTLTKDE YERHNSYTCEATHKTSTSPIVKS FNRNEC
2-B07	(SEQ ID NO: 73) QVQLVQSGAEVKKPGASVKVSCK ASGDTFTRHYVHWVRQAPGQGLE WMGIINPSGGYASYAQKFQGRVTM TRDTSTSTVYMELSSLRSEDVAVYY CAAGLFIWGQGTLLTVSSAKTTAPS VYPLAPVCGDITGSSVTLGCLVKG YFPEPVTLTWNSGSLSSGVHTFPAV LQSDLYTLSSSVTVTSSTWPSQSITC NVAHPASSTKVDKKIEPRGPTIKPC PPCKCPAPNAAGGPSVFIFPPKIKDV LMISLSPIVTCVVVDVSEDDPDVQIS WVFNNEVHTAQTQTHREDYNST LRVVSALPIQHQQDWMSGKEFKCKV NNKDLGAPIERTISKPKGSVRAPQV YVLPPPEEEMTKKQVTLTCMVTD FMPEDIYVEWTNNGKTELNYKNTEP VLDSGGSYFMYSKLRVEKKNWVE RNSYSCSVVHEGLHNHHTTKSFSRT PGK	(SEQ ID NO: 74) APTSSSTKKTQLQLEHLLLDLQ MILNGINNYKNPKLTRMLTFKF YMPKKATELKHLQCLEEELKPL EEVLNLAQSKNFHLRPRDLISNI NVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLTVP GVGVPGAGVPGVGVPGGGVPG VGVPGGGVPGAGVPGGGVPGV GVPGAGVPGVGVPGGGDIQMT QSPSSLSASVGDRVTITCRASQSI GRWLAWYQQKPGKAPKLLIYS ASNLETGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQANSFPV TFGPGTKVDIKRADAAPTVSIFP PSSEQLTSGGASVVCFLNMFYPK DINVKWKIDGSRQNGVLNSW TDQDSKDYSTYSMSSTLTLTKDE YERHNSYTCEATHKTSTSPIVKS FNRNEC
7-A04	(SEQ ID NO: 75)	(SEQ ID NO: 76)

Name	Heavy Chain Sequence SEQ ID NO	Light Chain Sequence SEQ ID NO
	QVQLVQSGAEVKKPGASVKVSCK ASGYTFTDYMHWRQAPGQGLE WMGIINPRAGYTSYALKFQGRVTM TRDTSTSTVYMELSSLRSEDTAVYY CTSGMDVWGQGTLLTVSSAKTTAP SVYPLAPVCGDTTGSSVTLGCLVK GYFPEPVTLTWNSGSLSSGVHTFPA VLQSDLYTLSSSVTVTSSTWPSQSIT CNVAHPASSTKVDKKIEPRGPTIKP CPPCKCPAPNAAGGPSVFIFPPKIKD VLMISLSPIVTCVVVDVSEDDPDVQ ISWVFNVEVHTAQTQTHREDYNS TLRVVSALPIQHQQDWMSGKEFKCK VNNKDLGAPIERTISKPKGSVRAPQ VYVLPPEEEMTKKQVTLTCMVTD FMPEDIYVEWTNNGKTELNYKNT PVLDSGYSYFMYSKLRVEKKNWV ERNSYSCSVVHEGLHNHHTTKSFSR TPGK	APTSSSTKKTQLQLEHLLLDLQ MILNGINNYKNPKLTRMLTFKF YMPKKATELKHLCLEELKPL EEVLNLAQSKNFHLRPRDLISNI NVIVLELKGSETTFMCEYADET ATIVEFLNRWITFCQSIISTLTVP GVGVPGAGVPGVGVPGGGVPG VGVPGGGVPGAGVPGGGVPGV GVPGAGVPGVGVPGGGDIQMT QSPSSLSASVGDRVTITCRASQSI STWLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTL TISSLPEDFATYYCQQSYSFPV TFGQGTKVEIKRADAAPTVSIFP PSSEQLTSGGASVVCFLNFPYK DINVKWKIDGSERQNGVLNSW TDQDSKDYSTYSMSSTLTTLTKDE YERHNSYTCEATHKTSTSPIVKS FNRNEC

[0114] The PD-1/IL-2 DBA-cytokine complexes were serially diluted in complete RPMI (+10% FBS, 2 mM L-glutamine, sodium pyruvate) and added to a 96-well plate. 2×10^5 human PBMCs were added to each well and plates were incubated at 37 °C for 20 minutes. An equal volume prewarmed fixation buffer (Biolegend) was then added to each well and plates were incubated at 37 °C for 10 minutes. Cells were then fixed in pre-chilled Perm Buffer III (BD Biosciences) for 30 minutes at 4 °C. Cells were washed with FACS wash buffer (PBS +2% FBS, 2 mM EDTA) and stained with fluorophore labeled antibodies directed against CD3, CD4, CD8, (BioLegend) and phospho-STAT5 (BD Biosciences) diluted 1:20 in FACS wash buffer. Cells were incubated 1 hour at 4 °C, washed with FACS wash buffer, and analyzed on a SA3800 Spectral Analyzer. In the absence of PD-1, the PD-1/IL-2 DBA/cytokine complexes induced less STAT5 phosphorylation in T cells compared to the monospecific control anti-HER2 IL-2 immunocytokine (**FIG. 3**).

EXAMPLE 4

General Method: Production of Complexes to Drive PD-1 Dependent IL-2 Activity In Human Cells

[0115] This example describes PD-1/IL-2 protein complexes for PD-1 dependent IL-2 activity in human cells, *in vitro* and *in vivo*. PD-1/IL-2 protein complexes comprise a PD-1 sensor domain (e.g., an anti-PD-1 antibody or an anti-PD-1 scFv) linked to an IL-2 cytokine therapeutic domain

via a linker, where the IL-2 cytokine is a therapeutic. In the absence of PD-1, the PD-1 sensor domain binds the IL-2 therapeutic domain, rendering the IL-2 therapeutic inert. In the presence of PD-1 (e.g., PD-1 is expressed on a cell, such as an immune cell), the PD-1 sensor domain binds PD-1, thereby unbinding the IL-2 therapeutic domain and allowing for IL-2 to exhibit therapeutic activity.

[0116] PD-1/IL-2 protein complexes are recombinantly expressed or chemically synthesized. PD-1/IL-2 protein complexes are administered *in vitro* to a human cell or *in vivo* to a mouse or to a human subject in need thereof. The human cell is a cell expressing PD-1. Administration to a mouse or to a human subject is performed intravenously, intramuscularly, subcutaneously, intradermally, intraperitoneally, or mucosally. In the absence of PD-1, the IL-2 therapeutic domain remains bound to the PD-1 sensor domain and no therapeutic efficacy is observed (e.g., cell activation *in vitro* and in the subject is unaltered). In the presence of PD-1, the PD-1 sensor domain binds PD-1 and unbinds the IL-2 therapeutic domain. Therapeutic efficacy is observed (e.g., cell activation is observed *in vitro* and, in the subject, *in vivo*). The subject has a disease. The disease is cancer. The cell may express PD-1 endogenously or after activation, or following introduction of a gene encoding PD-1. The therapeutic effect may be cell growth, differentiation, activation or induction of IL2-responsive genes. *In vitro*, if the cell is part of a mixture of cell types, any of these changes may be monitored for a responding cell population in the mixture.

EXAMPLE 5

PD-1/IL-2 DBA Cytokine Complex Induction of STAT5 Phosphorylation in a Lymphocyte Cell Line

[0117] This example describes PD-1/IL-2 DBA-cytokine complex induction of STAT5 phosphorylation in a lymphocytic cell line. To assess the dependence of PD-1/IL-2 DBA-cytokine complex activity on binding to PD-1, a PD-1-expressing variant is generated of an IL-2R⁺ T cell line such as Hut78 or Jurkat E6.1. The PD-1⁺ and PD-1⁻ variant cell lines are treated with titrating concentrations of a PD-1/IL-2 DBA-cytokine complex of this disclosure, and STAT5 phosphorylation is assessed by phospho-flow, TR-FRET, or other assays for measuring IL-2 signaling.

[0118] A HEK 293 IL-2 reporter cell line is engineered to express PD-1. The PD-1⁺ and PD-1⁻ variant cell lines are treated with titrating concentrations of PD-1/IL-2 DBA-cytokine complexes, and reporter activity is assessed as a measurement of IL-2 signaling. The PD-1/IL-2 DBA-cytokine complex exhibits increased potency on PD-1⁺ variant cell lines.

EXAMPLE 6

PD-1/IL-2 DBA Cytokine Complex Induction of STAT5 Phosphorylation and Other Markers of Activation, and Proliferation in Primary Lymphocytes

[0119] This example describes PD-1/IL-2 DBA-cytokine complex induction of STAT5 phosphorylation and other markers of activation and proliferation in primary lymphocytes. PBMCs are labeled with cell proliferation dye and incubated for 4 days with titrating concentrations of a PD-1/IL-2 DBA-cytokine complex of the present disclosure. PBMCs are stained with antibodies directed against immune cell phenotyping markers to distinguish CD4+ and CD8+ T cells, Treg cells, and natural killer (NK) cells and markers of cell activation, such as CD25. Dye dilution on immune cell subsets is examined by flow cytometry as a measurement of proliferation.

[0120] Total T cells are isolated from PBMCs using immunomagnetic negative selection (STEMCELL) and stimulated with plate-bound anti-CD3 and soluble anti-CD28 for 72 hours to induce expression of PD-1. The PD-1+ T cells are incubated for 20 minutes with titrating concentrations of PD-1/IL-2 DBA-cytokine complexes. STAT5 phosphorylation is measured in fixed and permeabilized T cells by flow cytometry. In some experiments, PD-1 may be blocked on T cells with anti-PD-1 prior to treatment with PD-1/IL-2 DBA-cytokine complexes to assess the dependence of PD-1/IL-2 DBA-cytokine complex activity on binding to PD-1. The PD-1/IL-2 DBA-cytokine complex induces minimal STAT5 phosphorylation when PD-1 is blocked, showing activity that is conditional on its ability to bind PD-1.

EXAMPLE 7

***In Vivo* PD-1/IL-2 DBA Cytokine Complex Signaling in Non-Tumor Peripheral Tissues**

[0121] This example describes PD-1/IL-2 DBA-cytokine complex pharmacokinetics in the blood of wild-type mice and the signaling of the complex in non-tumor peripheral tissue. The serum half-lives and peripheral tissue activities of PD-1/IL-2 DBA-cytokine complexes and suitable non-regulated controls such as anti-PD-1, anti-HER2-IL-2, or anti-PD-1-IL-2 were measured in mice dosed intravenously (i.v.) with the complexes. Blood, spleens, or both were collected at various timepoints after treatment and stained to identify CD8+ T cells and NK cells.

[0122] To examine the half-life of PD-1/IL-2 DBA-cytokine complex in circulation, wild-type C57BL/6 mice received a single 2.5 milligrams per kilogram intravenous dose of a PD-1/IL-2 DBA-cytokine complex (2B07 IL-2 mut; SEQ ID NO: 205-206), anti-HER2/IL-2-cytokine complex (Always-on IL-2 mut; SEQ ID NO: 64 and SEQ ID NO: 207), or anti-IL-2/IL-2-cytokine complex (Always-off IL-2 mut; ; SEQ ID SEQ ID NO: 208-209), as outlined in

TABLE 13. Mice were bled via retro-orbital sinus at 30 minutes, 4, 24, 48, 72, 96, and 168 hours post-dosing. The blood was collected into serum separator tubes, and the isolated serum was frozen at -80°C until analysis. To determine serum levels of the cytokine complexes, 96-well high-binding ELISA plates were coated with 1 µg/mL rabbit anti-hu IL-2 capture antibody (clone ab9618, Abcam) in carbonate-bicarbonate buffer overnight at 4°C. Plates were washed three times and blocked for 1 hour with SuperBlock blocking buffer (Thermo Scientific). Serum samples from the various timepoints and treatment groups were diluted in SuperBlock, added to the plates, and incubated 1 hr. To detect cytokine complexes, plates were incubated with goat anti-mouse Fc-HRP (Jackson ImmunoResearch) at 1:5000 in SuperBlock for 1 hour. The plates were then washed and developed with TMB substrate. Absorbance (OD) was measured using an EnVision 2105 microplate reader (PerkinElmer) at 450 nm. As shown in **FIG. 6**, at all timepoints examined the PD-1/IL-2 DBA-cytokine complex was detected at similar serum concentrations as the anti-IL-2/IL-2-cytokine complex. In contrast, the serum concentration of the non-regulated anti-HER2/IL-2-cytokine complex showed a greater decrease in serum concentration over time.

TABLE 12 – IgG PD-1/IL-2 DBA and control protein complexes

Protein Complexes	SEQ ID NO:	Sequence
2B07 IL-2 mut	SEQ ID NO: 205	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFAMPKK ATELKHLQCLEELKPLEEVLNGAQSKNFHLRPRDLISININIVLELKGSET TFMCEYADETATIVEFLNRWITFAQSIISTLTGGGGSGGGSGGGSGGG GGSQVQLVQSGAEVKKPGASVKVSCKASGDTFTRHYVHWVRQAPGQG LEWMGIINPSGGYASYAQKFQGRVTMTTRDTSTSTVYMELSSLRSEDTAV YYCAAGLFIWGQGTLTVTSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVK GYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSIT CNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKD VLMISLSPIVTCVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNS TLRVVSALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQ VYVLPPEEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNT PVLDSGGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRT PGK
	SEQ ID NO: 206	DIQMTQSPSSLSASVGDRVTITCRASQSIGRWLAWYQQKPGKAPKLLIYS ASNLETGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYESFPVTFGPG TKVDIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNFFPKDINVKWKID GSERQNGVLNSWTDQDSKDYSTMSSTLTLTKDEYERHNSYTCEATHKT STSPIVKSFNRENEC
anti-HER2/IL-2-cytokine complex	SEQ ID NO: 64	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIY SASFLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQ GTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNFFPKDINVKWKID GSERQNGVLNSWTDQDSKDYSTMSSTLTLTKDEYERHNSYTCEATHKT STSPIVKSFNRENEC

Protein Complexes	SEQ ID NO:	Sequence
	SEQ ID NO: 207	APASSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTAKFAMPKK ATELKHLQCLEEELKPLEEVLNGAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFAQSIISTLTGGGGSGGGSGGGSGGG GGSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLE WVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRWGGDGFYAMDYWGQGTLLTVSSAKTTAPSVYPLAPVCGDTTGSS VTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSS TWPSQSITCNVAHPASSTKVDKKIEPRGPTIKCPPCKCPAPNAAGGPSV FIFPPKIKDVLMLSLPIVTCVVDVSEDDPDVQISWVFNNEVHTAQTQ THREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTISKP KGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKT ELNYKNTEPVLDSGYSFMYSKLRVEKKNWVERNSYSCSVVHEGLHNH HTTKSFSRTPGK
Always-off IL-2 mut	SEQ ID NO: 208	APASSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTAKFAMPKK ATELKHLQCLEEELKPLEEVLNGAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFAQSIISTLTGGGGSGGGSGGGSGGG GGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSYTLAWVRQAPGKGLE WVAADSSSYTSPDVRGRFTISRDNKNSLYLQMNSLRAEDTAVYYCA RDSNWDALDYWGQGTLLTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGC LVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPS QSITCNVAHPASSTKVDKKIEPRGPTIKCPPCKCPAPNAAGGPSVFIFPP KIKDVLMLSLPIVTCVVDVSEDDPDVQISWVFNNEVHTAQTQTHRE DYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSV RAPQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTELNY KNTEPVLDSGYSFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKS FSRTPGK
	SEQ ID NO: 209	DIQMTQSPSSLSASVGDRVSITCKASQNVGTNVGWYQQKPGKAPKALY SASFRYSGVPSRFSGSGSGTDFTLTISLQPEDFATYFCQQYYTYPTFGG GTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKID GSERQNGVLNSWTDQDSKDYSTMSSTLTLTDEYERHNSYTCEATHKT STSPIVKSFNRENEC

[0123] To examine the activity of PD-1/IL-2 DBA-cytokine complexes in peripheral tissues, wild-type C57BL/6 mice received a single 2.5 milligrams per kilogram intravenous dose of PD-1/IL-2 DBA-cytokine complex (2B07 IL-2 mut; SEQ ID NO: 205-206), anti-HER2/IL-2-cytokine complex (Always-on IL-2 mut; SEQ ID NO: 64 and SEQ ID NO: 207), anti-IL-2/IL-2-cytokine complex (Always-off IL-2 mut; SEQ ID NO: 208-209), as shown in **TABLE 12** or PBS. Prior to dosing, the presence of intact IL-2 within each IL-2 cytokine complex was confirmed by ELISA as a means of verifying their potential for biological activity. Blood and spleens were collected 5 days following treatment and analyzed by flow cytometry to quantify the number of CD8⁺ T cells and NK cells per spleen and per microliter of blood. The PD-1/IL-2 DBA-cytokine complex did not induce expansion of CD8 T cells or NK cells, whereas the

HER2/IL-2-cytokine complex induced expansion of peripheral CD8⁺ T cells and NK cells (**FIG. 7A-D**).

EXAMPLE 8

PD-1/IL-2 DBA Cytokine Complex Modulation of Anti-Tumor Immunity in Syngeneic Tumor Models

[0124] This example describes PD-1/IL-2 DBA-cytokine complex modulation of anti-tumor immunity in a MC38 syngeneic mouse tumor model. A PD-1/IL-2 DBA-cytokine complex was assessed for the ability to drive anti-tumor immunity in vivo. 500,000 MC38 tumor cells were implanted subcutaneously in human PD-1 knock-in mice (GenOway). Tumors were measured twice weekly, and volumes calculated as $(\text{Length} \times \text{Width} \times \text{Width}/2)$. Mice were randomized into treatment groups, and treatments were initiated when tumors reached a volume of $\sim 100 \text{ mm}^3$. Mice were treated intravenously with PD-1/IL-2 DBA-cytokine complex (2B07 IL-2 mut; SEQ ID NO: 210-212), PD-1/IL-2 DBA lacking IL-2 (2B07; SEQ ID NO: 212-213), or an isotype control (SEQ ID NO: 214-215), as shown in **TABLE 13** below, at the indicated doses of 5 or 0.5 milligrams per kilogram on days 7, 10, and 13 post tumor implantation. The PD-1/IL-2 DBA-cytokine complex showed increased tumor growth inhibition compared to either the PD-1/IL-2 DBA lacking IL-2 or the isotype control (**FIG. 8**).

TABLE 13 – IgG PD-1/IL-2 DBA and control protein complexes

Protein Complex	SEQ ID NO:	Sequence
2B07 IL-2 mut	SEQ ID NO: 210	APASSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTAKFAMPKK ATELKHLQCLEELKPLEEVLNGAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFAQSIISTLTGGGGSGGGSGGGSGGG GGSQVQLVQSGAEVKKPGASVKVSCKASGDTFTRYVHVWRQAPGQG LEWMGIINPSGGYASYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAV YYCAAGLFIWGQGTLLTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVK GYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSIT CNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKD VLMISLSPIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNS TLRVVSALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQ VYVLPPEEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTE PVLDSGGSYFMYSDLRVEKKNWVERNSYSCSVVHEGLHNHHTTESFSRT PGK
	SEQ ID NO: 211	QVQLVQSGAEVKKPGASVKVSCKASGDTFTRYVHVWRQAPGQGLEW MGIINPSGGYASYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYC AAGLFIWGQGTLLTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYF PEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNV AHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLM ISLSPIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLR

Protein Complex	SEQ ID NO:	Sequence
		VVSALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTISKPGSVRAPQVYV LPPPEKEMTKKQVSLTCLVKDFMPEDIYVEWTNNGKTELNYKNTEPVLK SDGSYFMYSKLTVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK
	SEQ ID NO: 212	DIQMTQSPSSLSASVGDRVTITCRASQSIGRYLAWYQQKPGKAPKLLIYS ASNLETGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYNSFPVTFGPG TKVDIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNMFYPKDINVKWKID GSERQNGVLNSWTDQDSKDYSTMSSTLTITKDEYERHNSYTCEATHKT STSPIVKSFNRECE
PD-1/IL-2 DBA lacking IL-2	SEQ ID NO: 212	DIQMTQSPSSLSASVGDRVTITCRASQSIGRYLAWYQQKPGKAPKLLIYS ASNLETGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYNSFPVTFGPG TKVDIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNMFYPKDINVKWKID GSERQNGVLNSWTDQDSKDYSTMSSTLTITKDEYERHNSYTCEATHKT STSPIVKSFNRECE
	SEQ ID NO: 213	QVQLVQSGAEVKKPGASVKVSCKASGDTFTRYVHWVRQAPGQGLEWV MGIINPSGGYASYAQKFQGRVTMTSDTSTVYMELSSLRSEDTAVYYC AAGLFIWGQGTLLTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYF PEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNV AHPASSTKVDKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMI ISLSPITCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLR VVSALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTISKPGSVRAPQVYV LPPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTELNYKNTEPVL DSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK
isotype control	SEQ ID NO: 214	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWV ARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRSEDTAVYYCSR WGGDGFYAMDYWGQGTLLTVSSAKTTAPSVYPLAPVCGDTTGSSVTL GCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTW PSQSITCNVAHPASSTKVDKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIF PPKIKDVLMIISLSPITCVVVDVSEDDPDVQISWVFNNEVHTAQTQTH REDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTISKPG SVRAPQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTEL NYKNTEPVLDSGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHT TKSFSRTPGK
	SEQ ID NO: 215	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIY SASFLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQ GTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNMFYPKDINVKWKID GSERQNGVLNSWTDQDSKDYSTMSSTLTITKDEYERHNSYTCEATHKT STSPIVKSFNRECE

EXAMPLE 9**PD-1/IL-2 DBA Cytokine Complex Modulation of Anti-Tumor Immunity in Xenograft/Human Immune Cell Admixture Models**

[0125] This example describes PD-1/IL-2 DBA-cytokine complex modulation of anti-tumor immunity in xenograft/human immune cell admixture models. To examine the ability of PD-1/IL-2 DBA-cytokine complexes to drive anti-tumor immunity in an *in vivo* setting, an admixture system is used. Total human PBMCs or a combination of human T cells and monocyte-derived dendritic cells (moDCs) are mixed with human tumor cells (e.g., HPAC, A375, H441) at a 1:4 ratio and co-implanted subcutaneously into the flanks of NSG mice. One day later, treatment with a PD-1/IL-2 DBA-cytokine complex of the present disclosure, or suitable non-regulated controls such as anti-PD-1, anti-HER2-IL-2, or anti-PD-1-IL-2, is initiated. Tumors are measured at least twice weekly and volumes calculated as $(\text{Length} \times \text{Width} \times \text{Height})/2$. PD-1/IL-2 DBA-cytokine complexes exhibit increased anti-tumor efficacy compared to anti-PD-1 and anti-HER2-IL-2 and decreased off-tumor activity compared to anti-PD-1-IL-2.

[0126]

[0127]

EXAMPLE 10***General Method: In Vitro and In Vivo Characterization of Protein Complexes***

[0128] This example describes the evaluation of DBA-cytokine complexes for *in vitro* and *in vivo* stability. A protein complex of the present disclosure is recombinantly expressed or chemically synthesized. The protein complex includes a sensor domain linked to a therapeutic domain. The linker is a peptide linker. The sensor domain is capable of binding to the therapeutic domain and a marker. In the absence of the marker, the sensor domain binds the therapeutic domain rendering the therapeutic domain unable to bind to its target and unable to exert therapeutic activity. In the presence of the marker, the sensor domain binds the marker rendering the therapeutic domain free to bind to its target and able to exert therapeutic activity.

[0129] *In vitro*, the protein complexes are tested for stability and functionality at baseline or after incubation in conditions of stress, such as elevated temperature, pH changes, oxidative buffers, or serum/plasma, using methods of biophysical characterization to measure fragmentation, unfolding, or aggregation, and/or using methods to test for changes in functional activity. *In vivo*, the pharmacokinetic properties of the proteins are measured following dosing in a mammal, such as a mouse, rat, or non-human primate, and properties of distribution, clearance and degradation

are measured. These measurements are used to engineer or select the optimal therapeutic form of the DBA-protein complex.

EXAMPLE 11

Regulated IL-2 Receptor Signaling by a PD-1/IL-2 Dual Binding Antibody (DBA) Cytokine Complex

[0130] This example describes PD-1 regulated IL-2 activity in a HEK-Blue™ IL-2 reporter cell by PD-1/IL-2 DBA-cytokine complexes. The DBA-cytokine complexes and control antibody-cytokine complexes were produced in three formats shown in **FIGS. 2E, 2B AND 2H** by expression in mammalian cells using standard protocols. The wells of a 384-well ELISA plate were coated with constant concentration of PD-1-Fc or an IgG1 control protein captured with an anti-Fc antibody (Jackson ImmunoResearch, Prod. # 109-005-098). The cytokine complexes were serially diluted 1:4 for 8 points in growth media from a starting concentration of 6 nM and incubated briefly before addition of the HEK-Blue™ IL-2 reporter cells.

[0131] Results with a protein complexes comprising the structure shown in **FIG. 2E** are shown in **FIG. 9A-D**. As depicted in **FIG. 2E**, this symmetric format is comprised of one IL-2 linked to each antibody variable domain. The IL-2 activity of the PD-1/IL-2 DBA-IL-2 complex AF4379 comprising SEQ ID NO: 174-175 had an EC50 of 31 pM in the PD-1 coated wells versus 62 pM in the IgG1 coated wells, as shown in **FIG. 9A**, demonstrating PD-1 dependence. The IL-2 activity of antibody-cytokine complexes AF4377 comprising SEQ ID NO: 64 and 176 (anti-Her2 antibody) and AF4378 comprising SEQ ID NO: 177-178 (anti-IL-2 antibody) was unchanged in the presence of PD-1 (as shown in **FIG. 9B** and **FIG. 9C**, respectively), while the IL-2 activity of the anti-PD-1 antibody AF4376 comprising SEQ ID NO: 179-180 is reduced in the presence of PD-1, as shown in **FIG. 9D**. Sequences of the protein complexes are summarized in **TABLE 14** below.

TABLE 14 – IgG PD-1/IL-2 DBA with heavy chain IL-2 therapeutic domains, and control protein complexes

Protein Complex	SEQ ID NO:	Sequence
AF4379	SEQ ID NO: 174	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTDMLT FEFYMPKKATELKHLQCLERELKPLEEVNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTGGGGSGGGGSGGGGSGGGGSSQVQLVQSGAE VKKPGASVKVSKASGDTFTRYVHWVRQAPGQGLEWM GIINPSGGYASYAQKFQGRVTMTRDTSTSTVYMELSSLRSE DTAVYYCAAGLFIWGQGLTVTVSSAKTTAPSVYPLAPVCG DTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVL QSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEP RGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMSLSPIV

Protein Complex	SEQ ID NO:	Sequence
		TCVVVDVSEDDPDVQISWVNNVEVHTAQTQTHREDYNS TLRVVSALPIQHQDWMMSGKEFKCKVNNKDLGAPIERTISK PKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIY VEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKN WVERNSYSCSVVHEGLHNHHTTKSFSRTPGK
	SEQ ID NO: 175	DIQMTQSPSSLSASVGDRVTITCRASQSIGRYLAWYQQKPG KAPKLLIYSASNLETGVPSRFSGSGSGTDFTLTISLQPEDFA TYYCQQYNSEFPVTFGPGTKVDIKRADAAPTVSIFPPSSEQLT SGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTD QDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIV KSFNRNEC
AF4377	SEQ ID NO: 64	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKP GKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISLQPEDF ATYYCQQHYTTPPTFGQGTKVEIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWT DQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRENEC
	SEQ ID NO: 176	APTSSSTKKTKLQLEHLLDLQMILNGINNYKNPKLTDMLT FEFYMPKKATELKHLQCLERELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTGGGGSGGGGSGGGGSGGGGSEVQLVESGGGL VQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARI YPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDT AVYYCSRWGGDGFYAMDYWGQGTLLTVSSAKTTAPSVY PLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGV HTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTK VDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVL MISLSPIVTCVVVDVSEDDPDVQISWVNNVEVHTAQTQT HREDYNSTLRVVSALPIQHQDWMMSGKEFKCKVNNKDLGA PIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDF MPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKL RVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK
AF4378	SEQ ID NO: 177	APTSSSTKKTKLQLEHLLDLQMILNGINNYKNPKLTDMLT FEFYMPKKATELKHLQCLERELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTGGGGSGGGGSGGGGSGGGGSEVQLVESGGGL VKPGGSLRLSCAASGFTFSSYTLAWVRQAPGKGLEWVAI DSSSYTYSPTVVRGRFTISRDNANKSLYLQMNSLRAEDTA VYYCARDSDNWDALDYWGQGTLLTVSSAKTTAPSVYPLAP VCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFP AVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDK KIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVL MISLSPIVTCVVVDVSEDDPDVQISWVNNVEVHTAQTQTHRED YNSTLRVVSALPIQHQDWMMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPE DIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEK KNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK
	SEQ ID NO: 178	DIQMTQSPSSLSASVGDRVSITCKASQNVGTNVGWYQQKP GKAPKALIIYSASFRYSGVPSRFSGSGSGTDFTLTISLQPED

Protein Complex	SEQ ID NO:	Sequence
		FATYFCQQYYTYPTYTFGGGKLEIKRADAAPTVSIFPPSSE QLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNS WTDQDSKDYSTYSMSSTLTLTKDEYERHNSYTCEATHKTST SPIVKSFNRECE
AF4376	SEQ ID NO: 179	APTSSSTKKTKLQLEHLLDLQMLNGINNYKNPKLTDMLT FEFYMPKKATELKHLQCLERELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTGGGGSGGGGSGGGGSGGGGSGVQLVESGGG VVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVA VIWYDGSKRYYADSVKGRFTISRDNKNTLFLQMNSLRAE DTAVYYCATNDDYWGGTLVTVSSAKTTAPSVYPLAPVC GDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPA VLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMLSP IVTCVVDVSEDDPDVQISWVFNVEVHTAQTQTHREDY NSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTI SKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPED IYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKK NWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK
	SEQ ID NO: 180	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPG QAPRLLIYDASNRAITGIPARFSGSGSGTDFTLTISSELPEDFA VYYCQQSSNWPRTFGQGTKVEIKRADAAPTVSIFPPSSEQL TSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWT DQDSKDYSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRECE

[0132] Results with protein complexes comprising the structures depicted in **FIG. 2B**, are shown in **FIGS. 4A-F**. This format is composed of an asymmetric complex comprised of two antibody domains with a single IL-2 linked to one of the domains. The IL-2 activity of the PD-1/IL-2 DBA-IL-2 complexes AF4386 (comprising SEQ ID NO: 212 and 181-182, results shown in **FIG. 4A**), AF4387 (comprising SEQ ID NO: 183-185, results shown in **FIG. 4B**) and AF4389 (comprising SEQ ID NO: 186-188, results shown in **FIG. 4C**) had an EC₅₀ of 50 pM, 57 pM and 118 pM respectively in the PD-1 coated wells and 1.79 nM, 419 pM and 1.67 nM respectively in the IgG1 coated wells, demonstrating PD-1 dependence. The IL-2 activity of the anti-PD1 control protein AF4380 (comprising SEQ ID NO: 180, 189-190, results shown in **FIG. 4D**), the anti-Her2 control protein AF4383 (comprising SEQ ID NO: 64, 191-192, results shown in **FIG. 4E**), and the anti-IL-2 control protein AF4384 (comprising SEQ ID NO: 178, 193-194, results shown in **FIG. 4F**) were unchanged. Sequences of the protein complexes are summarized in **TABLE 15** below.

TABLE 15 – IgG PD-1/IL-2 PDA with single IL-2, and control protein complexes

Protein Complex	SEQ ID NO:	Sequence
AF4386	SEQ ID NO: 181	APTSSSTKKKTQLQLEHLLLDLQMILNGINNYKNPKLTDMLT FEFYMPKKATELKHLQCLERELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTGGGGSGGGGSGGGGSGGGGSGVQLVQSGAE VKKPGASVKVSCKASGDTFTRYVHWVRQAPGQGLEWM GIINPSGGYASYAQKFQGRVTMTRDTSTSTVYMELSSLRSE DTAVYYCAAGLFIWGQGTLLTVSSAKTTAPSVYPLAPVCG DTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVL QSDLTYLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEP RGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMSLSPIV TCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNS TLRVVSALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTISK PKGSVRAPQVYVLPPPEEEMTKKQVTLCMVTDMPEDIY VEWTNNGKTELNYKNTEPVLDSGGSYFMYSDLRVEKKN WVERNSYSCSVVHEGLHNHHTTESFSRTPGK
	SEQ ID NO: 182	QVQLVQSGAEVKKPGASVKVSCKASGDTFTRYVHWVR QAPGQGLEWMGIINPSGGYASYAQKFQGRVTMTRDTSTST VYMELSSLRSEDTAVYYCAAGLFIWGQGTLLTVSSAKTTA PSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSL SSGVHTFPAVLQSDLTYLSSSVTVTSSTWPSQSITCNVAHP ASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKI KDVLMSLSPIVTCVVVDVSEDDPDVQISWVFNNEVHTA QTQTHREDYNSLTRVVSALPIQHQQDWMSGKEFKCKVNNK DLGAPIERTISKPKGSVRAPQVYVLPPPEKEMTKKQVSLTC LVKDFMPEDIYVEWTNNGKTELNYKNTEPVLKSDGGSYFM YSKLTVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPG GGGSGGGSHHHHHH
	SEQ ID NO: 212	DIQMTQSPSSLSASVGDRVTITCRASQSIGRYLAWYQQKPG KAPKLLIYSASNLETGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQYNFPVTFGPGTKVDIKRADAAPTVSIFPPSSEQLT SGGASVVCFLNFPYKIDINVKWKIDGSERQNGVLNSWTD QDSKDSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPIV KSFNRNEC
AF4387	SEQ ID NO: 183	APTSSSTKKKTQLQLEHLLLDLQMILNGINNYKNPKLTDMLT FEFYMPKKATELKHLQCLERELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTGGGGSGGGGSGGGGSGGGGSGVQLVQSGAE VKKPGASVKVSCKASGYTFDYMHVVRQAPGQGLEWM GIINPRAGYTSYALKFQGRVTMTRDTSTSTVYMELSSLRSE DTAVYYCTSGWDVWGQGTLLTVSSAKTTAPSVYPLAPVC GDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPA VLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMSLSPIV TCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDY NSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTI SKPKGSVRAPQVYVLPPPEEEMTKKQVTLCMVTDMPEDIY VEWTNNGKTELNYKNTEPVLDSGGSYFMYSDLRVEKK NWVERNSYSCSVVHEGLHNHHTTESFSRTPGK

Protein Complex	SEQ ID NO:	Sequence
	SEQ ID NO: 184	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMHWVR QAPGQGLEWMGIINPRAGYTSYALKFQGRVTMTRDTSTST VYMESSLRSEDTAVYYCTSGWDVWGQGTLLTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAH PASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPK IKDVLMISSLPIVTCVVVDVSEDDPDVQISWVFNNEVHTA QTQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNK DLGAPIERTISKPKGSVRAPQVYVLPPEKEMTKKQVSLTC LVKDFMPEDIYVEWTNNGKTELNYKNTEPVLSKSDGSYFM YSKLTVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPG GGGSGGGSHHHHHH
	SEQ ID NO: 185	DIQMTQSPSSLSASVGDRVTITCRASQSISTWLAWYQQKPG KAPKLLIYAASSLDGVPSPRFSGSGSGTDFTLTISLQPEDF ATYYCQQSYSPVTFGQGTKVEIKRADAAPTVSIFPPSSEQL TSGGASVVCFLNFPYKIDINVKWKIDGSRQNGVLNSWT DQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRECE
AF4389	SEQ ID NO: 186	APTSSSTKKTKQLQLEHLLDLQMLNGINNYKNPKLTDMLT FEFYMPKKATELKHLLQCLERELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATVEFLNRWIT FCQSIISTLTGGGGSGGGGSGGGGSGGGGSGVQLVQSGAE VKKPGASVKVSCKASGHTFTRYMHWVRQAPGQGLEWM GIINPSGGYATYAQKFQGRVTMTRDTSTSTVYMESSLRSE DTAVYYCASGLFIWGQGTLLTVSSAKTTAPSVYPLAPVCG DTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVL QSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEP RGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISSLPIV TCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNS TLRVVSALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTISK PKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDMPEDIY VEWTNNGKTELNYKNTEPVLDSDGSYFMYSDLRVEKKN WVERNSYSCSVVHEGLHNHHTTESFSRTPGK
	SEQ ID NO: 187	QVQLVQSGAEVKKPGASVKVSCKASGHTFTRYMHWVR QAPGQGLEWMGIINPSGGYATYAQKFQGRVTMTRDTSTST VYMESSLRSEDTAVYYCASGLFIWGQGTLLTVSSAKTTA PSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSL SSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHP ASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKI KDVLMISSLPIVTCVVVDVSEDDPDVQISWVFNNEVHTA QTQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNK DLGAPIERTISKPKGSVRAPQVYVLPPEKEMTKKQVSLTC LVKDFMPEDIYVEWTNNGKTELNYKNTEPVLSKSDGSYFM YSKLTVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPG GGGSGGGSHHHHHH
	SEQ ID NO: 188	DIQMTQSPSSLSASVGDRVTITCRASQSINSWLAWYQQKPG KAPKLLIYATSTLESGVPSRFSGSGSGTDFTLTISLQPEDFA TYYCQQYYRFPVTFGQGTKVEIKRADAAPTVSIFPPSSEQL TSGGASVVCFLNFPYKIDINVKWKIDGSRQNGVLNSWT

Protein Complex	SEQ ID NO:	Sequence
		DQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRNEC
AF4380	SEQ ID NO: 180	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPG QAPRLLIYDASNRAATGIPARFSGSGSGTDFTLTISSELPEDFA VYYCQQSSNWPRTFGQGTKVEIKRADAAPTVSIFPPSSEQL TSGGASVVCFLNNFYPKDINVKWKIDGSRQNGVLNSWT DQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRNEC
	SEQ ID NO: 189	APTSSSTKKTKQLQLEHLLLDLQMLNGINNYKNPKLTDMLT FEFYMPKKATELKHLQCLERELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTGGGGSGGGGSGGGGSGGGGSGVQLVESGGG VVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVA VIWYDGSKRYYADSVKGRFTISRDN SKNTLFLQMNSLRAE DTAVYYCATNDDYWGQGT LVTVSSAKTTAPSVYPLAPVC GDTTGSSVTLGCLVKGYFPEPVTL TWNSGSLSSGVHTFPA VLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKK IEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVL MISLSP IVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDY NSTLRVVSALPIQH QDWMMSGKEFKCKVNNKDLGAPIERTI SKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPED IYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSDLRVEKK NWVERNSYSCSVVHEGLHNHHTTESFSRTPGK
	SEQ ID NO: 190	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQ APGKGLEWVA VIWYDGSKRYYADSVKGRFTISRDN SKNT LFLQMNSLRAEDTAVYYCATNDDYWGQGT LVTVSSAKTT APSVYPLAPVC GDTTGSSVTLGCLVKGYFPEPVTL TWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAH PASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPK IKDVL MISLSP IVTCVVVDVSEDDPDVQISWFVNNVEVHTA QTQTHREDY NSTLRVVSALPIQH QDWMMSGKEFKCKVNNK DLGAPIERTISKPKGSVRAPQVYVLPPEKEMTKKQVSLTC LVKDFMPEDIYVEWTNNGKTELNYKNTEPV LKSDGSYFM YSKLTVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPG GGGSGGGSHHHHHH
AF4383	SEQ ID NO: 64	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKP GKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDF ATYYCQQHYTTPPTFGQGTKVEIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSRQNGVLNSWT DQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRNEC
	SEQ ID NO: 191	APTSSSTKKTKQLQLEHLLLDLQMLNGINNYKNPKLTDMLT FEFYMPKKATELKHLQCLERELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTGGGGSGGGGSGGGGSGGGGSGEVQLVESGGGL VQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARI YPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDT AVYYCSRWGGDGFYAMDYWGQGT LVTVSSAKTTAPSVY PLAPVC GDTTGSSVTLGCLVKGYFPEPVTL TWNSGSLSSGV

Protein Complex	SEQ ID NO:	Sequence
		HTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTK VDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVL MISLSPIVTCVVVDVSEDDPDVQISWVFVNNVEVHTAQTQT HREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLGA PIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTD FMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSDL RVEKKNWVERNSYSCSVVHEGLHNHHTTESFSRTPGK
	SEQ ID NO: 192	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKG LEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLR AEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVSSAKTTAPSVY PLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFP AVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEP RGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVL MISLSPIVTCVV VDVSEDDPDVQISWVFVNNVEVHTAQTQTHREDYNSTLRVVSAL PIQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQVYV LPPPEKEMTKKQVSLTCLVKDFMPEDIYVEWTNNGKTELNYKN TEPVLSKSDGSYFMYSKLTVEKKNWVERNSYSCSVVHEGLHNHH TTKSFSRTPGGGGSGGGSHHHHHH
AF4384	SEQ ID NO: 178	DIQMTQSPSSLSASVGDVRSITCKASQNVGTNVGWYQQKP GKAPKALIYSASFRYSGVPSRFSGSGSGTDFLTISLQPED FATYFCQQYYTYPTYTFGGGTKLEIKRADAAPTVSIFPPSSE QLTSGGASVVCFLNFPYKPDINVKWKIDGSRQNGVLNS WTDQDSKDYSTYSMSSTLTLTKDEYERHNSYTCEATHKTST SPIVKSFNRECE
	SEQ ID NO: 193	APTSSSTKKTKLQLEHLLDLQMILNGINNYKNPKLTDMLT FEFYMPKKA TELKHLQCLERELKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FCQSIISTLTGGGGSGGGGSGGGGSGGGGSEVQLVESGGGL VKPGGSLRLSCAASGFTFSSYTLAWVRQAPGKGLEWVA AI DSSSYTYSPD TVRGRFTISRDN AKNSLYLQMNSLRAEDTA VYYCARD SNWDALDYWGQGTLLTVSSAKTTAPSVYPLAP VCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFP AVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDK KIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVL MISL SPIVTCVVVDVSEDDPDVQISWVFVNNVEVHTAQTQTHRED YNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLGAPIERT ISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPE DIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSDLRVEK KNWVERNSYSCSVVHEGLHNHHTTESFSRTPGK
	SEQ ID NO: 194	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYTLAWVRQA PGKLEWVA AIDSSSYTYSPD TVRGRFTISRDN AKNSLYLQ MNSLRAEDTAVYYCARD SNWDALDYWGQGTLLTVSSAK TTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWN SGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNV AHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIF PPKIKDVL MISLSPIVTCVVVDVSEDDPDVQISWVFVNNVEV HTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKV NNKDLGAPIERTISKPKGSVRAPQVYVLPPEKEMTKKQVS LTCLVKDFMPEDIYVEWTNNGKTELNYKNTEPVLSKSDGSY

Protein Complex	SEQ ID NO:	Sequence
		FMYSKLTVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSR TPGGGSGGGSHHHHHH

[0133] Results with protein complexes comprising the structures depicted in **FIG. 2H** are shown in **FIGS. 5A-H**. As depicted in **FIG. 2H**, these complexes are asymmetric and comprised of two identical monospecific Fab arms with a single IL-2 attached to one Fc domain by flexible linker and a single scFv attached to the other Fc domain by a flexible linker. The active PD-1/IL-2 DBA complexes, AF4403 comprising SEQ ID NO: 180, 195, 199 and AF4404 comprising SEQ ID NO: 180, 196, 199, are composed of anti-PD-1 domains in the Fab arms and a PD-1/IL-2 DBA scFv on the Fc arm. The control antibody-cytokine complexes are composed of a) antibody-cytokine complexes with an irrelevant antibody on the Fab arms with the DBA scFv on the Fc (AF4395 comprising SEQ ID NO: 64, 197, 202 and AF4396 comprising SEQ ID NO: 64, 198, 202), b) antibody-cytokine complexes with a non-DBA scFv on the Fc arm (AF4400 comprising SEQ ID NO: 180, 199-200 and AF4401 comprising SEQ ID NO: 180, 199, 201), and c) antibody-cytokine complexes with non-DBA antibodies in both the Fab and scFv domains (AF4392 comprising SEQ ID NO: 64, 202-203 and AF4393 comprising SEQ ID NO: 64, 202, 204). As shown in **FIGS. 5B** and **5D**, the IL-2 activity of the DBA-cytokine complexes AF4403 and AF4404 had an EC₅₀ of 31 pM and 26 pM respectively in the PD-1 coated wells and 62 pM and 64 pM respectively in the control wells, demonstrating PD-1 dependence of the IL-2 activity. None of the control proteins AF4395, AF4396, AF4400, AF4401, AF4392 and AF4393 described above showed a lower EC₅₀ on PD-1 coated wells than on wells coated with the IgG1 protein, as shown in **FIGS. 5A, 5C** and **5E-H**. Sequences of the protein complexes are summarized in **TABLE 16** below.

TABLE 16 – IgG PD-1 with C-terminal scFv and IL-2, and control protein complexes

Protein Complex	SEQ ID NO:	Sequence
AF4403	SEQ ID NO: 180	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPG QAPRLLIYDASNRAITGIPARFSGSGSGTDFTLTISSELPEDFA VYYCQQSSNWPRTFGQGTKVEIKRADAAPTVISIFPPSSEQL TSGGASVVCFLNFPKDIVKWKIDGSRQNGVLNSWT DQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRNEC
	SEQ ID NO: 195	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQ APGKGLEWVAIVYDGSKRYYADSVKGRFTISRDNKNT LFLQMNSLRAEDTAVYYCATNDDYWGQGTLLTVSSAKTT APSVYPLAPVCGDITGSSVTLGCLVKGYFPEPVTLTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAH

Protein Complex	SEQ ID NO:	Sequence
		PASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPK IKDVLMISSLPIVTCVVVDVSEDDPDVQISWVFNNEVHTA QTQTHREDYNSTLRVVSALPIQHQDWMMSGKEFKCKVNNK DLGAPIERTISKPKGSVRAPQVYVLPPPEKEMTKKQVSLTC LVKDFMPEDIYVEWTNNGKTELNYKNTEPVLKSDGSYFM YSKLTVEKKNWVERNNSYSCSVVHEGLHNHHTTKSFSRTPG GGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASV KVSCKASGDTFTRYVHWVRQAPGQGLEWMGIINPSGGY ASYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDNAVYYC AAGLFIWGQGLTVTVSSASGGGGSGGGGSGGGGSHASDIQ MTQSPSSLSASVGDRVTITCRASQSIGRYLAWYQQKPGKA PKLLIYSASNLETGVPSTRFSGSGSGTDFTLTISLQPEDFATY YCQQYNSEFPVTFGPSTKVDIKGGGSGGGSHHHHHH
	SEQ ID NO: 199	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQ APGKGLEWVAWIYDGSKRYYADSVKGRFTISRDNKNT LFLQMNSLRAEDNAVYYCATNDDYWGQGLTVTVSSAKTT APSVYPLAPVCGDITGSSVTLGCLVKGYFPEPVTLTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAH PASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPK IKDVLMISSLPIVTCVVVDVSEDDPDVQISWVFNNEVHTA QTQTHREDYNSTLRVVSALPIQHQDWMMSGKEFKCKVNNK DLGAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTC MVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSGDSYFM YSDLRVEKKNWVERNNSYSCSVVHEGLHNHHTTESFSRTPG GGGGSGGGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQMIL NGINNYKNPKLTDMLTFEFYMPKKATELKHLQCLERELKP LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATVEFLNRWITFCQSIISTLT
AF4404	SEQ ID NO: 180	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPG QAPRLLIYDASNRAITGIPARFSGSGSGTDFTLTISLQPEDFA VYYCQQSSNWPRITFGQGTKVEIKRADAAPTVSIFPPSSEQL TSGGASVVCFLNFPKDIQVKKIDGSERQNGVLNSWT DQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRECE
	SEQ ID NO: 196	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQ APGKGLEWVAWIYDGSKRYYADSVKGRFTISRDNKNT LFLQMNSLRAEDNAVYYCATNDDYWGQGLTVTVSSAKTT APSVYPLAPVCGDITGSSVTLGCLVKGYFPEPVTLTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAH PASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPK IKDVLMISSLPIVTCVVVDVSEDDPDVQISWVFNNEVHTA QTQTHREDYNSTLRVVSALPIQHQDWMMSGKEFKCKVNNK DLGAPIERTISKPKGSVRAPQVYVLPPPEKEMTKKQVSLTC LVKDFMPEDIYVEWTNNGKTELNYKNTEPVLKSDGSYFM YSKLTVEKKNWVERNNSYSCSVVHEGLHNHHTTKSFSRTPG GGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASV KVSCKASGYTFDYYMHWRQAPGQGLEWMGIINPRAG YTSYALKFQGRVTMTRDTSTSTVYMELSSLRSEDNAVYYC TSGWDVWGQGLTVTVSSASGGGGSGGGGSGGGGSHASDI

Protein Complex	SEQ ID NO:	Sequence
		QMTQSPSSLSASVGDRVITITCRASQSISTWLAWYQQKPGK APKLLIYAASSLD SGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCQQSYSPVTFGQGTKVEIKGGGSGGGSHHHHHH
	SEQ ID NO: 199	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQ APGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDN SKNT LFLQMNSLRAEDTAVYYCATNDDYWGQGT LVTVSSAKTT APSVYPLAPVCGD TTGSSVT LGCLVKGYFPEPVLTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAH PASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPK IKDVL MISLSPIVTCVVVDVSEDDPDVQISW FVNNVEVHTA QTQTHREDYNSTLRVVSALPIQH QDWMMSGKEFKCKVNNK DLGAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTC MVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFM YSDLRVEKKNWVERNSYSCSVVHEGLHNHHTTESFSRTPG GGGGSGGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQMIL NGINNYKNPKLTDMLTFEFYMPKKATEL KHLQCLERELKP LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFCQSIISTLT
AF4395	SEQ ID NO: 64	DIQMTQSPSSLSASVGDRVITITCRASQDVNTAVAWYQQKP GKAPKLLIYASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDF ATYYCQQHYTTPPTFGQGTKVEIKRADAAPT VSIFFPSSEQ LTSGGASVVCFLNNFY PKDINVKWKIDG SERQNGVLNSWT DQDSKDSTYSMSSTLT LTKDEYERHNSYTCEATHKTSTSPI VKSFNRENC
	SEQ ID NO: 197	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAY LQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT LVT VSSAKTTAPSVYPLAPVCGD TTGSSVT LGCLVKGYFPEPV LTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSI TCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP SVFIFPPKIKDVL MISLSPIVTCVVVDVSEDDPDVQISW FVN NVEVHTAQTQTHREDYNSTLRVVSALPIQH QDWMMSGKEF KCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPPEKEMT KKQVSLTCLVKDFMPEDIYVEWTNNGKTELNYKNTEPV L KSDGSYFMYSKL TVEKKNWVERNSYSCSVVHEGLHNHHT TKSFSRTPGGGGSGGGGSGGGGSGGGGSQVQLVQSGAE VKKPGASVKVSC KASGDTFTRYVHWVRQAPGQGLEWM GIINPSGGYASYAQKFQGRVTMTRDTSTSTVYMELSSLRSE DTAVYYCAAGLFIWGQGT LVTVSSASGGGGSGGGGSGGG GSHASDIQMTQSPSSLSASVGDRVITITCRASQSIGRYLAWY QQKPGKAPKLLIYASNL ETGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQYNSFPVTFGP GTKVDIKGGGSGGGSHHH HHH
	SEQ ID NO: 202	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAY LQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT LVT VSSAKTTAPSVYPLAPVCGD TTGSSVT LGCLVKGYFPEPV LTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSI TCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP

Protein Complex	SEQ ID NO:	Sequence
		SVFIFPPKIKDVLMI SLSPIVTCVVVDVSEDDPDVQISW FVN NVEVHTAQTQTHREDYNSTLRVVSALPIQH QDWMSGKEF KCKVNNKDLGAPIERTISKPKGSVRAPQVYVLP PPEEEMTK KQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLD SDGSYFMYSDLRVEKKNWVERN SYSCSVVHEGLHNHHTT ESFSRTPGGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEHLL LDLQMILNGINNYKNPKLTDMLTFEFYMPKKATELKHLQC LERELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFCQSIISTLT
AF4396	SEQ ID NO: 64	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKP GKAPKLLIYSASFLYSGVPSRFSGSRSGTDFLTISLQPEDF ATYYCQQHYTTPPTFGQGTKVEIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWT DQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRNEC
	SEQ ID NO: 198	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAY LQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT LVT VSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVT LTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSI TCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP SVFIFPPKIKDVLMI SLSPIVTCVVVDVSEDDPDVQISW FVN NVEVHTAQTQTHREDYNSTLRVVSALPIQH QDWMSGKEF KCKVNNKDLGAPIERTISKPKGSVRAPQVYVLP PPEKEMT KKQVSLTCLVKDFMPEDIYVEWTNNGKTELNYKNTEPV L KSDGSYFMYSKLTVEKKNWVERN SYSCSVVHEGLHNHHT TKSFSRTPGGGGGSGGGGSGGGGSGGGGSGVQLVQSGAE VKKPGASVKVSKASGYTFDYYMHWRQAPGQGLEWM GIINPRAGYTSYALKFQGRVTMTRDTSTSTVYMESSLRSE DTAVYYCTSGWDVWGQGT LVTVSSASGGGGSGGGGSGG GGSHASDIQMTQSPSSLSASVGDRVTITCRASQSISTWLAW YQQKPGKAPKLLIYAASSLD SGVPSRFSGSGSGTDFLTIS LQPEDFATYYCQQSY SFPVTFGQGTKVEIKGGGSGGGSHH HHHH
	SEQ ID NO: 202	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAY LQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT LVT VSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVT LTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSI TCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP SVFIFPPKIKDVLMI SLSPIVTCVVVDVSEDDPDVQISW FVN NVEVHTAQTQTHREDYNSTLRVVSALPIQH QDWMSGKEF KCKVNNKDLGAPIERTISKPKGSVRAPQVYVLP PPEEEMTK KQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLD SDGSYFMYSDLRVEKKNWVERN SYSCSVVHEGLHNHHTT ESFSRTPGGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEHLL LDLQMILNGINNYKNPKLTDMLTFEFYMPKKATELKHLQC LERELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFCQSIISTLT

Protein Complex	SEQ ID NO:	Sequence
AF4400	SEQ ID NO: 180	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRAATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCQQSSNWPRTFGQGTKVEIKRADAAPTIVSIFPPSSEQLTSGGASVVCFLNFPKDIVKWKIDGSRQNGVLNSWTDQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRENC
	SEQ ID NO: 199	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAIVWYDGSKRYYADSVKGRFTISRDNKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLLTVSSAKTTAPSVYPLAPVCGDITGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISSLPIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSGGSYFMYSDLRVEKKNWVERNSYSCSVVHEGLHNHHTTESFSRTPGGGGSGGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTDMLTFEFYMPKKATELKHLQCLERELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
	SEQ ID NO: 200	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAIVWYDGSKRYYADSVKGRFTISRDNKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLLTVSSAKTTAPSVYPLAPVCGDITGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISSLPIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPEKEMTKKQVSLTCLVKDFMPEDIYVEWTNNGKTELNYKNTEPVLKSDGSYFMYSKLTVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGGGGSGGGSGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFSNIKDTYIHWRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVSSASGGGGSGGGSGGGGSHASDIQMTQSPSSLSASVGDRTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGGSGGGSHHHH
AF4401	SEQ ID NO: 180	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRAATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCQQSSNWPRTFGQGTKVEIKRADAAPTIVSIFPPSSEQLTSGGASVVCFLNFPKDIVKWKIDGSRQNGVLNSWTDQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRENC
	SEQ ID NO: 199	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAIVWYDGSKRYYADSVKGRFTISRDNKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLLTVSSAKTT

Protein Complex	SEQ ID NO:	Sequence
		APSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAH PASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPK IKDVLMISSLPIVTCVVVDVSEDDPDVQISWVFNVEVHTA QTQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNK DLGAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTC MVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFM YSDLRVEKKNWVERNNSYSCSVVHEGLHNHHTTESFSRTPG GGGGSGGGSGGGGSAPTSSSTKKTQLQLEHLLLDLQML NGINNYKNPKLTDMLTFEFYMPKKATELKHLQCLERELKP LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEY ADETATIVEFLNRWITFCQSIISTLT
	SEQ ID NO: 201	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQ APGKGLEWVAVIWYDGSKRYADSVKGRFTISRDNKNT LFLQMNSLRAEDTAVYYCATNDDYWGQGLTVTVSSAKTT APSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAH PASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGPSVFIFPPK IKDVLMISSLPIVTCVVVDVSEDDPDVQISWVFNVEVHTA QTQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNK DLGAPIERTISKPKGSVRAPQVYVLPPPEKEMTKKQVSLTC LVKDFMPEDIYVEWTNNGKTELNYKNTEPVLKSDGSYFM YSKLTVEKKNWVERNNSYSCSVVHEGLHNHHTTKSFSRTPG GGGGSGGGSGGGSGGGGSEVQLVESGGGLVKPGGSLR LSCAASGFTFSSYTLAWVRQAPGKGLEWVAIDSSSYTYS PDTVRGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARDS NWDALDYWGQGLTVTVSSASGGGGSGGGSGGGGSHAS DIQMTQSPSSLSASVGDRVSITCKASQNVGTNVGWYQQKP GKAPKALIYSASFRYSGVPSRFSGSGSGTDFTLTISSLQPED FATYFCQQYYTYPYTFGGGKLEIKGGGSGGGSHHHHHH
AF4392	SEQ ID NO: 64	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKP GKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDF ATYYCQQHYTTPTFGQGTKVEIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSRQNGVLNSWT DQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRNEC
	SEQ ID NO: 202	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAY LQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVT VSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVT LTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSI TCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP SVFIFPPKIKDVLMISSLPIVTCVVVDVSEDDPDVQISWVFN NVEVHTAQTQTHREDYNSTLRVVSALPIQHQQDWMSGKEF KCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPPEEEMTK KQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLD SDGSYFMYSDLRVEKKNWVERNNSYSCSVVHEGLHNHHTT ESFSRTPGGGGSGGGSGGGGSAPTSSSTKKTQLQLEHLL LDLQMLNGINNYKNPKLTDMLTFEFYMPKKATELKHLQC

Protein Complex	SEQ ID NO:	Sequence
		LERELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFCQSIISTLT
	SEQ ID NO: 203	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAY LQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVT VSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVT LTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSI TCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP SVFIFPPKIKDVLMSLSPIVTCVVVDVSEDDPDVQISWVFN NVEVHTAQTQTHREDYNSTLRVVSALPIQHQQDWMSGKEF KCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPEKEMT KKQVSLTCLVKDFMPEDIYVEWTNNGKTELNYKNTEPVL KSDGSYFMYSKLTVEKKNWVERNSYSCSVVHEGLHNHHT TKSFSRTPGGGGGSGGGGSGGGGSGGGGSEVQLVESGGGL VQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARI YPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDT AVYYCSRWGGDGFYAMDYWGQGTLVTVSSASGGGGSGG GGSGGGGSHASDIQMTQSPSSLSASVGDRVITICRASQDV NTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGT DFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGG SGGGSHHHHHH
AF4393	SEQ ID NO: 64	DIQMTQSPSSLSASVGDRVITICRASQDVNTAVAWYQQKPG KAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISLQPEDF ATYYCQQHYTTPPTFGQGTKVEIKRADAAPTVSIFPPSSEQ LTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWT DQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRECE
	SEQ ID NO: 202	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAY LQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVT VSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVT LTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSI TCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP SVFIFPPKIKDVLMSLSPIVTCVVVDVSEDDPDVQISWVFN NVEVHTAQTQTHREDYNSTLRVVSALPIQHQQDWMSGKEF KCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPEEEMTK KQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVL SDGSYFMYSDLRVEKKNWVERNSYSCSVVHEGLHNHHTT ESFSRTPGGGGGSGGGGSGGGGSAPTSSSTKKTQLQLEHLL LDLQMILNGINNYKNPKLTDMLTFEFYMPKKATELKHLQC LERELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFCQSIISTLT
	SEQ ID NO: 204	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAY LQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVT VSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVT LTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSI TCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNAAGGP SVFIFPPKIKDVLMSLSPIVTCVVVDVSEDDPDVQISWVFN

Protein Complex	SEQ ID NO:	Sequence
		NVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEF KCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPPEKEMT KKQVSLTCLVKDFMPEDIYVEWTNNGKTELNYKNTEPVL KSDGSYFMYSKLTVEKKNWVERNYSYSCSVVHEGLHNHHT TKSFSRTPGGGGGSGGGGSGGGGSGGGGSEVQLVESGGGL VKPGGSLRLSCAASGFTFSSYTLAWVRQAPGKGLEWVAAI DSSSYTSPDTPVRGRFTISRDNANKNSLYLQMNSLRAEDTA VYYCARDSDNWDALDYWGQGLTVTVSSASGGGGSGGGGS GGGGSHASDIQMTQSPSSLSASVGDRVSITCKASQNVGTN VGWYQQKPGKAPKALISASFRYSGVPSRFSGSGSGTDFT LTISSLQPEDFATYFCQQYYTYPTYTFGGGTKLEIKGGGSGG GSHHHHHH

EXAMPLE 12

Binding of additional Dual Binding Antibodies to PD1 and IL2

[0134] Having observed the effectiveness of dual binding antibodies (DBAs) in previous examples, additional dual binding antibody scFvs were generated (see **TABLE 17**, **TABLE 18**, and **TABLE 19** below). This example describes the measurement of binding of PD1 and IL2 to these dual binding antibody scFvs. The scFv DNA sequence for each clone was synthesized as a gBlock (Integrated DNA Technologies, Inc.) with a T7 promoter, a translation initiation site, the coding sequence for the scFv sequence with cMyc and V5 tags, and a T7 terminator sequence. Proteins from each of the gBlock fragments were expressed using a cell-free transcription/translation system (Cosmo Bio USA, Inc., PUREfrex2.1, Product # GFK-PF213 with DS Supplement, Prod. # GFK-PF005). The scFv samples were subjected to ELISA analysis to detect PD1 and IL2 binding. In these experiments, wells of a 384-well plate were coated with an anti-V5 antibody (Sv5-Pk1, BioRad) at 1 µg/ml overnight at 4 degrees. After washing, wells were blocked with SuperBlock (ThermoFisher, 37515) followed by addition of saturating levels of scFvs in SuperBlock. After washing, antigens were added and plates incubated for one hour. To detect PD1 binding, a biotinylated recombinant PD1 was used (PD1-HisAvi, Acro Biosystems, PD1-H82E4). To detect IL2 binding, IL2 (R&D, 202-IL) was preincubated with biotinylated-anti-IL2 mAb (mab202, biotinylated using standard methods) at a ratio of 2:1 in SuperBlock buffer. Biotinylated antigens were detected using streptavidin HRP using standard methods. Varying amounts of labelled test antigen were added to show binding and to estimate relative affinities of the different scFvs. **TABLE 17A-C** shows the EC₅₀ values for PD1 and IL2 binding for three sets of scFvs, demonstrating dual binding of these antibodies.

[0135] The data indicate that all the antibodies in **TABLE 17A-C** except for the control antibodies AB000694, AB000719, AB000880 are able to effectively bind both PD1 and IL2.

TABLE 17A: Binding affinities for additional DBAs to PD1 and IL2 according to the disclosure as assessed by ELISA in a first experiment

antibodyID	Protein name	PD1 EC50	IL2 EC50
AB002293_2B07v2	AF003676	0.2	6
AB002353_2B07v9	AF003736	0.9	30
AB002342_2B07v5	AF003725	0.2	7
AB002328_2B07v4	AF003711	0.2	0.4
AB002326_2B07v3	AF003709	0.2	30
AB002345_2B07v6	AF003728	0.2	60
AB002348_2B07v8	AF003731	0.3	100
AB002022_2B07v1	AF002829	0.6	10
AB002347_2B07v7	AF003730	0.1	8

TABLE 17B: Binding affinities for additional DBAs to PD1 and IL2 according to the disclosure as assessed by ELISA in a second experiment

antibodyID	Protein name	PD1 EC50	IL2 EC50
AB000694 (anti-PD1 control)	AF005039	0.7	-
AB000719 (anti-PD1 control)	AF005040	0.4	-
AB000880 (anti-PD1 control)	AF000327	4	-
AB003021_2A11v7	AF005103	0.6	40
AB002417_2A11v4	AF005148	4	50
AB002413_2A11v3	AF005147	2	2
AB002427_2A11v5	AF005149	3	0.9
AB003200_2B05v1	AF005131	0.5	weak

AB003209_2B05v4	AF005140	0.4	weak
AB003201_2B05v2	AF005132	0.5	weak
AB003202_2B05v3	AF005133	0.5	weak
AB003155_2B07v10	AF005042	0.7	0.4
AB002328_2B07v4	AF005041	2	1
AB003174_2B07v11	AF005062	0.6	7
AB003180_2B07v13	AF005068	0.6	0.8
AB003183_2B07v14	AF005071	0.3	40
AB003178_2B07v12	AF005066	1	0.3
AB003007_7A04v6	AF005087	1	10
AB002365_7A04v2	AF005095	1	8
AB003012_7A04v7	AF005092	1	7
AB003005_7A04v5	AF005085	4	3
AB002864_2A11v6	AF005116	0.4	60

TABLE 17C: Binding affinities for additional DBAs to PD1 and IL2 according to the disclosure as assessed by ELISA in a third experiment

antibodyID	Protein name	PD1 EC50	IL2 EC50
AB000880 (anti-PD1 control)	AF000327	8	-
AB002360_7A04v1	AF003765	1	70
AB002365_7A04v2	AF003770	1	30
AB002370_7A04v3	AF003775	0.7	30
AB002381_7A04v4	AF003786	0.7	30
AB002410_2A11v2	AF003815	3	100
AB002413_2A11v3	AF003818	3	3
AB002417_2A11v4	AF003822	5	400
AB002427_2A11v5	AF003832	3	0.8
AB002022_2B07v1	AF002829	5	30
AB001938	AF002745	10	30

TABLE 18: Sequences of V_H and V_L regions for DBAs described in Example 12

ID	antibodyID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
AB000694_nivo	AB000694	QVQLVESGGGVVQPGRSLRLD CKASGITFSNSGMHWVRQAPG KGLEWVAVIWYDGSKRYYADS VKGRFTISRDN SKNTLFLQMNS LRAEDTAVYYCATNDDYWGQ GTLVTVSS	EIVLTQSPATLSLSPGERATLSCRASQS VSSYLAWYQQKPGQAPRLLIYDASNR ATGIPARFSGSGGTDFTLTISSLEPED FAVYYCQQSSNWPRTFGQGTKVEIK	329	330
AB000880_PD1_R04_C10	AB000880	QVQLVQSGAEVKKPGASVKVS CKASGYTFTTYYMHWVRQAPG QGLEWMGIINPSGGGTLYAQKF QGRVTMTRDTSTSTVYMELSSL RSEDTA VYYCAAGLFIWGQGT TVTVSS	DIVMTQSPDSLAVSLGERATINCKAS QSLHSSSNKNYLAWYQQKPGQPPK LLIYWASTRESGVPDFRFGSGSGTDFT LTISSLQAEDVAVYYCQQYYSTPITFG PGTKVDIK	331	332
AB002022_2B07v1	AB002022	QVQLVQSGAEVKKPGASVKVS CKASGDTFTRHYVHWVRQAPG QGLEWMGIINPSGGYASYAQKF QGRVTMTRDTSTSTVYMELSSL	DIQMTQSPSSLSASVGDRTVTITCRASQ SIGRWLAWYQQKPGKAPKLLIYSASN LETGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQYESFPVTFGPGTKVDIK	333	334

ID	antibody ID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		RSEDTAVYYCAAGLFIWGQGT L VTVSS			
AB002293_2B07v2	AB00229 3	QVQLVQSGAEVKKPGASVKVS CKASGDTFTIRYYVHWVRQAPG QGLEWMGIINPSSGGYASYAQKF QGRVTMTTRDTSTSTVYMESSL RSEDTAVYYCAAGLFIWGQGT L VTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ SIGRWLA WYQQKPGKAPKLLIYSASN LETGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQYNFPVTFGPGTKVDIK	335	336
AB002326_2B07v3	AB00232 6	QVQLVQSGAEVKKPGASVKVS CKASGDTFTIRYYVHWVRQAPG QGLEWMGIINPSSGGYASYAQKF QGRVTMTTRDTSTSTVYMESSL RSEDTAVYYCAAGLFIWGQGT L VTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ SIGRWLA WYQQKPGKAPKLLIYSASN LETGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQYNFPVTFGPGTKVDIK	337	338
AB002328_2B07v4	AB00232 8	QVQLVQSGAEVKKPGASVKVS CKASGDTFTIRYYVHWVRQAPG QGLEWMGIINPSSGGYASYAQKF QGRVTMTTRDTSTSTVYMESSL	DIQMTQSPSSLSASVGDRVTITCRASQ SIGRWLA WYQQKPGKAPKLLIYSASN LETGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQYNFPVTFGPGTKVDIK	339	340

ID	antibody ID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		RSEDTAVYYCAAGLFIWGQGT L VTVSS			
AB002342_2B07v5	AB00234 2	QVQLVQSGAEVKKPGASVKVS CKASGDTFTIRYYVHWVRQAPG QGLEWMGIINPSGGYASYAQKF QGRVTMTTRDTSTSTVYME LSSL RSEDTAVYYCAAGLFIWGQGT L VTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ SIGRWLA WYQQKPKGAPKLLIYSASN LETGVPSRFSGSGGTDFTLTIS SLQPE DFATYYCQQYERFPVTFGPGTKVDIK	341	342
AB002345_2B07v6	AB00234 5	QVQLVQSGAEVKKPGASVKVS CKASGDTFTIRYYVHWVRQAPG QGLEWMGIINPSGGYASYAQKF QGRVTMTTRDTSTSTVYME LSSL RSEDTAVYYCAAGLFIWGQGT L VTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ SIGRWLA WYQQKPKGAPKLLIYSASN LETGVPSRFSGSGGTDFTLTIS SLQPE DFATYYCQQYNSFPVTFGPGTKVDIK	343	344
AB002347_2B07v7	AB00234 7	QVQLVQSGAEVKKPGASVKVS CKASGDTFTIRYYVHWVRQAPG QGLEWMGIINPSGGYASYAQKF QGRVTMTTRDTSTSTVYME LSSL	DIQMTQSPSSLSASVGDRVTITCRASQ SIGRWLA WYQQKPKGAPKLLIYSASN LETGVPSRFSGSGGTDFTLTIS SLQPE DFATYYCQQYNSFPVTFGPGTKVDIK	345	346

ID	antibody ID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		RSEDTAVYYCAAGLFIWGQGT LVTVSS			
AB002348_2B07v8	AB002348	QVQLVQSGAEVKKPGASVKVS CKASGDTFTIRYYVHWVRQAPG QGLEWMGIINPSSGGYASYAQKF QGRVTMTTRDTSTSTVYMESSL RSEDTAVYYCAAGLFIWGQGT LVTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ SIGRYLAWYQQKPGKAPKLLIYYASN LETGVPSRFSGSGGTDFLTITSSLPQE DFATYYCQQYNFPVTFGGGTKVDIK	347	348
AB002353_2B07v9	AB002353	QVQLVQSGAEVKKPGASVKVS CKASGDTFTIRYYVHWVRQAPG QGLEWMGIINPSSGGYASYAQKF QGRVTMTTRDTSTSTVYMESSL RSEDTAVYYCAAGLFIWGQGT LVTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ SIGRWLAWYQQKPGKAPKLLIYYASN LETGVPSRFSGSGGTDFLTITSSLPQE DFATYYCQQYESFPVTFGGGTKVDIK	349	350
AB002360_7A04v1	AB002360	QVQLVQSGAEVKKPGASVKVS CKASGYTFTDYYMHWRQAPG QGLEWMGIINPRAGYTSYALKF QGRVTMTTRDTSTSTVYMESSL	DIQMTQSPSSLSASVGDRVTITCRASQ SISTWLAWYQQKPGKAPKLLIYAASS LDGVPSPRFSGSGGTDFLTITSSLPQE DFATYYCQQSYSPVTFGGGTKVEIK	351	352

ID	antibody ID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		RSED _{TA} VYYCTSGWDVWGQGT LVT _V SS			
AB002365_7A04v2	AB002365	QVQLVQSGAEVKKPGASVKVS CKASGYTFT _{RY} YMHWVRQAPG QGLEWMGIINPRAGYTSYALKF QGRVTMT _{RD} TSTSTVYME _L SSL RSED _{TA} VYYCTSGWDVWGQGT LVT _V SS	DIQMTQSPSSLSASVGD _{RV} TITCRASQ SISTWLA _W YQQKPGKAPKLLIYAASS LD _{SG} VPSRFSGSGGTDF _{TL} TISS _{LQ} PE DFATYYCQQSYSPVTFGQGT _K VEIK	353	354
AB002370_7A04v3	AB002370	QVQLVQSGAEVKKPGASVKVS CKASGYTFT _{TY} YMHWVRQAPG QGLEWMGIINPRAGYTSYALKF QGRVTMT _{RD} TSTSTVYME _L SSL RSED _{TA} VYYCTSGWDVWGQGT LVT _V SS	DIQMTQSPSSLSASVGD _{RV} TITCRASQ SISTWLA _W YQQKPGKAPKLLIYAASS LD _{SG} VPSRFSGSGGTDF _{TL} TISS _{LQ} PE DFATYYCQQSYSPVTFGQGT _K VEIK	355	356
AB002381_7A04v4	AB002381	QVQLVQSGAEVKKPGASVKVS CKASGDTFT _{DY} YMHWVRQAPG QGLEWMGIINPRAGYTSYALKF QGRVTMT _{RD} TSTSTVYME _L SSL	DIQMTQSPSSLSASVGD _{RV} TITCRASQ SISTWLA _W YQQKPGKAPKLLIYAASS LD _{SG} VPSRFSGSGGTDF _{TL} TISS _{LQ} PE DFATYYCQQSYSPVTFGQGT _K VEIK	357	358

ID	antibody ID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		RSED _{TA} VYYCTSGWDVWGQGT LVT _V SS			
AB002410_2A11v2	AB002410	QVQLVQSGAEVKKPGASVKVS CKASGHTFTTRY _Y MHWV _R QAPG QGLEWMGIINPSGGYATYAQKF QGRVTMT _R DTSTSTVYME _L SSL RSED _{TA} VYYC _{AS} GW _{FW} GQGT LVT _V SS	DIQMTQSPSSLSASVGD _R VTITCRASQ SIN _{SW} LA _W YQ _Q KPGKAPKLLIYDTST LESGVPSR _F SGSGGTDFTLT _{IS} SLQPE DFATYYCQ _Q SYSP _F VTFGQG _T KVEIK	359	360
AB002413_2A11v3	AB002413	QVQLVQSGAEVKKPGASVKVS CKASGHTFTTRY _Y MHWV _R QAPG QGLEWMGIINPSGGYATYAQKF QGRVTMT _R DTSTSTVYME _L SSL RSED _{TA} VYYC _{AS} GFIW _G QGT VT _V SS	DIQMTQSPSSLSASVGD _R VTITCRASQ SIN _{SW} LA _W YQ _Q KPGKAPKLLIYATST LESGVPSR _F SGSGGTDFTLT _{IS} SLQPE DFATYYCQ _Q YYR _F PVTFGQG _T KVEIK	361	362
AB002417_2A11v4	AB002417	QVQLVQSGAEVKKPGASVKVS CKASGHTFTTRY _Y MHWV _R QAPG QGLEWMGIINPSGGYATYAQKF QGRVTMT _R DTSTSTVYME _L SSL	DIQMTQSPSSLSASVGD _R VTITCRASQ SIN _{SW} LA _W YQ _Q KPGKAPKLLIYATST LESGVPSR _F SGSGGTDFTLT _{IS} SLQPE DFATYYCQ _Q YYSP _F VTFGQG _T KVEIK	363	364

ID	antibodyID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		RSEDTAVYYCASGLFIWGGTGL VTVSS			
AB002427_2A11v5	AB002427	QVQLVQSGAEVKKPGASVKVS CKASGHTFTTRYMHWVRQAPG QGLEWMGIINPSGGYATYAQKF QGRVTMTTRDTSTSTVYMESSL RSEDTAVYYCASGLFIWGGTGL VTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ SINSWLAWYQQKPKGAPKLLIYATST LESGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQSYRFPVTFGGQGTKVEIK	365	366
AB002520_7A04v8	AB002520	QVQLVQSGAEVKKPGASVKVS CKASGYTFTDYYMHWVRQAPG QGLEWMGIINPRAGYTSYALKF QGRVTMTTRDTSTSTVYMESSL RSEDTAVYYCTSGWDVWGQGT LVTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ SISTWLAWYQQKPKGAPKLLIYAASS LD SGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQSYRFPVTFGGQGTKVEIK	367	368
AB002521_2A11v1	AB002521	QVQLVQSGAEVKKPGASVKVS CKASGHTFTTRYMHWVRQAPG QGLEWMGIINPSGGYATYAQKF QGRVTMTTRDTSTSTVYMESSL	DIQMTQSPSSLSASVGDRVTITCRASQ SINSWLAWYQQKPKGAPKLLIYATST LESGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQSYRFPVTFGGQGTKVEIK	369	370

ID	antibody ID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		RSEDTAVYYCASGLDIWGQGT LVTSS			
AB002829_knd_A08	AB00282 9	QVQLVQSGAEVKKPGASVKVS CKASGYIFNGYDIHWVRQAPGQ GLEWMGWMNPDNGNTGLAQK FQGRVTITADESTSTAYMELSSL RSEDTAVYYCARGMATRFPYY YYGMDVWGQGTLLVTVSS	DIVMTQSPSLPVTGPASISCRSSQS LLHSNGYNLYLDWYLQKPGQSPQLLIY LGSNRASGVDPDRFSGSGGTDFTLKIS RVEAEDVGVVYCMQGTTHWPPTFGQ GTKVEIK	371	372
AB002864_2A11v6	AB00286 4	QVQLVQSGAEVKKPGASVKVS CKASGHTFTTRYMHWVRQAPG QGLEWMGVNPSGGYATYAQK FQGRVTMTRDTSTSTVYMELSS LRSEDTAVYYCASGWDVWGQ GTLVTVSS	DIQMTQSPSSLASVGDRTVTITCRASQ SINSLAWYQQKPKGAPKLLIYATST LESGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQSYSSPVTFGGQGTKVEIK	373	374
AB003005_7A04v5	AB00300 5	QVQLVQSGAEVKKPGASVKVS CKASGYTFTRYMHWVRQAPG QGLEWMGIQPRAGYTSYALKF QGRVTMTRDTSTSTVYMELSSL	DIQMTQSPSSLASVGDRTVTITCRASQ SISTWLAWYQQKPKGAPKLLIYAASS LDEGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQSYSPVTFGGQGTKVEIK	375	376

ID	antibody ID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		RSEDTAVYYCTSGWDVWGQGT LVTVSS			
AB003007_7A04v6	AB003007 7	QVQLVQSGAEVKKPGASVKVS CKASGYTFTRYMHWRQAPG QGLEWMGIINPRAGYTSYALKF QGRVTMTTRDTSTSTVYMELSSL RSEDTAVYYCTSGWEVWGQGT LVTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ SISTWLAWYQQKPKGAPKLLIYAASS LDEGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQSYSPVTFGGQGTKVEIK	377	378
AB003012_7A04v7	AB003012 2	QVQLVQSGAEVKKPGASVKVS CKASGYTFTRYMHWRQAPG QGLEWMGIINPRAGYTSYALKF QGRVTMTTRDTSTSTVYMELSSL RSEDTAVYYCTSGWDVWGQGT LVTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ SISTWLAWYQQKPKGAPKLLIYAASS LDEGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQSYSPVTFGGQGTKVEIK	379	380
AB003021_2A11v7	AB003021 1	QVQLVQSGAEVKKPGASVKVS CKASGYTFTRYMHWRQAPG QGLEWMGVINPSGGYATYAQK FQGRVTMTTRDTSTSTVYMELSSL	DIQMTQSPSSLSASVGDRVTITCRASQ SISSWLAWYQQKPKGAPKLLIYATST LESGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQSYSSPVTFGGQGTKVEIK	381	382

ID	antibody ID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		LRSEDTAVYYCASGWDVWGQ GTLVTVSS			
AB003155_2B07v10	AB00315 5	QVQLVQSGAEVKKPGASVKVS CKASGETFTTRYVYVHWVRQAPG QGLEWMGIINPSGGYASYAQKF QGRVTMTTRDTSTSTVYMESSL RSEDTAVYYCAAGLFIWGQGT LTVTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ SIGRWLAWYQQKPKGAPKLLIYSASN LETGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQYNRFPVTFGPGTKVDIK	383	384
AB003174_2B07v11	AB00317 4	QVQLVQSGAEVKKPGASVKVS CKASGDTFTTRYVYVHWVRQAPG QGLEWMGIINPSGGYASYAQKF QGRVTMTTRDTSTSTVYMESSL RSEDTAVYYCAAGLFIWGQGT LTVTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ SIGRELAWYQQKPKGAPKLLIYSASN LETGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQYNRFPVTFGPGTKVDIK	385	386
AB003178_2B07v12	AB00317 8	QVQLVQSGAEVKKPGASVKVS CKASGDEFTRYVYVHWVRQAPG QGLEWMGIINPSGGYASYAQKF QGRVTMTTRDTSTSTVYMESSL	DIQMTQSPSSLSASVGDRVTITCRASQ SIGRWLAWYQQKPKGAPKLLIYSASN LETGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQYNRFPVTFGPGTKVDIK	387	388

ID	antibody ID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		RSEDTAVYYCAAGLFIWGQGT LVTSS			
AB003180_2B07v13	AB003180	QVQLVQSGAEVKKPGASVKVS CKASGDEFTYYVHWVRQAPG QGLEWMGIQNPSSGGYASYAQK FQGRVTMTRDTSTSTVYME LSS LRSEDTAVYYCAAGLFIWGQGT LVTSS	DIQMTQSPSSLSASVGDRVTITCRASQ SIGRWLA WYQQKPGKAPKLLIYSASN LETGVPSRFSGSGGTDFTLTISSLP QE DFATYYCQQYNRFPVTFPGTKVDIK	389	390
AB003183_2B07v14	AB003183	QVQLVQSGAEVKKPGASVKVS CKASGDEFTYYVHWVRQAPG QGLEWMGIQNPSSGGYASYAQK FQGRVTMTRDTSTSTVYME LSS LRSEDTAVYYCAAGLFIWGQGT LVTSS	DIQMTQSPSSLSASVGDRVTITCRASQ SIGRELA WYQQKPGKAPKLLIYDASN LETGVPSRFSGSGGTDFTLTISSLP QE DFATYYCQQYNRFPVTFPGTKVDIK	391	392
AB003200_2B05v1	AB003200	QVQLVQSGAEVKKPGASVKVS CKASGETFTNYYIHWVRQAPG QGLEWMGVNPRAGYTSYALK FQGRVTMTRDTSTSTVYME LSS	DIQMTQSPSSLSASVGDRVTITCRISQS ISSWLA WYQQKPGKAPKLLIYSASSL QSGVPSRFSGSGGTDFTLTISSLP QPED FATYYCQQQSFTSPITFGQGT RLEIK	393	394

ID	antibody ID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		LRSEDTAVYYCAGGWEDWGQ GTLVTVSS			
AB003201_2B05v2	AB003201	QVQLVQSGAEVKKPGASVKVS CKASGGTFTNYYIHWVRQAPG QGLEWMGIIDPRAGYTSYALKF QGRVTMTTRDTSTSTVYMELSSL RSEDTAVYYCAGGWEDWGQ TLVTVSS	DIQMTQSPSSLSASVGDRTITCRISQS ISSWLAWYQQKPGKAPKLLIYSASSL QSGVPSRFSGSGGTDFTLTISSLQPED FATYYCQQSFTSPITFGQGRLEIK	395	396
AB003202_2B05v3	AB003202	QVQLVQSGAEVKKPGASVKVS CKASGGTFTNYYIHWVRQAPGQ GLEWMGIIDPRAGYTSYALKFQ GRVTMTTRDTSTSTVYMELSSLR SEDTAVYYCAGGWEDWGQGT LVTVSS	DIQMTQSPSSLSASVGDRTITCRISQS ISSFLAWYQQKPGKAPKLLIYSASSLQ SGVPSRFSGSGGTDFTLTISSLQPEDF ATYYCQQSFTSPITFGQGRLEIK	397	398
AB003209_2B05v4	AB003209	QVQLVQSGAEVKKPGASVKVS CKASGETFTNYYIHWVRQAPG QGLEWMGIIDPRAGYTSYALKF QGRVTMTTRDTSTSTVYMELSSL	DIQMTQSPSSLSASVGDRTITCRISQS ISSFLAWYQQKPGKAPKLLIYSASSLQ SGVPSRFSGSGGTDFTLTISSLQPEDF ATYYCQQSFTSPITFGQGRLEIK	399	400

ID	antibody ID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		RSEDTAVYYCAGGWEDWGQ TLVTVSS			
AB003232_2B05v5	AB00323 2	QVQLVQSGAEVKKPGASVKVS CKASGTTFTNYYIHWRQAPG QGLEWMGVNPRAGYTSYALK FQGRVTMTRDTSTSTVYMELSS LRSEDTAVYYCTGGWLDWGQ GTLVTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ SISSWLAWYQQKPKGAPKLLIYSASS LQSGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQSVTMPVTFGGQTRLEIK	401	402
AB003422_2B05v5	AB00342 2	QVQLVQSGAEVKKPGASVKVS CKASGYTFTNYYIHWRQAPG QGLEWMGVNPRAGYTSYALK FQGRVTMTRDTSTSTVYMELSS LRSEDTAVYYCAGGWLDWGQ GTLVTVSS	DIQMTQSPSSLSASVGDRVTITCRASQ RISSWLAWYQQKPKGAPKLLIYDASS LQSGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQSFTMPVTFGGQTRLEIK	403	404
AB003465_2A11v9	AB00346 5	QVQLVQSGAEVKKPGASVKVS CKASGFTFTRYMHWRQAPG QGLEWMGVNPSGGYATYAQK FQGRVTMTRDTSTSTVYMELSS	DIQMTQSPSSLSASVGDRVTITCRASQ SISSWLAWYQQKPKGAPKLLIYATST LESGVPSRFSGSGGTDFTLTISSLQPE DFATYYCQQQSYFPPTFGGQTKVEIK	405	406

ID	antibody ID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		LRSEDTAVYYCAGWDVWGQ GTLVTVSS			
AB003466_2B07v10	AB003466 6	QVQLVQSGAEVKKPGASVKVS CKASGETFTTRYVYVHWVRQAPG QGLEWMGIINPSSGGYASYAQKF QGRVTMTTRDTSTSTVYMESSL RSEDTAVYYCAAGLFIWGQGT LVTVSS	DIQMTQSPSSLSASVGDRTVTITCRASQ SIGRWLAWYQQKPKGAPKLLIYSASN LETGVPSRFSGSGGTDFTLTISLQPE DFATYYCQQYNFPTFGPGTKVDIK	407	408
AB003467_7A04v9	AB003467 7	QVQLVQSGAEVKKPGASVKVS CKASGYTFTTRYVYVHWVRQAPG QGLEWMGIINPSSGGYASYALKF QGRVTMTTRDTSTSTVYMESSL RSEDTAVYYCTSGWDVWGQGT LVTVSS	DIQMTQSPSSLSASVGDRTVTITCRASQ SISTWLAWYQQKPKGAPKLLIYAASS LDTGVPSRFSGSGGTDFTLTISLQPE DFATYYCQQSYSPVTFGGQGTKVEIK	409	410
AB003470_knd_A04	AB003470 0	QVQLVQSGAEVKKPGSSVKVS CKASGYTFTAYYIHWVRQAPG QGLEFMGWHPYSGGTNYAQK FQGRVTITADESTSTAYMESSL	DIQMTQSPSSLSASVGDRTVTITCRASQ TISRYLNWYQQKPKGAPKLLIYAASS LQSGVPSRFSGSGGTDFTLTISLQPE DFATYYCQQSYSTPRTFGGQGTKLEIK	411	412

ID	antibodyID	V _H	V _L	V _H SEQ ID NO:	V _L SEQ ID NO:
		RSEDTA VYYCAIGYYGKFDY WGQGTLVTVSS			

TABLE 19: Sequences of V_H and V_L CDRs for DBAs described in Example 12

ID	antibodyID	V _H CDR1	V _H CDR2	V _H CDR3	V _L CDR1	V _L CDR2	V _L CDR3	V _L CDR1 SEQ ID NO:	V _L CDR2 SEQ ID NO:	V _L CDR3 SEQ ID NO:
AB002022_2B07v1	AB002022_2B07v1	GDTFTR HYVH	IINPSGGYASY AQKFQG	AAGLFI	RASQSIGRW LA	RASQSIGRW LA	QQYES FPV	431	432	433
AB002293_2B07v2	AB002293_2B07v2	GDTFTR YYVH	IINPSGGYASY AQKFQG	AAGLFI	RASQSIGRW LA	RASQSIGRW LA	QQYN SFPV	437	438	439
AB002326_2B07v3	AB002326_2B07v3	GDTFTR YYVH	IINPSGGYASY AQKFQG	AAGLFI	RASQSIGW LA	RASQSIGW LA	QQYN SFPV	443	444	445
AB002328_2B07v4	AB002328_2B07v4	GDTFTR YYVH	IINPSGGYASY AQKFQG	AAGLFI	RASQSIGRW LA	RASQSIGRW LA	QQYN RFPV	449	450	451
AB002342_2B07v5	AB002342_2B07v5	GDTFTR YYVH	IINPSGGYASY AQKFQG	AAGLFI	RASQSIGRW LA	RASQSIGRW LA	QQYE RFPV	455	456	457

ID	antibo dyID	V_H_cdr1	V_H_cdr2	V_H_cdr3	V_L_cdr1	V_L_cdr r2	V_L_cdr 3	V_H_ cdr1 SEQ ID NO:	V_H_ cdr2 SEQ ID NO:	V_H_ cdr3 SEQ ID NO:	V_L_ cdr1 SEQ ID NO:	V_L_ cdr2 SEQ ID NO:	V_L_ cdr3 SEQ ID NO:
AB002345_ 2B07v6	AB00 2345	GDTFTR YYVH	IINPSGGYASY AQKFQG	AAGLFI	RASQSIGRY LA	SASN LET	QQYN SFPV	461	462	463	464	465	466
AB002347_ 2B07v7	AB00 2347	GDTFTR YYVH	IINPSGGYASY AQKFQG	AAGLFI	RASQSIGRY LA	SASN LET	QQYN RFPV	467	468	469	470	471	472
AB002348_ 2B07v8	AB00 2348	GDTFTR YYVH	IINPSGGYASY AQKFQG	AAGLFI	RASQSIGRY LA	YAS NLET	QQYN SFPV	473	474	475	476	477	478
AB002353_ 2B07v9	AB00 2353	GDTFTR YYVH	IINPSGGYASY AQKFQG	AAGLFI	RASQSIGRW LA	SASN LET	QQYES FPV	479	480	481	482	483	484
AB002360_ 7A04v1	AB00 2360	GYTFTD YYMH	IINPRAGYTSY ALKFQG	TSGWDV	RASQSIGRW LA	AASS LDS	QQSYS FPV	485	486	487	488	489	490
AB002365_ 7A04v2	AB00 2365	GYTFTD YYMH	IINPRAGYTSY ALKFQG	TSGWDV	RASQSIGRW LA	AASS LDS	QQSYS FPV	491	492	493	494	495	496
AB002370_ 7A04v3	AB00 2370	GYTFTT YYMH	IINPRAGYTSY ALKFQG	TSGWDV	RASQSIGRW LA	AASS LDS	QQSYS FPV	497	498	499	500	501	502
AB002381_ 7A04v4	AB00 2381	GDTFTD YYMH	IINPRAGYTSY ALKFQG	TSGWDV	RASQSIGRW LA	AASS LDS	QQSYS FPV	503	504	505	506	507	508
AB002410_ 2A11v2	AB00 2410	GHTFTR YYMH	IINPSGGYATY AQKFQG	ASGWV	RASQSIGRW LA	DTST LES	QQSYS FPV	509	510	511	512	513	514

ID	antibo dyID	V _{H_cdr1}	V _{H_cdr2}	V _{H_cdr3}	V _{L_cdr1}	V _{L_cdr2}	V _{L_cdr3}	V _{H_L} cdr1 SEQ ID NO:	V _{H_L} cdr2 SEQ ID NO:	V _{H_L} cdr3 SEQ ID NO:	V _{L_c} dr1 SEQ ID NO:	V _{L_c} dr2 SEQ ID NO:	V _{L_c} dr3 SEQ ID NO:
AB002413_ 2A11v3	AB00 2413	GHTFTR YYMH	IINPSGGYATY AQKFQG	ASGLFI	RASQSINSW LA	ATST LES	QQYY RFPV	515	516	517	518	519	520
AB002417_ 2A11v4	AB00 2417	GHTFTR YYMH	IINPSGGYATY AQKFQG	ASGLFI	RASQSINSW LA	ATST LES	QQYY SFPV	521	522	523	524	525	526
AB002427_ 2A11v5	AB00 2427	GHTFTR YYMH	IINPSGGYATY AQKFQG	ASGLFI	RASQSINSW LA	ATST LES	QQSY RFPV	527	528	529	530	531	532
AB002520_ 7A04v8	AB00 2520	GYTFTD YYMH	IINPRAGYTSY ALKFQG	TSGWDV	RASQSISTW LA	AASS LDS	QQSY RFPV	533	534	535	536	537	538
AB002521_ 2A11v1	AB00 2521	GHTFTR YYMH	IINPSGGYATY AQKFQG	ASGLDI	RASQSINSW LA	ATST LES	QQSY RFPV	539	540	541	542	543	544
AB002829_ knd_A08	AB00 2829	GYIFNG YDIH	WMNPDNGNT GLAQKFQG	ARGMATRFPY YYYGMDV	RSSQSLLHS NGYNYLD	LGSN RAS	MQGT HWPP	545	546	547	548	549	550
AB002864_ 2A11v6	AB00 2864	GHTFTR YYMH	VINPSGGYAT YAQKFQG	ASGWDV	RASQSINSW LA	ATST LES	QQSYS SPV	551	552	553	554	555	556
AB003005_ 7A04v5	AB00 3005	GYTFTTR YYMH	IIQPRAGYTSY ALKFQG	TSGWDV	RASQSISTW LA	AASS LDE	QQSYS FPV	557	558	559	560	561	562
AB003007_ 7A04v6	AB00 3007	GYTFTTR YYMH	IINPRAGYTSY ALKFQG	TSGWEV	RASQSISTW LA	AASS LDE	QQSYS FPV	563	564	565	566	567	568

ID	antibo dyID	V _{H_cdr1}	V _{H_cdr2}	V _{H_cdr3}	V _{L_cdr1}	V _{L_cdr2}	V _{L_cdr3}	V _{H_cdr1} SEQ ID NO:	V _{H_cdr2} SEQ ID NO:	V _{H_cdr3} SEQ ID NO:	V _{L_cdr1} SEQ ID NO:	V _{L_cdr2} SEQ ID NO:	V _{L_cdr3} SEQ ID NO:
AB003012_7A04v7	AB003012_3012	GYTFTR YYMH	IINPRAGYTSY ALKFQG	TSGWDV	RASQISSTW LA	AASS LDE	QQSYS FPV	569	570	571	572	573	574
AB003021_2A11v7	AB003021_3021	GFTFTR YYMH	VINPSGGYAT YAQKFQG	ASGWDV	RASQISSW LA	ATST LES	QQSYS SPV		576	577	578	579	580
AB003155_2B07v10	AB003155_3155	GETFTR YYVH	IINPSGGYASY AQKFQG	AAGLFI	RASQIGRW LA	SASN LET	QQYN RFPV	581	582	583	584	585	586
AB003174_2B07v11	AB003174_3174	GDTFTR YYVH	IINPSGGYASY AQKFQG	AAGLFI	RASQIGRE LA	SASN LET	QQYN RFPV	587	588	589	590	591	592
AB003178_2B07v12	AB003178_3178	GDEFTR YYVH	IINPSGGYASY AQKFQG	AAGLFI	RASQIGRW LA	SASN LET	QQYN RFPV	593	594	595	596	597	598
AB003180_2B07v13	AB003180_3180	GDEFTR YYVH	IQNPSGGYASY AQKFQG	AAGLFI	RASQIGRW LA	SASN LET	QQYN RFPV	599	600	601	602	603	604
AB003183_2B07v14	AB003183_3183	GDEFTR YYVH	IQNPSGGYASY AQKFQG	AAGLFI	RASQIGRE LA	DAS NLET	QQYN RFPV	605	606	607	608	609	610
AB003200_2B05v1	AB003200_3200	GETFTN YYIH	VINPRAGYTSY ALKFQG	AGGWED	RISQISSWL A	SASS LQS	QQSFT SPI	611	612	613	614	615	616
AB003201_2B05v2	AB003201_3201	GGTFTN YYIH	IIDPRAGYTSY ALKFQG	AGGWED	RISQISSWL A	SASS LQS	QQSFT SPI	617	618	619	620	621	622

ID	antibo dyID	V _{H_cdr1}	V _{H_cdr2}	V _{H_cdr3}	V _{L_cdr1}	V _{L_cdr2}	V _{L_cdr3}	V _{L_cdr1} SEQ ID NO:	V _{H_cdr1} SEQ ID NO:	V _{L_cdr1} SEQ ID NO:	V _{L_cdr2} SEQ ID NO:	V _{L_cdr3} SEQ ID NO:
AB003202_2B05v3	AB00 3202	GSTFTN YYIH	IIDPRAGYTSY ALKFQG	AGGWED	RISQSISSFL A	SASS LQS	QQSFT SPI	623	624	625	626	627
AB003209_2B05v4	AB00 3209	GETFTN YYIH	IIDPRAGYTSY ALKFQG	AGGWED	RISQSISSFL A	SASS LQS	QQSFT SPI	629	630	631	632	633
AB003232_2B05v5	AB00 3232	GTFTFN YYIH	VINPRAGYTSY ALKFQG	TGGWLD	RASQSISSW LA	SASS LQS	QQSVT MPV	635	636	637	638	640
AB003422_2B05v5	AB00 3422	GYTFTN YYIH	VINPRAGYTSY ALKFQG	AGGWLD	RASQSISSW LA	DASS LQS	QQSFT MPV	641	642	643	644	645
AB003465_2A11v9	AB00 3465	GFTFTR YYMH	VINPSGGYAT YAQKFQG	ASGWDV	RASQSISSW LA	ATST LES	QQSYS FPP	647	648	649	650	652
AB003466_2B07v10	AB00 3466	GETFTR YYVH	IINPSGGYASY AQKFQG	AAGLFI	RASQSIGRW LA	SASN LET	QQYN SFPV	653	654	655	656	658
AB003467_7A04v9	AB00 3467	GYTFTR YYMH	IINPRAGYTSY ALKFQG	TSGWDV	RASQSISSW LA	AASS LDT	QQSYS FPV	659	660	661	662	664
AB003470_knd_A04	AB00 3470	GYTFTA YYIH	WIHPYSGGTN YAQKFQG	AIGYYGKFD Y	RASQTSRY LN	AASS LQS	QQSYS TPR	665	666	667	668	670

EXAMPLE 13**Regulated IL-2 Receptor Binding by PD-1/IL-2 Cytokine Complexes containing additional Dual Binding Antibody (DBA) domains *in vitro***

[0136] This example describes PD-1 regulated IL-2 receptor binding by PD-1/IL-2 DBA-cytokine complexes including the additional DBA binding elements described in **Example 12**. Anti PD-1/IL-2 DBA-cytokine complexes were analyzed along with suitable non-regulated controls such as anti-PD-1, anti Her2, anti PDL-1 or anti IL-2 cytokine complexes. The DBA-cytokine complexes and control antibody-cytokine complexes were produced in two formats: (1) “Symmetric” immunocytokine (shown in **FIG. 2E**); or (2) “Asymmetric” immunocytokine (shown in **FIG. 2B**) by expression in mammalian cells and purified using standard protocols. An ELISA assay was performed with a constant amount of the antibody-cytokine construct coated on each well probed with biotinylated IL-2 receptor beta gamma heterodimer Fc (IL-2RBG; Acro Cat #: ILG-H5254) in the presence of varying amounts of PD-1-Fc or hIgG1-Fc. To perform the assay, 384-well ELISA plates were coated with anti-Fc antibody at 1 micrograms/ml in 100 mM bicarbonate solution pH 9.0 overnight at 4°C and washed twice with SuperBlock. Antibody-cytokine complexes were then added to each well at a constant concentration of 6nM and allowed to incubate for 1 hour and washed three times in PBS plus 0.05% Tween 20 (PBST). Titrated concentrations of PD-1 Fc or an IgG1 control Fc were added and allowed to incubate for 15 minutes before the addition of a constant amount of biotinylated IL-2RBG at 10nM. The plates were incubated for an additional 30 minutes, washed and biotinylated IL-2RBG detection was performed using streptavidin-HRP and standard ELISA protocols.

Symmetric Designs

[0137] Results with protein complexes comprising the structure shown in **FIG. 2E** are shown in **FIG. 9**. As depicted in **FIG. 2E**, this symmetric format is comprised of one IL-2 linked to each antibody variable domain. As can be seen for the behavior of these constructs in **FIG. 9**, IL-2RBG binding increased in a dose dependent manner with the addition of PD-1 Fc (but not with the addition of negative control hIgG1 Fc protein) for the DBA-cytokine complexes AF3247, AF3644, AF3651, AF3652, AF3653, AF3657, AF3930, AF3931, AF3933, AF3934, and AF3935 comprising the peptide IDs noted in **TABLE 35** below (the sequences of the referenced peptides can be found in Table 2A). IL-2RBG binding for the control monospecific antibody-cytokine complexes including anti Her2 (AF3243) and anti IL-2 (AF3246) did not change with the addition of PD-1 Fc protein.

TABLE 20: Multiprotein Components of “Symmetric” Immunocytokine designs and controls tested in Example 13

Protein Complex	peptide1	peptide2	peptide3	Antibody1
AF003232	PEP003639	PEP003648	PEP003642	AB002022_2B07v1
AF003740	PEP004243	PEP004247	PEP004159	AB002293_2B07v2
AF003747	PEP004243	PEP004247	PEP004162	AB002345_2B07v6
AF003749	PEP004243	PEP004247	PEP004163	AB002348_2B07v8
AF003753	PEP004243	PEP004247	PEP003642	AB002353_2B07v9
AF003941	PEP004251	PEP004443	PEP000169	AB001054_PDL1_DB02_D10 control
AF003945	PEP004243	PEP004438	PEP004421	AB002326_2B07v3
AF003947	PEP004243	PEP004438	PEP004420	AB002342_2B07v5
AF003951	PEP004433	PEP004442	PEP004427	AB002360_7A04v1
AF003952	PEP004435	PEP004445	PEP004427	AB002365_7A04v2
AF003953	PEP004436	PEP004446	PEP004427	AB002370_7A04v3
AF003955	PEP004431	PEP004440	PEP004423	AB002413_2A11v3
AF003956	PEP004433	PEP004442	PEP004426	AB002520_7A04v8
AF003243	PEP003655	PEP000113		AB001203_trastuzumab control
AF003246	PEP003654	PEP003641		AB001923_Anti-IL2 control

Asymmetric Designs

[0138] Results with protein complexes comprising the structures depicted in **FIG. 2B**

(“asymmetric” immunocytokine design) and described in **TABLE 21** below are shown in **FIG. 10**. This format is composed of an asymmetric complex comprised of two antibody domains with a single IL-2 linked to one of the domains. IL-2RBG binding increased in a dose dependent manner with the addition of PD-1 Fc but not with the addition of an IgG1 control Fc protein for the DBA-cytokine complexes AF3232, AF3740, AF3747, AF3749, AF3753, AF3945, AF3947, AF3951, AF3952, AF3953, AF3955, and AF3956 comprising the peptide IDs noted in **TABLE 21** below (the sequences of the referenced peptides can be found in **Table 2A**). IL-2RBG binding for the control monospecific antibody-cytokine complex anti PDL-1 (AF3941) did not change with the addition of PD-1 Fc protein.

TABLE 21: Multiprotein Components of “Asymmetric” Immunocytokine designs and controls tested in Example 13

Protein Complex	peptide1	peptide2	peptide2	Antibody
AF003232	PEP003639	PEP003648	PEP003642	AB002022_2B07v1
AF003740	PEP004243	PEP004247	PEP004159	AB002293_2B07v2
AF003747	PEP004243	PEP004247	PEP004162	AB002345_2B07v6
AF003749	PEP004243	PEP004247	PEP004163	AB002348_2B07v8
AF003753	PEP004243	PEP004247	PEP003642	AB002353_2B07v9
AF003945	PEP004243	PEP004438	PEP004421	AB002326_2B07v3
AF003947	PEP004243	PEP004438	PEP004420	AB002342_2B07v5
AF003951	PEP004433	PEP004442	PEP004427	AB002360_7A04v1
AF003952	PEP004435	PEP004445	PEP004427	AB002365_7A04v2
AF003953	PEP004436	PEP004446	PEP004427	AB002370_7A04v3
AF003955	PEP004431	PEP004440	PEP004423	AB002413_2A11v3
AF003956	PEP004433	PEP004442	PEP004426	AB002520_7A04v8
AF003941	PEP004251	PEP004443	PEP000169	AB001054_PDL1_DB02_D10 control

EXAMPLE 14**Inhibition of Two Forms of IL-2 Binding to IL-2RBG to PD-1/IL-2 Cytokine Complexes *in vitro***

[0139] This example describes two forms of IL-2 in PD-1/IL-2 DBA-cytokine complexes binding to IL-2RBG. Antibody-cytokine complexes in the format depicted in **FIG. 2H** were generated with wild-type (WT) IL-2 or IL-2 3x (an IL-2 variant with reduced binding to IL-2Ralpha and having R38D, K43E, and E61R mutations, see e.g. Vazquez-Lombardi et al. Nat Commun. 8:15371 2017, which is incorporated by reference in its entirety herein). The DBA-cytokine complexes and control antibody-cytokine complexes were produced by expression in mammalian cells and purified using standard protocols. To examine the ability of DBA binding domains to block IL-2 from binding to IL-2RBG, an ELISA assay was performed with a constant amount of the antibody-cytokine construct coated on each well probed with biotinylated IL-2 receptor beta gamma heterodimer Fc (IL-2RBG; Acro Cat #: ILG-H5254. In these experiments, 384-well ELISA plates were coated with anti-Fc antibody (Jackson ImmunoResearch) at 1 micrograms/ml in 100 mM bicarbonate solution pH 9.0 overnight at 4°C and washed twice with SuperBlock (ThermoFisher, 37515). Antibody-cytokine complexes were then added to each well at a constant concentration of 6nM and allowed to incubate for 1 hour

and washed three times in PBS plus 0.05% Tween 20 (PBST). Titrating concentrations of biotinylated IL-2RBG were added and the plates were incubated for an additional 45 minutes. After washing, the biotinylated IL-2RBG detection was performed using streptavidin-HRP and standard ELISA protocols.

[0140] Results with protein complexes described in **TABLE 22** below are shown in **FIG. 11**. As depicted in **FIG. 2H**, these complexes are asymmetric and comprised of two identical monospecific Fab arms with a single IL-2 (either WT or 3x) attached to one Fc domain by flexible linker and a single scFv attached to the other Fc domain by a flexible linker. The antibody-cytokine complexes comprise an anti-PD-1 domain in the Fab arms and a PD-1/IL-2 DBA scFv on the Fc arm. The control antibody-cytokine complexes comprise the same anti-PD-1 domain in the Fab arms and an anti HER2 monospecific scFv on the Fc arm. In **FIG. 11** panel A, the antibody-cytokine complexes contain the WT IL-2 form on the Fc domain and in **FIG. 11** panel B, the antibody-cytokine complexes contain the 3x IL-2 form on the Fc. As shown in **FIG. 11** panel A, the PD-1/IL-2 DBA complexes AF5418 and AF5419 have reduced IL-2RBG binding compared to the anti Her2 non DBA control complex AF5416 demonstrating the ability of the DBA domains to block receptor binding to WT IL-2. As shown in **FIG. 11** panel B, the PD-1/IL-2 DBA complexes AF4695 and AF4696 have reduced IL-2RBG binding compared to the anti Her2 non DBA control complex AF4693 demonstrating the ability of the DBA domains to block receptor binding to IL-2 3x.

TABLE 22: Multiprotein Components of “Cterm” Immunocytokine designs and controls tested in Example 14

Peptide ID	peptide1	peptide2	peptide3	Antibody1	Antibody2
AF0046 93	PEP0049 67	PEP0050 90	PEP0047 29	AB001203_trastuzu mab control	AB000694_PD1_nivol umab control
AF0046 95	PEP0049 67	PEP0050 91	PEP0047 29	AB002328_2B07v4	AB000694_PD1_nivol umab control
AF0046 96	PEP0049 67	PEP0050 92	PEP0047 29	AB002365_7A04v2	AB000694_PD1_nivol umab control
AF0054 16	PEP0061 77	PEP0050 90	PEP0047 29	AB001203_trastuzu mab control	AB000694_PD1_nivol umab control
AF0054 18	PEP0061 77	PEP0050 91	PEP0047 29	AB002328_2B07v4	AB000694_PD1_nivol umab control
AF0054 19	PEP0061 77	PEP0050 92	PEP0047 29	AB002365_7A04v2	AB000694_PD1_nivol umab control

EXAMPLE 15**Regulated IL-2 Receptor Signaling by PD-1/IL-2 Dual Binding Antibody (DBA) Cytokine Complexes in Cellular Assays**

[0141] This example describes PD-1 regulated IL-2 activity in a HEK-Blue™ IL-2 reporter cell by PD-1/IL-2 DBA-cytokine complexes. Anti PD-1/IL-2 DBA-cytokine complexes were analyzed along with suitable non-regulated controls such as anti Her2, anti PDL-1 or anti IL-2 cytokine complexes. The DBA-cytokine complexes and control antibody-cytokine complexes were produced in five formats shown in **FIG. 2B, 2D, 2E, 2G, 2H, and 2I** by expression in mammalian cells and purified using standard protocols; the peptide components of these complexes are outlined in **TABLE 23** below (where sequences of individual components can be found in Table 2A). A cell-based reporter assay was performed for each of the five formats in the presence of varying amounts of PD-1-Fc or hIgG1-Fc.

TABLE 23: Multiprotein Components of Immunocytokine designs and controls tested in Example 15

Protein Complex	Format (Figure)	Chain 1	Chain 2	Chain 3	Antibody1	Antibody2
AF003 232	2B	PEP003 639	PEP003 648	PEP003 642	AB002022_2B07v1	-
AF003 744	2B	PEP004 243	PEP004 247	PEP004 158	AB002328_2B07v4	-
AF003 747	2B	PEP004 243	PEP004 247	PEP004 162	AB002345_2B07v6	-
AF003 941	2B	PEP004 251	PEP004 443	PEP000 169	AB001054_PDL1_D B02_D10 control	-
AF003 946	2B	PEP004 243	PEP004 438	PEP004 158	AB002328_2B07v4	-
AF003 948	2B	PEP004 243	PEP004 438	PEP004 162	AB002345_2B07v6	-
AF003 952	2B	PEP004 435	PEP004 445	PEP004 427	AB002365_7A04v2	-
AF003 955	2B	PEP004 431	PEP004 440	PEP004 423	AB002413_2A11v3	-
AF003 956	2B	PEP004 433	PEP004 442	PEP004 426	AB002520_7A04v8	-
AF003 345	2D	PEP003 791	PEP003 786		AB002022_2B07v1	-
AF003 243	2E	PEP003 655	PEP000 113		AB001203_trastuzum ab control	-

Protein Complex	Format (Figure)	Chain 1	Chain 2	Chain 3	Antibody1	Antibody2
AF003 246	2E	PEP003 654	PEP003 641		AB001923_Anti-IL2 control	-
AF003 247	2E	PEP003 657	PEP003 642		AB002022_2B07v1	-
AF003 341	2E	PEP003 780	PEP003 782		AB002022_2B07v1	-
AF003 644	2E	PEP004 153	PEP004 159		AB002293_2B07v2	-
AF003 651	2E	PEP004 153	PEP004 162		AB002345_2B07v6	-
AF003 657	2E	PEP004 153	PEP003 642		AB002353_2B07v9	-
AF003 934	2E	PEP004 418	PEP004 427		AB002365_7A04v2	-
AF003 873	2G	PEP004 361	PEP003 642		AB002022_2B07v1	AB000880_PD1_4 C10 control
AF003 876	2G	PEP004 363	PEP004 158		AB002328_2B07v4	AB000880_PD1_4 C10 control
AF003 877	2G	PEP004 363	PEP004 162		AB002345_2B07v6	AB000880_PD1_4 C10 control
AF003 864	2H	PEP003 626	PEP004 356	PEP000 243	AB001923_Anti-IL2 control	AB000881_PD1_control
AF003 871	2H	PEP004 360	PEP000 113	PEP004 360	AB001203_trastuzumab control	AB000880_PD1_4 C10 control
AF003 872	2H	PEP004 359	PEP003 641	PEP004 359	AB001923_Anti-IL2 control	AB000880_PD1_4 C10 control
AF003 913	2H	PEP003 626	PEP004 395	PEP000 243	AB002022_2B07v1	AB000881_PD1_control
AF003 918	2H	PEP003 626	PEP004 398	PEP000 243	AB002328_2B07v4	AB000881_PD1_control
AF003 923	2H	PEP003 626	PEP004 406	PEP000 243	AB002360_7A04v1	AB000881_PD1_control
AF003 927	2H	PEP003 626	PEP004 404	PEP000 243	AB002413_2A11v3	AB000881_PD1_control
AF004 502	2H	PEP005 089	PEP005 090	PEP004 729	AB000694_PD1_nivolumab control	AB001203_trastuzumab control
AF004 503	2H	PEP003 628	PEP005 088	PEP000 113	AB001203_trastuzumab control	AB001203_trastuzumab control
AF004 504	2H	PEP005 089	PEP005 091	PEP004 729	AB002328_2B07v4	AB000694_PD1_nivolumab control
AF004 505	2H	PEP005 089	PEP005 092	PEP004 729	AB002365_7A04v2	AB000694_PD1_nivolumab control
AF004 892	2H	PEP000 288	PEP005 631	PEP000 244	AB002328_2B07v4	AB000881_PD1_control
AF004 893	2H	PEP000 288	PEP005 634	PEP000 244	AB002365_7A04v2	AB000881_PD1_control

Protein Complex	Format (Figure)	Chain 1	Chain 2	Chain 3	Antibody1	Antibody2
AF003632	2I	PEP004130	PEP004129	PEP000244	AB000881_PD1_control	-
AF003634	2I	PEP004134	PEP004133	PEP003782	AB002022_2B07v1	-

[0142] In the experiments shown in **FIGs. 12A, 12B, 13A and 25**, the antibody-cytokine complexes were diluted to a final concentration of 100pM (a concentration previously shown to have a strong reporter signal for the always on control but little to no signal for the always off control) into wells of a 384 well TC treated plate (Corning 3701) in complete DMEM (+10% FBS, 2 mM L-glutamine, sodium pyruvate) along with titrated concentrations of PD-1 Fc or an IgG1 control Fc. After a 15-minute incubation HEK-Blue™ IL-2 reporter cells (12,500 cells) were added to each well and incubated overnight. Five microliters from each well was transferred to a new plate containing 45 microliters of QuantiBlue solution (Invivogen Product # rep-qbs). After 30 to 60 minutes the absorbance at 630 nm was determined using a Perkin-Elmer Envision.

[0143] In an alternative experiment format shown in **FIGs. 13B, 14A, 14B, 20 and 22**, the wells of a 384-well ELISA plate were coated with constant concentration of PD-1-Fc or an IgG1 Fc control protein captured with an anti-Fc antibody (Jackson ImmunoResearch, Prod. # 109-005-098). The cytokine complexes were serially diluted 1:4 for 8 points in growth media from a starting concentration of 6 nM and incubated briefly before addition of the HEK-Blue™ IL-2 reporter cells.

[0144] Results with protein complexes comprising the structure shown in **FIG. 2E** are shown in **FIGs. 12A and 12B**. As depicted in **FIG. 2E**, this symmetric format is comprised of one IL-2 linked to each antibody heavy chain variable domain. IL-2 activity increased in a dose dependent manner with the addition of PD-1 Fc but not with the addition of hIgG1 Fc protein for the DBA-cytokine complexes AF3247, AF3644, AF3651, AF3657, and AF3934. IL-2 activity for the control anti-Her2 AF3243 and anti-IL-2 AF3246 monospecific antibody-cytokine complexes did not change with the addition of PD-1 Fc protein. The symmetric format in **FIG. 12B** is similar to the DBA-cytokine complexes in **FIG. 12A** however the IL-2 is conjugated to the heavy chain variable domain for AF3341 and the light chain variable domain for AF3345. Both symmetric formats demonstrate increased IL-2 activity with the addition of PD-1 Fc but not with the addition of hIgG1 Fc.

[0145] Results with protein complexes comprising the structures depicted in **FIG. 2B** are shown in **FIGS. 13A and 13B**. This format is composed of an asymmetric complex comprised of two antibody domains with a single IL-2 linked to one of the domains. In **FIG. 13A**, IL-2 activity increased in a dose dependent manner with the addition of PD-1 Fc but not with the addition of an IgG1 control Fc protein for the DBA-cytokine complexes AF3232, AF3744, and AF3747. In **FIG. 13B**, results with the asymmetric constructs in the alternative assay format where PD-1 Fc or hIgG1 Fc is captured to the plate are shown. The DBA-cytokine complexes AF3946, AF3948, AF3952, AF3955 and AF3956 demonstrate increased IL-2 activity in the wells coated with PD-1 compared to wells coated with hIgG1 Fc. IL-2 activity for the control anti-PDL-1 monospecific antibody-cytokine complex AF3941 did not change with the addition of PD-1 Fc protein.

[0146] Results with protein complexes comprising the structures depicted in **FIG. 2H** are shown in **FIGS. 14A and 14B**. As depicted in **FIG. 2H**, these complexes are asymmetric and comprised of two identical monospecific Fab arms with a single IL-2 attached to one Fc domain by flexible linker and a single scFv attached to the other Fc domain by a flexible linker.

[0147] In **FIG. 14A**, the PD-1/IL-2 DBA complexes are composed of an anti-PD-1 domain in the Fab arms (a PD1-nivolumab control) and a PD-1/IL-2 DBA scFv on the Fc arm. The control antibody-cytokine complexes are composed of the same anti-PD-1 domain in the Fab arm and a non-DBA scFv on the Fc arm. When titrating amounts of the cytokine complexes are added to the cells, the PD-1/IL-2 DBA containing cytokine complexes AF4504 and AF4505 in the upper graph show decreased reporter activation compared to equimolar amounts of the control anti-HER2 IL-2 immunocytokines AF4502 and AF4503. The same titrating amounts of the cytokine complexes were added to wells coated with PD-1 Fc or human IgG1 Fc control in the lower graphs. Both AF4504 and AF4505 demonstrate increased IL-2 activity in the PD-1 coated wells compared to the wells coated with the human IgG1 Fc. IL-2 activity for the control anti-HER2 IL-2 immunocytokines AF4502 and AF4503 did not change in the wells coated with PD1 Fc compared to the hIgG1 Fc control. In **FIG. 14B**, the PD-1/IL-2 DBA complexes are composed of a different anti-PD-1 domain in the Fab arms (AB000881_PD1_control) and PD-1/IL-2 DBA scFvs on the Fc arm. The PD-1/IL-2 DBA containing cytokine complexes AF3913, AF3918, AF3923 and AF3927 demonstrate increased IL-2 activity in the wells coated with PD-1 Fc compared to wells coated with hIgG1 Fc. IL-2 activity for the anti-IL-2 non-DBA scFv control antibody-cytokine complex AF3864 did not change in the wells coated with PD-1.

[0148] Results with protein complexes comprising the structure depicted in **FIG. 2G** are shown in **FIG. 15**. As depicted in **FIG. 2G**, this symmetric format is comprised of one IL-2 linked to

each antibody heavy chain variable domain in the Fab arm and one scFv attached to each Fc domain. The antibody-cytokine complexes are composed of a PD-1/IL-2 DBA in the Fabs arms and an anti-PD-1 scFv (AB000880_PD1_4C10_control) on the Fc domain. The control antibody-cytokine complexes are composed of the same anti-PD-1 domain in the Fc domain and a non-DBA in the Fab arm. AF3871 has the non-DBA anti-Her2 antibody on the Fab arm and AF3872 has the non-DBA anti-IL-2 antibody on the Fab arm. The PD-1/IL-2 DBA containing complexes AF3873, AF3876 and AF3877 demonstrate increased IL-2 reporter activity in the wells coated with PD-1 Fc compared to the wells coated with hIgG1. IL-2 activity for the two control antibody-cytokine complexes AF3871 and AF3872 did not change in the wells coated with PD-1 Fc.

[0149] Results with protein complexes comprising the structure depicted in **FIG. 2I** are shown in **FIG. 16**. As depicted in **FIG. 2I**, these complexes are asymmetric and comprised of a PD-1/IL-2 DBA in the Fab arms with a single IL-2 attached to one Fc domain by flexible linker. The hinge region of the antibody is a hybrid of the hinge sequence of an IgG1 and IgG3 with the disulfide bridges removed to provide increased flexibility between the Fab arm and the IL-2 cytokine on the Fc domain. The control antibody-cytokine complex is composed of a monospecific anti-PD-1 domain in the Fab arms and the same IL-2 on the Fc domain. IL-2 activity increased in a dose dependent manner with the addition of PD-1 Fc but not with the addition of hIgG1 Fc protein for the DBA-cytokine complex AF3634. IL-2 activity for the control anti-PD-1 monospecific antibody-cytokine complex AF3632 did not change with the addition of PD-1 Fc protein.

[0150] Results with protein complexes comprising the structures depicted in **FIG. 2H** are shown in **FIG. 17**. As depicted in **FIG. 2H**) and similar to the constructs in **FIGs. 14A and 14B**, these complexes are asymmetric and comprised of two identical monospecific Fab arms with a single IL-2 attached to one Fc domain by flexible linker and a single scFv attached to the other Fc domain by a flexible linker. Unique to the constructs in **FIG. 17**, the Fc portion of the construct is a human IgG1 isotype. The two constructs depicted in **FIG. 17** are composed of an anti-PD-1 domain in the Fab arms (AB000881_PD1_control) and a PD-1/IL-2 DBA scFv on the Fc arm. Both PD-1/IL2 DBA containing cytokine complexes AF4892 and AF4893 demonstrate increased IL-2 activity in the wells coated with PD-1 Fc compared to wells coated with mIgG2a Fc control.

EXAMPLE 16**Regulated IL-2 3x Receptor Signaling by PD-1/IL-2 3x Dual Binding Antibody (DBA)****Cytokine Complexes *in vitro***

[0151] This example describes PD-1 regulated IL-2 3x (an IL-2 variant with reduced binding to IL-2Ralpha, Lombardi et al, 2017) activity in a HEK-Blue™ IL-2 reporter cell by PD-1/IL-2 3x DBA-cytokine complexes. Anti PD-1/IL-2 3x DBA-cytokine complexes were analyzed along with suitable non-regulated controls such as anti Her2, anti PD-1 or anti IL-2 cytokine complexes. The DBA-cytokine complexes and control antibody-cytokine complexes were produced in two formats shown in **FIG. 2B and 2H** by expression in mammalian cells and purified using standard protocols. A cell-based reporter assay was performed for each of the two formats in the presence of plate bound PD-1-Fc or hIgG1-Fc. 384-well ELISA plates (Corning 3700) were coated with anti-Fc antibody (Jackson ImmunoResearch) at 1 micrograms/ml in 100 mM bicarbonate solution pH 9.0 overnight at 4°C and washed twice with SuperBlock (ThermoFisher). PD1-Fc or IgG1 control Fc were then added to each well at a constant concentration of 6nM and allowed to incubate for 1 hour and washed three times in PBS plus 0.05% Tween 20 (PBST). The antibody-cytokine complexes were serially diluted 1:4 for 8 points in complete DMEM (+10% FBS, 2 mM L-glutamine, sodium pyruvate) from a starting concentration of 6 nM. After a 15-minute incubation HEK-Blue™ IL-2 reporter cells (12,500 cells) were added to each well and incubated overnight. Five microliters from each well were transferred to a new plate containing 45 microliters of QuantiBlue solution (Invivogen Product # rep-qbs). After 30 to 60 minutes the absorbance at 630 nm was determined using a Perkin-Elmer Envision.

[0152] Results with protein complexes comprising the structures depicted in **FIG. 2B** are shown in **FIG. 18**. This format is composed of an asymmetric complex comprised of two antibody domains with a single IL-2 3x linked to one of the domains. The DBA-cytokine complexes AF4385, AF4386, AF4387, AF4388, and AF4389 demonstrate increased IL-2 3x activity in the wells coated with PD-1 Fc compared to wells coated with hIgG1 Fc. IL-2 3x activity for the control anti-PD-1 monospecific antibody-cytokine complex AF4380 and the anti-IL-2 monospecific antibody-cytokine complex AF4384 did not change with the addition of PD-1 Fc protein.

[0153] Results with protein complexes comprising the structures depicted in **FIG. 2H** are shown in **FIGs. 19A, 19B, and 19C**. As depicted in **FIG. 2H**, these complexes are asymmetric and comprise two identical monospecific Fab arms with a single IL-2 3x attached to one Fc domain by flexible linker and a single scFv attached to the other Fc domain by a flexible linker.

[0154] In **FIG. 19A**, the PD-1/IL-2 DBA complexes are composed of an anti-PD-1 domain in the Fab arms (AB000694_nivo) and a PD-1/IL-2 DBA scFv on the Fc arm. The control antibody-cytokine complexes are composed of the same anti-PD-1 domain in the Fab arms and a non-DBA scFv on the Fc arm. The PD-1/IL-2 3x DBA containing cytokine complexes AF4404, AF4405, AF4695 and AF4696 demonstrate increased IL-2 3x activity in the wells coated with PD-1 Fc compared to wells coated with hIgG1 Fc. IL-2 3x activity for the anti-IL-2 non-DBA scFv control antibody-cytokine complex AF4401 and the anti-Her2 non-DBA scFv control antibody-cytokine complex AF4694 did not change in the wells coated with PD-1 Fc. In **FIG. 19B**, the PD-1/IL-2 DBA complexes are composed of a different anti-PD-1 domain in the Fab arms (AB000880_PD1_R04_C10,) and PD-1/IL-2 DBA scFvs on the Fc arm. The PD-1/IL-2 3x DBA containing cytokine complexes AF4413, AF4414, AF4415 and AF4416 demonstrate increased IL-2 3x activity in the wells coated with PD-1 Fc compared to wells coated with hIgG1 Fc. IL-2 3x activity for the anti-IL-2 non-DBA scFv control antibody-cytokine complex AF4412 did not change in the wells coated with PD-1 Fc. In **FIG. 19C**, the PD-1/IL-2 DBA complexes are composed of anti-PD-1 domains in the Fab arms that do not block PDL-1 binding to PD-1 nor block the binding of nivolumab to PD-1. Both PD-1/IL-2 DBA containing complexes AF4771 and AF4773 demonstrate increased IL-2 3x activity in the wells coated with PD-1 Fc compared to wells coated with hIgG1 Fc.

TABLE 24: Multiprotein Components of Immunocytokine designs and controls tested in Example 16

Protein Complex	Format (Figure)	Chain 1	Chain 2	Chain 3	Antibody1	Antibody2
AF004385	2B	PEP004957	PEP004438	PEP004420	AB002342_2B07v5	-
AF004386	2B	PEP004957	PEP004438	PEP004162	AB002345_2B07v6	-
AF004387	2B	PEP004959	PEP004442	PEP004427	AB002360_7A04v1	-
AF004388	2B	PEP004960	PEP004446	PEP004427	AB002370_7A04v3	-
AF004389	2B	PEP004958	PEP004440	PEP004423	AB002413_2A11v3	-
AF004404	2H	PEP004967	PEP004974	PEP004729	AB002360_7A04v1	AB000694_PD1_nivolumab control
AF004405	2H	PEP004967	PEP004973	PEP004729	AB002413_2A11v3	AB000694_PD1_nivolumab

						mab control
AF004413	2H	PEP004978	PEP004981	PEP000245	AB002342_2B07v5	AB000880_PD1_4C10 control
AF004414	2H	PEP004978	PEP004982	PEP000245	AB002345_2B07v6	AB000880_PD1_4C10 control
AF004415	2H	PEP004978	PEP004984	PEP000245	AB002360_7A04v1	AB000880_PD1_4C10 control
AF004416	2H	PEP004978	PEP004983	PEP000245	AB002413_2A11v3	AB000880_PD1_4C10 control
AF004695	2H	PEP004967	PEP005091	PEP004729	AB002328_2B07v4	AB000694_PD1_nivolu mab control
AF004696	2H	PEP004967	PEP005092	PEP004729	AB002365_7A04v2	AB000694_PD1_nivolu mab control
AF004771	2H	PEP005470	PEP005471	PEP005469	AB002328_2B07v4	AB002829_knd_A04_PD1 antibody
AF004773	2H	PEP005474	PEP005475	PEP005473	AB002328_2B07v4	AB003470_knd_A08_PD1 antibody

EXAMPLE 17

Regulated IL-2 Receptor Signaling by PD-1/IL-2 Dual Binding Antibody (DBA) Cytokine Complexes with Variable Linker Lengths in cells

[0155] This example describes PD-1 regulated IL-2 activity in a HEK-Blue™ IL-2 reporter cell model by PD-1/IL-2 DBA-cytokine complexes with varying linker lengths. The DBA-cytokine complexes were produced in the format shown in **FIG. 2B** where the Glycine-Serine (GS) linker connecting the IL-2 cytokine to the DBA domain is varied from 5 GS repeats to 25 GS repeats. The DBA-cytokine complexes were expressed in mammalian cells and purified using standard protocols. A cell-based reporter assay was performed in the presence of plate bound PD-1-Fc or hIgG1-Fc. In this experiment, 384-well ELISA plates (Corning 3700) were coated with anti-Fc antibody (Jackson ImmunoResearch) at 1 micrograms/ml in 100 mM bicarbonate solution pH 9.0 overnight at 4°C and washed twice with SuperBlock (ThermoFisher). PD1-Fc or IgG1 control Fc were then added to each well at a constant concentration of 6nM and allowed to

incubate for 1 hour and washed three times in PBS plus 0.05% Tween 20 (PBST). The antibody-cytokine complexes were serially diluted 1:4 for 8 points in complete DMEM (+10% FBS, 2 mM L-glutamine, sodium pyruvate) from a starting concentration of 6 nM. After a 15-minute incubation HEK-Blue™ IL-2 reporter cells (12,500 cells) were added to each well and incubated overnight. Five microliters from each well were transferred to a new plate containing 45 microliters of QuantiBlue solution (Invivogen Product # rep-qbs). After 30 to 60 minutes the absorbance at 630 nm was determined using a Perkin-Elmer Envision.

[0156] Results with the protein complexes depicted in **FIG. 2B** with varied linker lengths, are shown in **FIG. 20**. The asymmetric complex is comprised of two antibody domains with a single IL-2 linked to one of the domains. Varying linker lengths were chosen from GS5 – GS25 to test for linker length dependence on PD-1 regulation. The cytokine complexes containing the PD-1/IL-2 DBA domain 2B07 variants in AF4262, AF4273, AF4284, and AF4295 all demonstrated increased IL-2 activity in the wells coated with PD-1 Fc compared to wells coated with hIgG1 Fc. Similar results were observed in the cytokine complexes containing the PD-1/IL-2 DBA domain 7A04 variants in AF4265, AF4276, AF4287 and AF4298. These data demonstrate that PD-1 regulation is possible with multiple cytokine antibody linker lengths varying from GS5 to GS25.

EXAMPLE 18

PD-1-Dependent Induction of STAT5 Phosphorylation by PD-1/IL-2 DBA-Cytokine Complexes in Human Primary CD8+ T Cells

[0157] This example describes PD-1/IL-2 DBA-cytokine complex induction of STAT5 phosphorylation in primary human CD8+ T cells. The DBA-cytokine complexes were produced in the format shown in **FIG. 2H**. CD8+ T cells were isolated from human PBMCs using immunomagnetic negative selection (STEMCELL) and stimulated with plate-bound anti-CD3 and soluble anti-CD28 for 72 hours to induce expression of PD-1. The stimulated CD8+ T cells were incubated 1 hour with an anti-PD-1 blocking antibody or and isotype control antibody. Titration concentrations of PD-1/IL-2 DBA cytokine complex (PD-1-regulated IL-2) or anti-HER2/IL-2-cytokine complex (Always-on IL-2) were then added to the CD8+ T cells and incubated at 37C for 20 minutes. The CD8+ T cells were fixed with Perm Buffer III (BD Biosciences), washed, and stained with antibodies directed against CD8, CD45RA, CD45RO, and pSTAT5. STAT5 phosphorylation within the CD45RA+ and CD45RO+ T cell populations was assessed by flow cytometry. The results of this experiment are depicted in **FIGs. 21A and 21B**. In CD8+CD45RA+ T cells, which are largely PD-1 negative, the PD-1/IL-2 DBA cytokine

complex induces a lower frequency of STAT5 phosphorylation-positive CD8⁺ T cells compared to the non-regulated anti-HER2/IL-2-cytokine complex control. In CD8⁺CD45RO⁺ T cells, which are largely PD-1 positive, the PD-1/IL-2 DBA cytokine complex induces an equivalent frequency of STAT5 phosphorylation-positive CD8⁺ T cells compared to the non-regulated anti-HER2/IL-2-cytokine complex control. Furthermore, CD8⁺CD45RO⁺ T cells that were pre-treated with an anti-PD-1 blocking antibody had a lower frequency of STAT5 phosphorylation-positive cells following treatment with the PD-1/IL-2 DBA cytokine complex showing the dependence of activity on PD-1 binding. Taken together, these data show that PD-1/IL-2 DBA cytokine complex shows decreased activity on PD-1 negative cells. On PD-1 positive cells, PD-1/IL-2 DBA cytokine complex activity is diminished by PD-1 blockade.

EXAMPLE 19

PD-1/IL-2 DBA-Cytokine Complex Modulation of Human T Cell Activation in a Mixed Lymphocyte Reaction

[0158] This example describes PD-1/IL-2 DBA-cytokine complex modulation of human CD4⁺ T cell activation in a mixed lymphocyte reaction (MLR) model. In this model, we assessed the activation of T cells against foreign antigen-presenting cells and the ability of our immunocytokine constructs to modulate that activation. CD4⁺CD25⁻ T cells were isolated from human PBMCs using immunomagnetic negative selection (STEMCELL) and labeled with CellTrace Violet proliferation dye (ThermoFisher) following the manufactures protocol. To generate monocyte-derived dendritic cells (MDDCs), monocytes were isolated from PBMCs of a different donor using immunomagnetic negative selection (STEMCELL) and cultured in the presence of GM-CSF (100ng/mL) and IL-4 (50ng/mL). The culture media was replaced after 3 days, and MDDCs were collected on day 7. 10,000 MDDCs were added to each well of a 96-well round-bottom plate followed by the addition of 50,000 proliferation dye-labeled CD4⁺ T cells. A dilution series of each immunocytokine complex was generated and added to the cell cultures. After 5 days at 37C, cells were stained with a live/dead viability dye (ThermoFisher) and then then incubated in fixation/permeabilization buffer (BD Biosciences) for 20 minutes at 4C. Cells were then stained with fluorophore-conjugated antibodies directed against CD4 and granzyme B (BD Bioscience), and the frequency of granzyme B-expressing cells amongst proliferating CD4⁺ T cells was assessed by flow cytometry. The results of this experiment are depicted in **FIG. 22A** and **FIG. 22B**. While HER2-IL2 induced minimal specific activation of the T cells (as expected because the T cells do not carry the HER2 molecule), the non-regulated and regulated PD1-IL2 constructs were able to stimulate the T cell activation, as indicated by

dose-dependent granzyme B expression. These data demonstrate the PD-1 binding-dependent activity of the regulated PD1-IL2 complexes (via their ability to activate T-cells). Furthermore, the degree of granzyme B induction observed was comparable between the regulated and non-regulated PD1-IL2 complexes, indicating that the addition of the regulating moieties to IL2 does not diminish the activity of IL2 under properly permissive conditions.

EXAMPLE 20

PD-1/IL-2 DBA-Cytokine Complex Modulation of Anti-Tumor Immunity in a Syngeneic Tumor Model

[0159] This example describes PD-1/IL-2 DBA-cytokine complex modulation of anti-tumor immunity in the MC38 syngeneic mouse tumor model. PD-1/IL-2 DBA-cytokine complex was assessed for the ability to drive anti-tumor immunity in vivo. 500,000 MC38 tumor cells were implanted subcutaneously in human PD-1 knock-in mice (GenOway). Tumors were measured twice weekly, and volumes calculated as $(\text{Length} \times \text{Width} \times \text{Width}/2)$. Mice were randomized into treatment groups, and therapy initiated when tumors reached a volume of $\sim 100 \text{ mm}^3$. Mice were treated intravenously with PD-1/IL-2 DBA-cytokine complex, non-regulated anti-PD1-IL2, anti-HER2-IL2, anti-PD-1, or anti-HER2 at 0.5 milligrams per kilogram of body weight on days 7, 10, and 13 post tumor implantation. The results of this experiment are presented in **FIG. 23A** and **23B**. The PD-1/IL-2 DBA-cytokine complexes showed comparable tumor growth inhibition compared to the non-regulated PD1-IL2 and superior tumor growth inhibition compared to anti-PD1 and anti-HER antibodies.

EXAMPLE 21

PD1-IL2 enhancement of T cell bispecific engager activity (prophetic)

[0160] PD-1-regulated immunocytokines can be generated in which the PD1-regulated IL2 is used to enhance the activity of T cell bispecific antibodies (TCBs). In this example, PD1-IL2 TCBs can be generated in which a PD1-IL2 DBA scFv is fused to the C-term of one heavy chain and an IL-2 variant is fused to the C-term of the opposing heavy chain of a TCB. The N-term variable regions of the PD1-IL2 TCB can be directed against CD3 and a tumor associated antigen such as PSMA, HER2, CD20, or against CD3 and an irrelevant antigen. To assess PD1-IL2 TCB activity, human T cells are isolated from fresh PBMCs and co-cultured with tumor cell lines expressing various levels of tumor-associated antigens. Titrating concentrations of naked TCBs or PD1-IL2 TCBs are added to the T cell:tumor cell co-cultures. Tumor killing as well as T cell activation and cytokine production is assessed at various timepoints.

EXAMPLE 22**PD-1-regulated IL-2 activity targeted to T cell-associated antigens (prophetic)**

[0161] PD-1-regulated immunocytokines can be generated in which the PD1-regulated IL2 is targeted to T cells using any T cell marker. For example, PD1-IL2 DBA scFv can be fused to the C-term of one heavy chain and an IL-2 variant can be fused to the C-term of the opposing heavy chain of an antibody directed against a T cell-expressed marker including but not limited to CD28, CD28H, OX40, GITR, CD137, CD27, HVEM, CTLA-4, PD-1, TIM-3, BTLA, VISTA, LAG-3, TIGIT, CD244, ICOS, CD40L, CD4, CD8, KLRG1, FasL, and CD7. T cells can be activated under various conditions to induce the expression of a given T cell marker. Titrating concentrations of PD1-IL2 DBA-containing immunocytokines directed against the marker of interest or an irrelevant marker may then be added. STAT5 phosphorylation may then be assessed as a measurement of targeted IL-2 activity. In some experiments, a blocking antibody against the marker of interest may be added prior to treatment with the PD1-IL2 DBA-containing immunocytokine to show specificity.

[0162] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A complex comprising:
 - (a) a therapeutic domain comprising an IL-2 peptide;
 - (b) a linker; and
 - (c) a sensor domain comprising an antibody, wherein said sensor domain is configured to bind PD-1 and IL-2 in a mutually exclusive manner,wherein the therapeutic domain is linked to the sensor domain by the linker.
2. The complex of claim 1, wherein the sensor domain is configured: (i) to bind IL-2 in the absence of PD-1; and (ii) to not bind IL-2 in the presence of PD-1.
3. The complex of claim 1 or 2, wherein the antibody is an antibody fragment or an antibody derivative.
4. The complex of any one of claims 1-3, wherein the sensor domain comprises a single dual binding antibody (DBA) configured to bind PD-1 and IL-2.
5. The complex of any one of claims 1-4, wherein the DBA comprises a heavy chain CDR3 having at least 80% identity to any one of SEQ ID NOs: 11-20, 154-156, 168-173, 114-119, 415, 421, 433, 439, 445, 451, 457, 463, 469, 475, 481, 487, 493, 499, 505, 511, 517, 523, 529, 535, 541, 547, 553, 559, 565, 571, 577, 583, 589, 595, 601, 607, 613, 619, 625, 631, 637, 643, 649, 655, 661, or 667.
6. The complex of any one of claims 1-4, wherein the DBA comprises a heavy chain CDR1, CDR2, or CDR3 comprising a sequence having at least 80% identity to any of the sequences recited in Table 3, Table 7, Table 8, or Table 19.
7. The complex of any one of claims 1-4, wherein the DBA comprises a V_H or a V_L comprising a sequence having at least 80% identity to any of the sequences recited in Table 18.
8. The complex of any one of claims 1-7, wherein the complex comprises an Fc domain.
9. The complex of claim 8, wherein the Fc domain is homodimeric.
10. The complex of claim 8, wherein the Fc domain is heterodimeric.
11. The complex of claim 10, wherein the Fc domain comprises: (a) a first polypeptide comprising a knob mutation and (b) a second polypeptide comprising a hole mutation.
12. The complex of claim 11, wherein the knob mutation comprises or the hole mutation comprises mutations of any one of following pairs of residues relative to IgG: 366 and 407, 405 and 394, or 407 and 366.

13. The complex of claim 12, wherein the knob mutation comprises an arginine residue, a phenylalanine residue, a tyrosine residue or a tryptophan residue and the hole mutation comprises an alanine residue, a serine residue, a threonine residue, or a valine residue.
14. The complex of any one of claims 1-12, wherein the complex comprises a sensor domain comprising a full-length DBA, wherein the IL-2 peptide is linked to an N-terminus of a heavy chain of said full-length DBA or wherein the IL-2 peptide is linked to an N-terminus of a light chain of said full-length DBA.
15. The complex of any one of claims 1-12, wherein the complex comprises a sensor domain comprising a full-length DBA, wherein the IL-2 peptide is linked to a C-terminus of a heavy chain of said full-length DBA.
16. The complex of any one of claims 1-9 or 13, wherein the complex comprises:
 - (a) a first polypeptide according to N-[IL-2]-[linker]-[V_H]-[C_H]-[hinge]-Fc-C; and
 - a second polypeptide according to N-[V_L]-[C_L]-C, or
 - (b) a first polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc-C; and
 - a second polypeptide according to N-[IL-2]-[linker]-[V_L]-[C_L]-C.wherein N- denotes a peptide N-terminus, C- denotes a peptide C-terminus, [linker] denotes said linker, V_H indicates a heavy chain variable domain of said DBA, C_H indicates a heavy chain constant domain of an immunoglobulin, V_L denotes a light chain variable domain of said DBA, [hinge] denotes a hinge region of an immunoglobulin, Fc denotes an Fc region of an immunoglobulin, and C_L denotes a light chain constant domain of an immunoglobulin.
17. The complex of claim 16, wherein the complex comprises any one of AF003345, AF003243, AF003246, AF003247, AF003341, AF003644, AF003651, AF003657, or AF003934.
18. The complex of any one of claims 1-8, 10-12, or 13, wherein the complex comprises:
 - (a) a first polypeptide according to N-[IL-2]-[linker]-[V_H]-[C_H]-[hinge]-Fc[knob]-C;
 - a second polypeptide according to N-[V_L]-[C_L]-C; and
 - a third polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc[hole]-C, or
 - (b) a first polypeptide according to N-[IL-2]-[linker]-[V_H]-[C_H]-[hinge]-Fc[hole]-C;
 - a second polypeptide according to N-[V_L]-[C_L]-C; and
 - a third polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc[knob]-C,wherein N- denotes a peptide N-terminus, C- denotes a peptide C-terminus, [linker] denotes said linker, V_H indicates a heavy chain variable domain of said DBA, C_H indicates a heavy chain constant domain of an immunoglobulin, V_L denotes a light chain variable domain of said DBA, [hinge] denotes a hinge region of an immunoglobulin, Fc[knob] denotes an Fc of an immunoglobulin comprising a knob mutation, Fc[hole] denotes an Fc region of an

immunoglobulin comprising a hole mutation, and C_L denotes a light chain constant domain of an immunoglobulin.

19. The complex of claim 18, wherein the knob mutation or the hole mutation comprises mutations of any one of following pairs of residues relative to IgG: 366 and 407, 405 and 394, or 407 and 366.
20. The complex of any one of claims 18-19, wherein the complex comprises any one of AF003229, AF003230, AF003232, AF003740, AF003747, AF003749, AF003753, AF003945, AF003947, AF003951, AF003952, AF003953, AF003955, AF003956, or AF003941. The complex of any one of claims 1-9 or 15, wherein the complex comprises:
 - (a) a first polypeptide according to N-[IL-2]-[linker]-[V_H]-[C_H]-[hinge]-Fc-[scFv]-C; and
 - (b) a second polypeptide according to N-[V_L]-[C_L]-C, orwherein N- denotes a peptide N-terminus, C- denotes a peptide C-terminus, [linker] denotes said linker, V_H indicates a heavy chain variable domain of an anti-PD-1 monoselective antibody, C_H indicates a heavy chain constant domain of an immunoglobulin, V_L denotes a light chain variable domain of an anti-PD-1 monoselective antibody, [hinge] denotes a hinge region of an immunoglobulin, Fc denotes an Fc region of an immunoglobulin, C_L denotes a light chain constant domain of an immunoglobulin, and [scFv] denotes an scFv comprising V_H and V_L domains of said DBA.
21. The complex of claim 20, wherein said scFv is oriented according to N-[V_H]-[linker2]-[V_L]-C.
22. The complex of claim 20 or 21 wherein said scFv comprising V_H and V_L domains of said DBA comprises:
 - (a) a V_H domain comprising a sequence having at least 80% identity to a V_H domain of any one of AB002022_2B07v1, AB002328_2B07v4, AB002360_7A04v1, AB002413_2A11v3, AB002342_2B07v5, AB002345_2B07v6, or AB002365_7A04v2; or
 - (b) a V_L domain comprising a sequence having at least 80% identity to a V_L domain of any one of AB002022_2B07v1, AB002328_2B07v4, AB002360_7A04v1, AB002413_2A11v3, AB002342_2B07v5, AB002345_2B07v6, or AB002365_7A04v2.
23. The complex of claim 20 or 21, wherein said scFv comprising V_H and V_L domains of said DBA comprises:
 - (a) heavy chain CDRs of any one of AB002022_2B07v1, AB002328_2B07v4, AB002360_7A04v1, AB002413_2A11v3, AB002342_2B07v5, AB002345_2B07v6, or AB002365_7A04v2; or

- (b) light chain CDRs of any one of AB002022_2B07v1, AB002328_2B07v4, AB002360_7A04v1, AB002413_2A11v3, AB002342_2B07v5, AB002345_2B07v6, or AB002365_7A04v2.
24. The complex of any one of claims 20-22, wherein the complex comprises any one AF003864, AF003871, AF003872, AF003913, AF003918, AF003923, AF003927, AF004502, AF004503, AF004504, AF004505, AF004892, or AF004893.
25. The complex of any one of claims 1-8, 10-12, or 15, wherein the complex comprises:
- (a) a first polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc[knob]-[linker]-[IL-2]-C;
a second polypeptide according to N-[V_L]-[C_L]-C; and
a third polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc[hole]-[linker]-[scFv]-C, or
- (b) a first polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc[hole]-[linker]-[IL-2]-C;
a second polypeptide according to N-[V_L]-[C_L]-C; and
a third polypeptide according to N-[V_H]-[C_H]-[hinge]-Fc[knob]-[linker]-[scFv]-C;
- wherein N- denotes a peptide N-terminus, C- denotes a peptide C-terminus, [linker] denotes said linker, V_H indicates a heavy chain variable domain of said DBA, C_H indicates a heavy chain constant domain of an immunoglobulin, V_L denotes a light chain variable domain of said DBA, [hinge] denotes a hinge region of an immunoglobulin, Fc[knob] denotes an Fc of an immunoglobulin comprising a knob mutation, Fc[hole] denotes an Fc region of an immunoglobulin comprising a hole mutation, C_L denotes a light chain constant domain of an immunoglobulin, and [scFv] denotes an scFv of said DBA.
26. The complex of claim 25, wherein the complex comprises any one AF004693, AF004695, AF004696, AF005416, AF005418, or AF005419.
27. The complex of any one of claims 1-8, 10-12, or 15, wherein the complex comprises:
- (a) a first polypeptide according to N-[V_H]-[C_H]-[het-hinge]-Fc[knob]-[linker]-[IL-2]-C;
a second polypeptide according to N-[V_L]-[C_L]-C; and
a third polypeptide according to N-[V_H]-[C_H]-[het-hinge]-Fc[hole]-C, or
- (b) a first polypeptide according to N-[V_H]-[C_H]-[het-hinge]-Fc[hole]-[linker]-[IL-2]-C;
a second polypeptide according to N-[V_L]-[C_L]-C; and
a third polypeptide according to N-[V_H]-[C_H]-[het-hinge]-Fc[knob]-C,
- wherein N- denotes a peptide N-terminus, C- denotes a peptide C-terminus, [linker] denotes said linker, V_H indicates a heavy chain variable domain of said DBA, C_H indicates a heavy chain constant domain of an immunoglobulin, V_L denotes a light chain variable domain of said DBA, [het hinge] denotes a hinge region heterologous to said Fc region, Fc[knob] denotes an Fc of an immunoglobulin comprising a knob mutation, Fc[hole] denotes an Fc

region of an immunoglobulin comprising a hole mutation, and C_L denotes a light chain constant domain of an immunoglobulin.

28. The complex of claim 27, wherein said hinge region heterologous to said Fc region is: (a) a hinge region derived from an IgG3 antibody, or (b) a G4S-based linker
29. The complex of claim 27 or 28, wherein said complex comprises AF003632 or AF003634.
30. The complex of any one of claims 1-29, wherein the IL-2 peptide comprises a wild-type human IL-2 peptide.
31. The complex of claim 30, wherein the IL-2 peptide comprises a sequence having at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or substantially 100% sequence identity to human IL-2.
32. The complex of claim 31, wherein the complex comprises any one of AF003232, AF003243, AF003246, AF003247, AF003341, AF003345, AF003632, AF003634, AF003644, AF003651, AF003652, AF003653, AF003657, AF003740, AF003744, AF003747, AF003749, AF003753, AF003864, AF003873, AF003876, AF003877, AF003913, AF003918, AF003923, AF003927, AF003930, AF003931, AF003933, AF003934, AF003935, AF003941, AF003945, AF003946, AF003947, AF003948, AF003951, AF003952, AF003953, AF003955, AF003956, AF004262, AF004265, AF004273, AF004276, AF004284, AF004287, AF004295, AF004298, AF004385, AF004386, AF004387, AF004388, AF004389, AF004404, AF004405, AF004413, AF004414, AF004415, AF004416, AF004504, AF004505, AF004693, AF004695, AF004696, AF004771, AF004773, AF004892, or AF004893.
33. A method of enhancing T-cell reactivity to heterologous cells, comprising administering the complex of any one of claims 1-32 to a subject in need thereof.
34. The method of claim 33, wherein the heterologous cells are cancer cells.
35. A method of treating a subject in need thereof comprising administering the complex of any one of claims 1-32 to the subject in need thereof.
36. The method of claim 35, wherein the administering comprises intravenous, intramuscular, or subcutaneous administration.
37. The method of any one of claims 35-36, wherein the subject in need thereof has cancer
38. The method of any one of claims 35-37, wherein the therapeutic domain treats the subject in need thereof.

39. The method of any one of claims 35-38, wherein the subject in need thereof is a mammal.
40. The method of claim 39, wherein the subject in need thereof is a human.
41. A composition comprising a recombinant nucleic acid encoding the complex of any of claims 1-32.
42. A pharmaceutical composition comprising the complex of any of claims 1-32 and a pharmaceutically acceptable excipient.

FIG. 1A

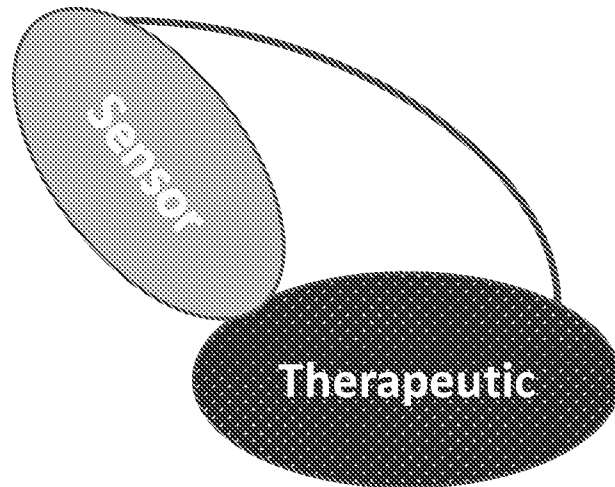


FIG. 1B

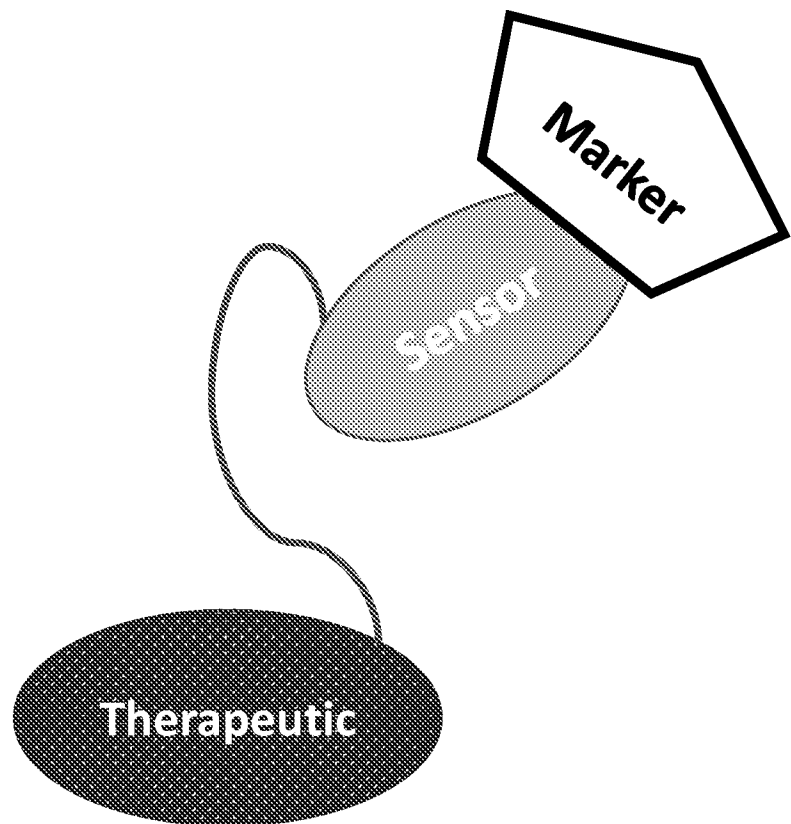
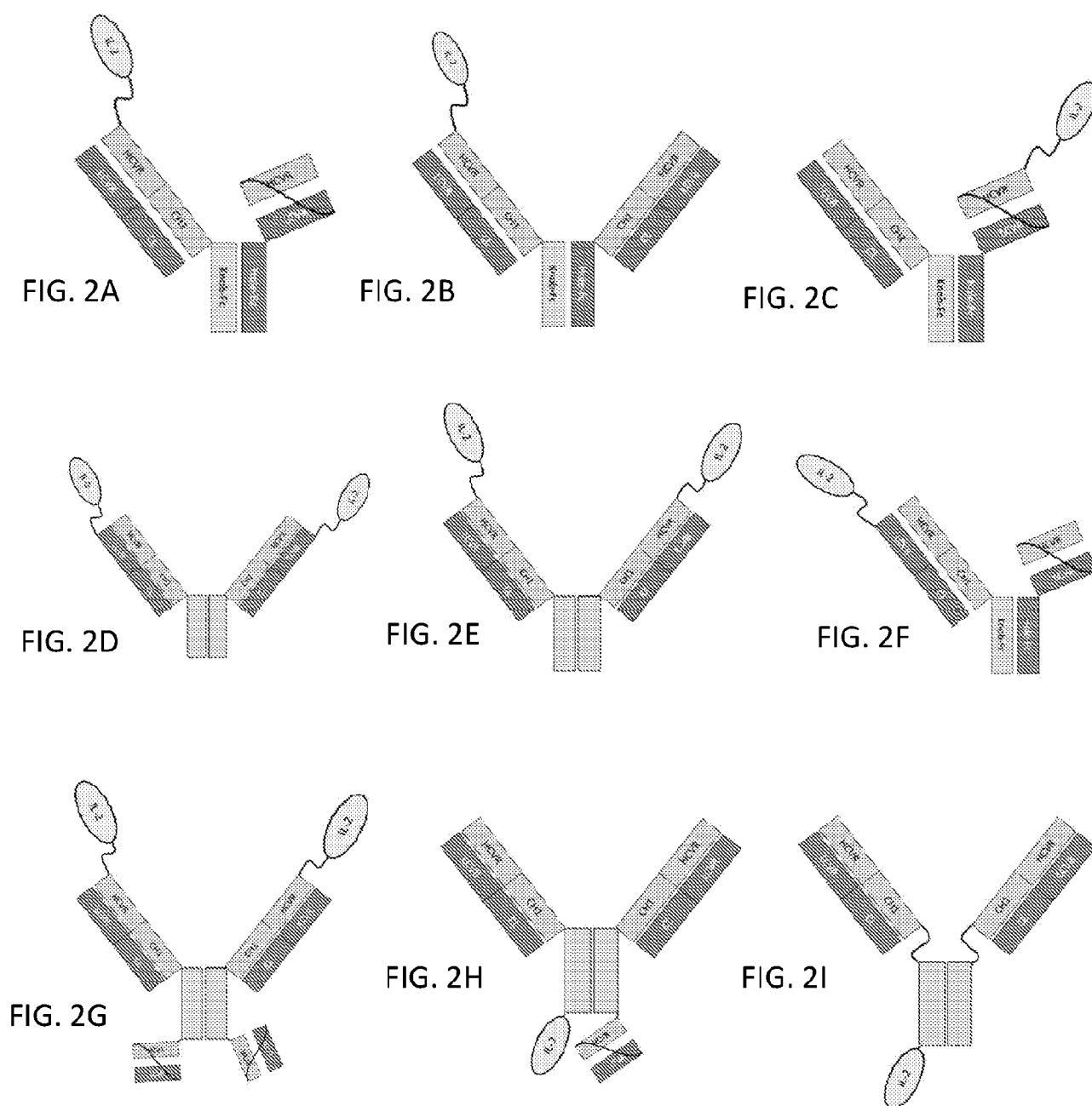


FIG. 1



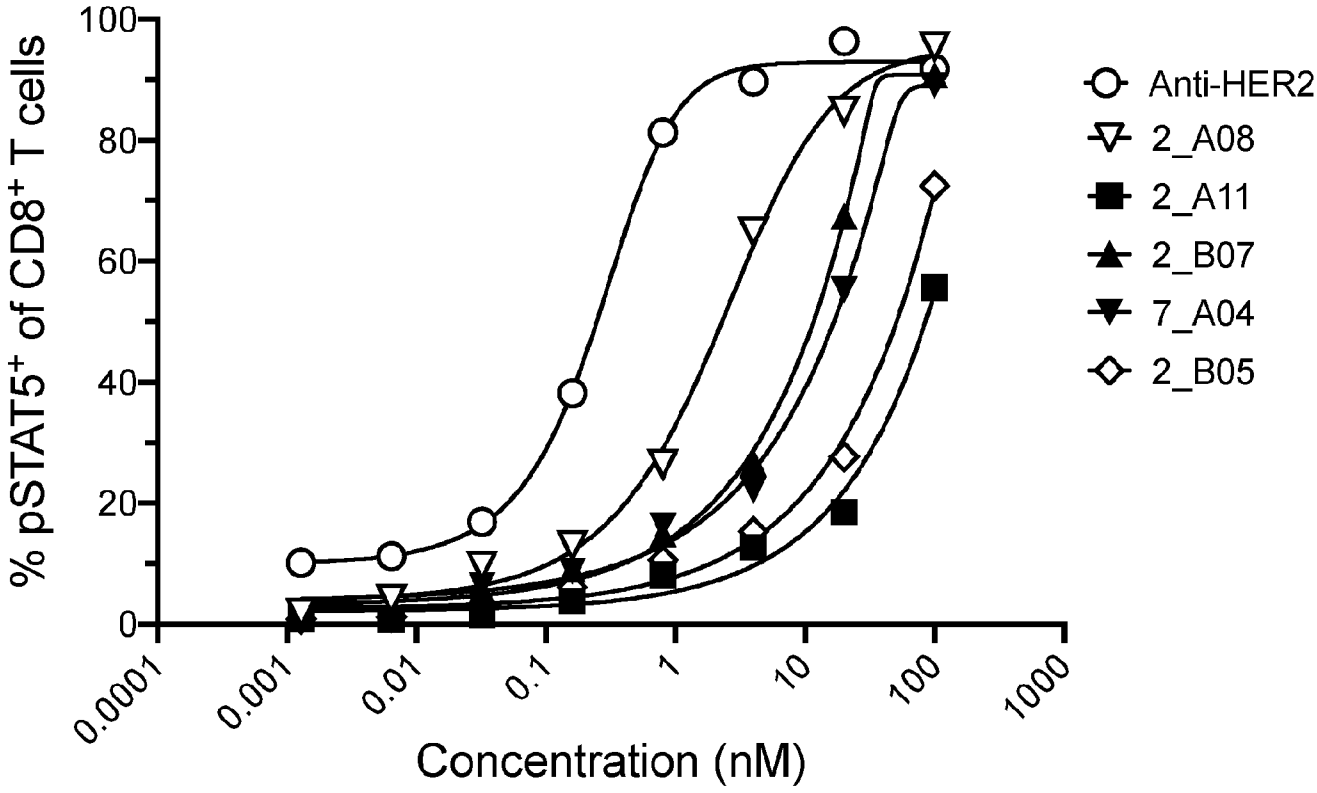


FIG. 3

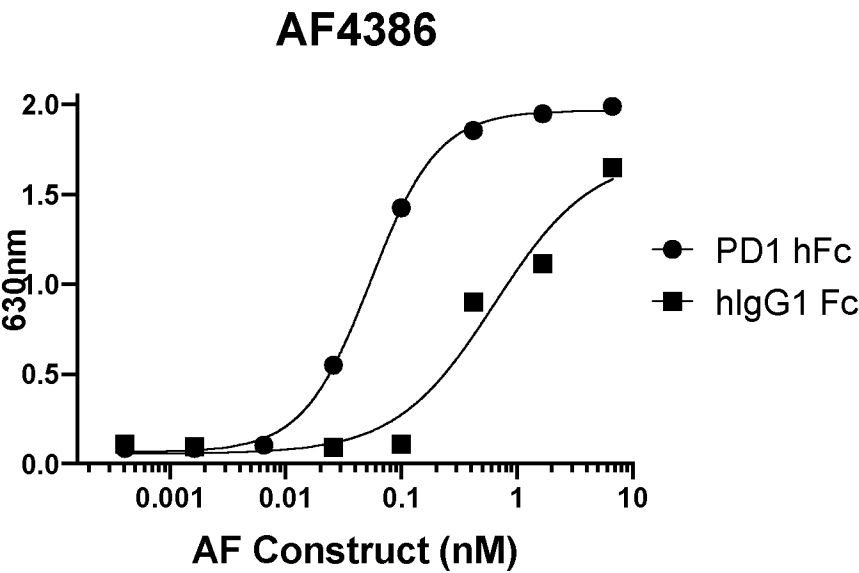


FIG. 4A

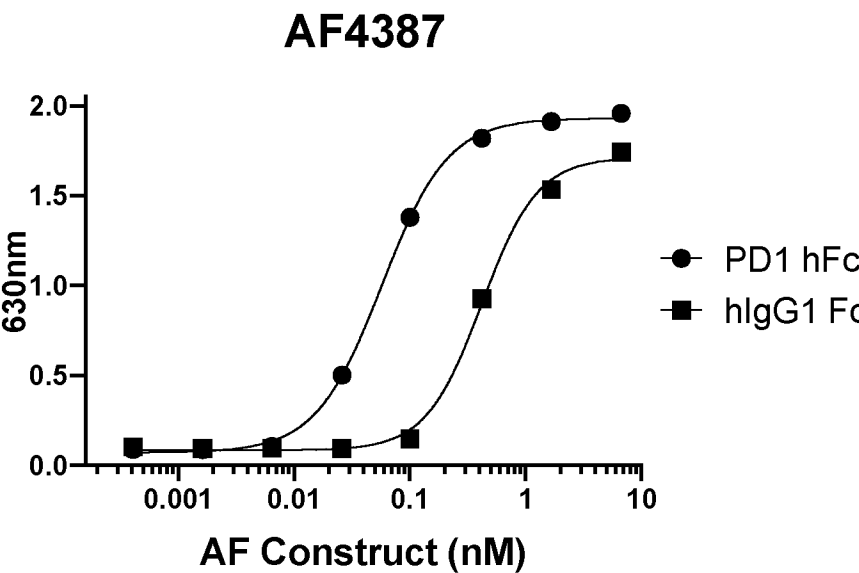


FIG. 4B

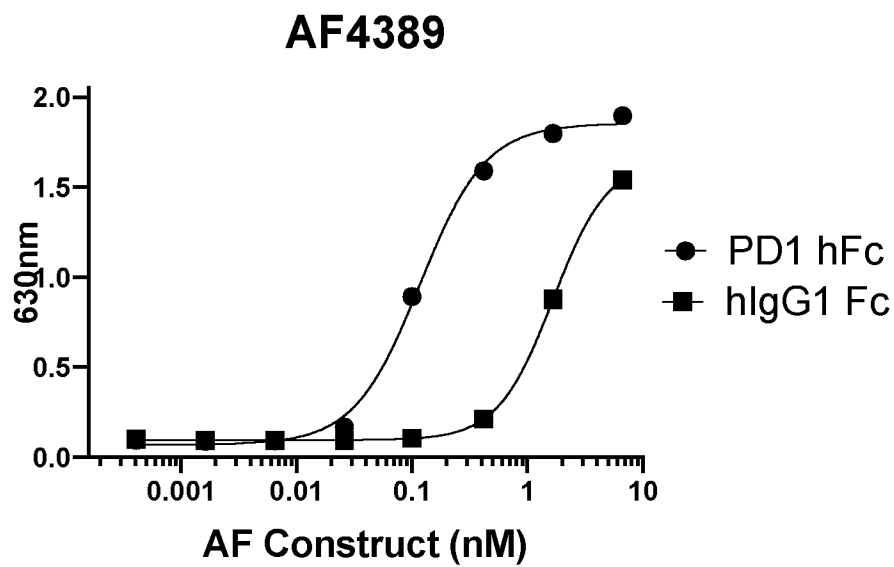


FIG. 4C

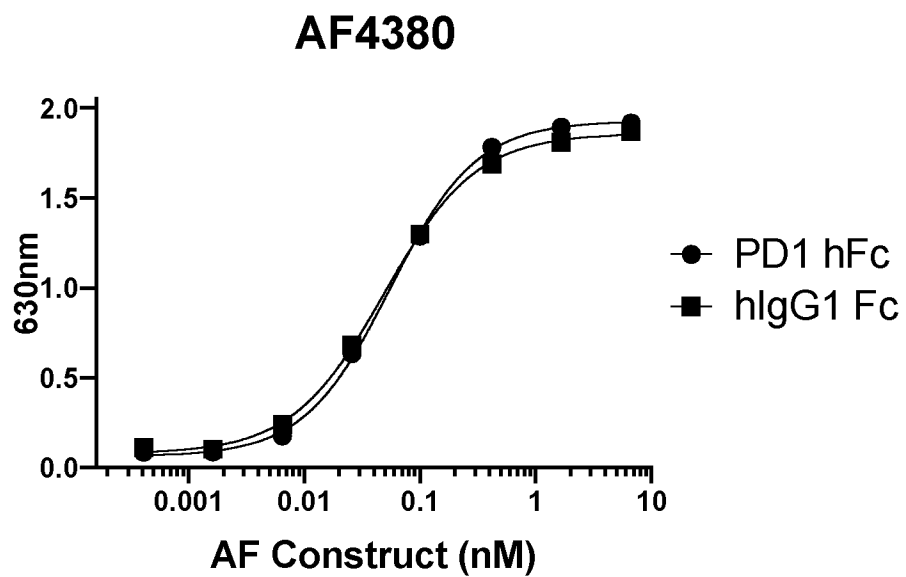


FIG. 4D

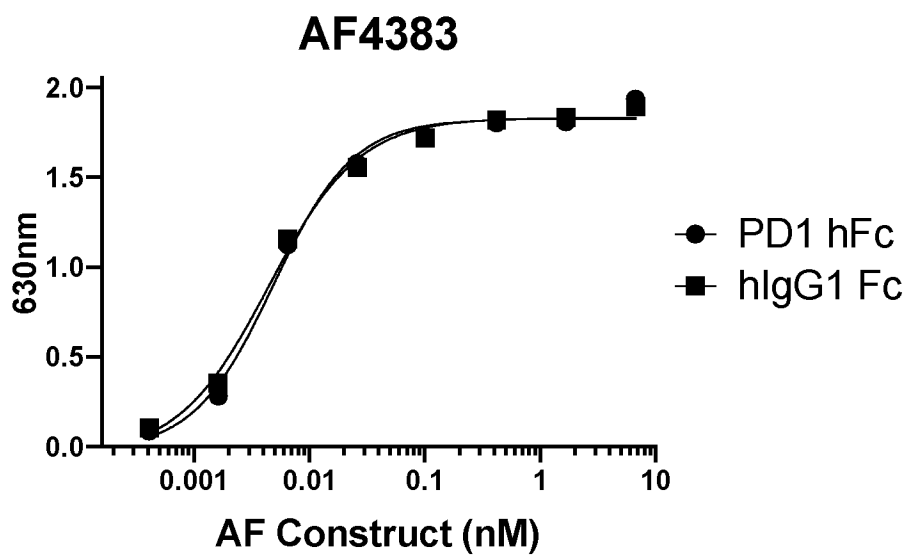


FIG. 4E

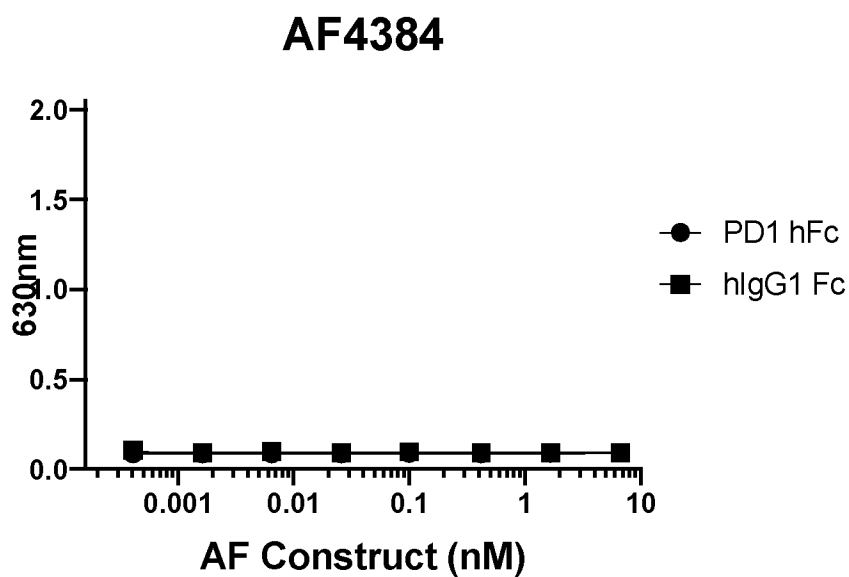


FIG. 4F

Fab:

Her2

PD1

scFv

PD1/IL2 DBA
2B07 variant

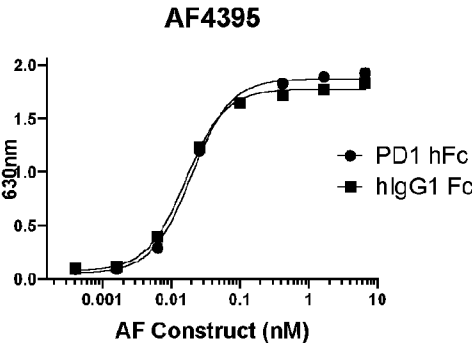


FIG. 5A

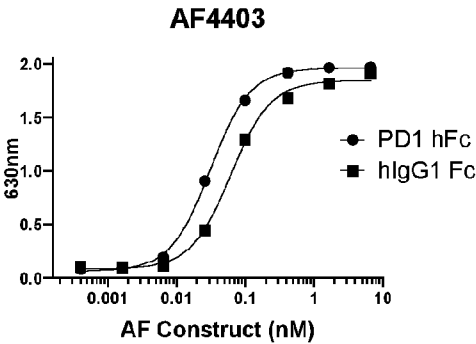


FIG. 5B

PD1/IL2 DBA
7A04 variant

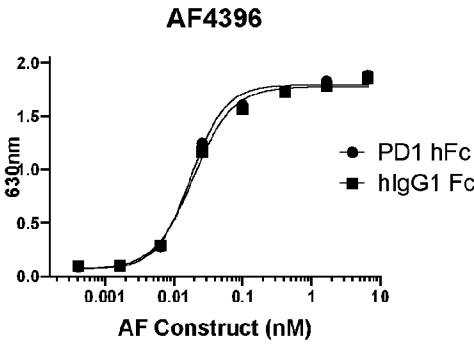


FIG. 5C

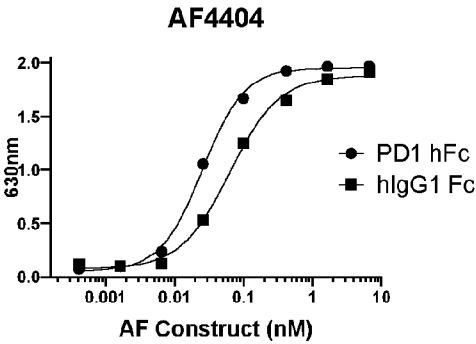


FIG. 5D

Fab:

Her2

PD1

scFv

Her2

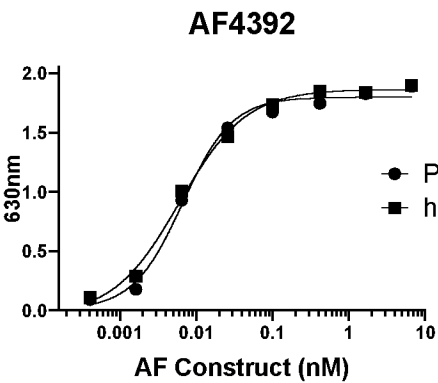


FIG. 5E

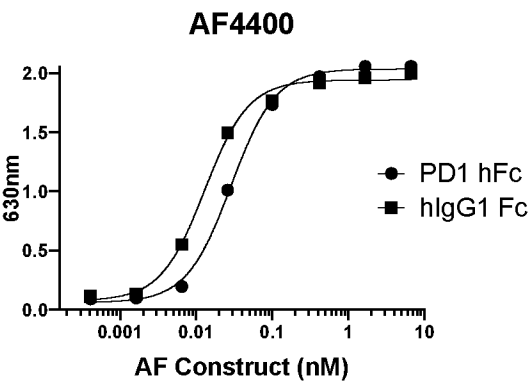


FIG. 5F

IL-2

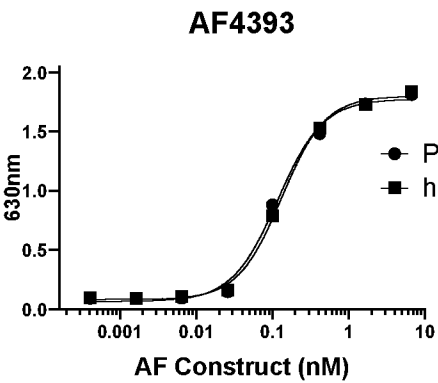


FIG. 5G

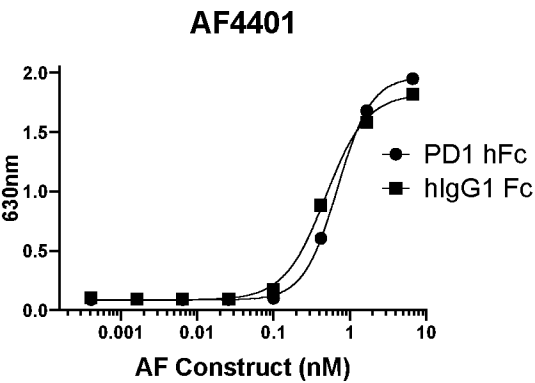


FIG. 5H

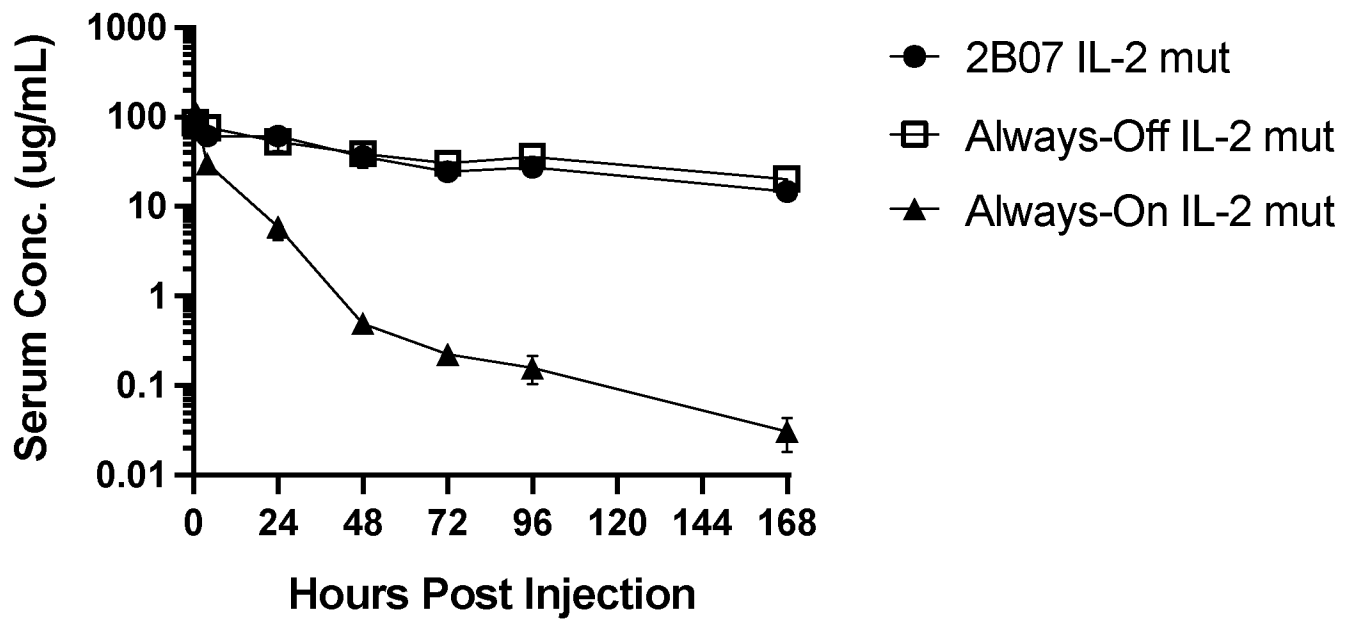


FIG. 6

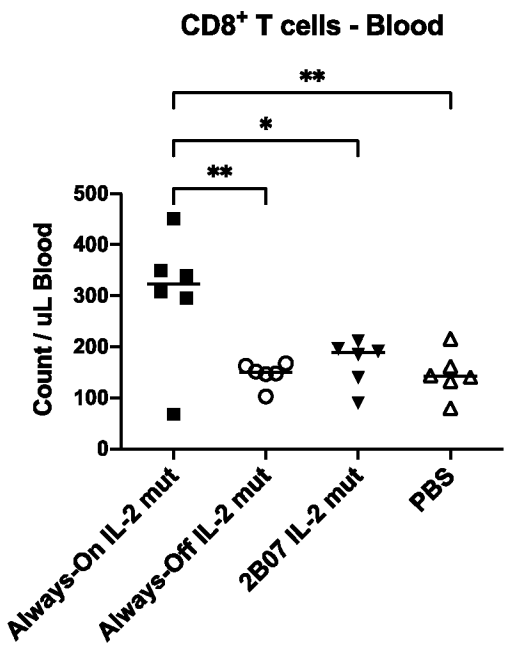


FIG. 7A

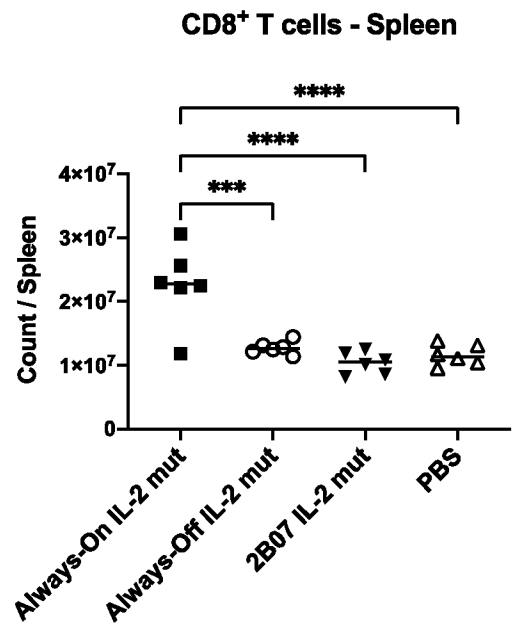


FIG. 7B

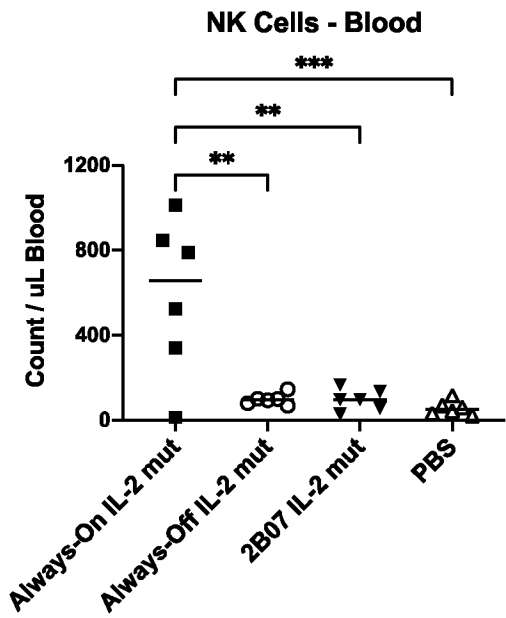


FIG. 7C

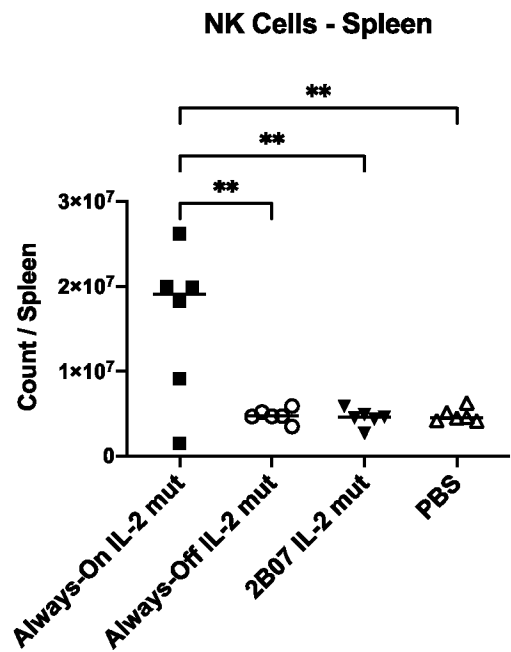


FIG. 7D

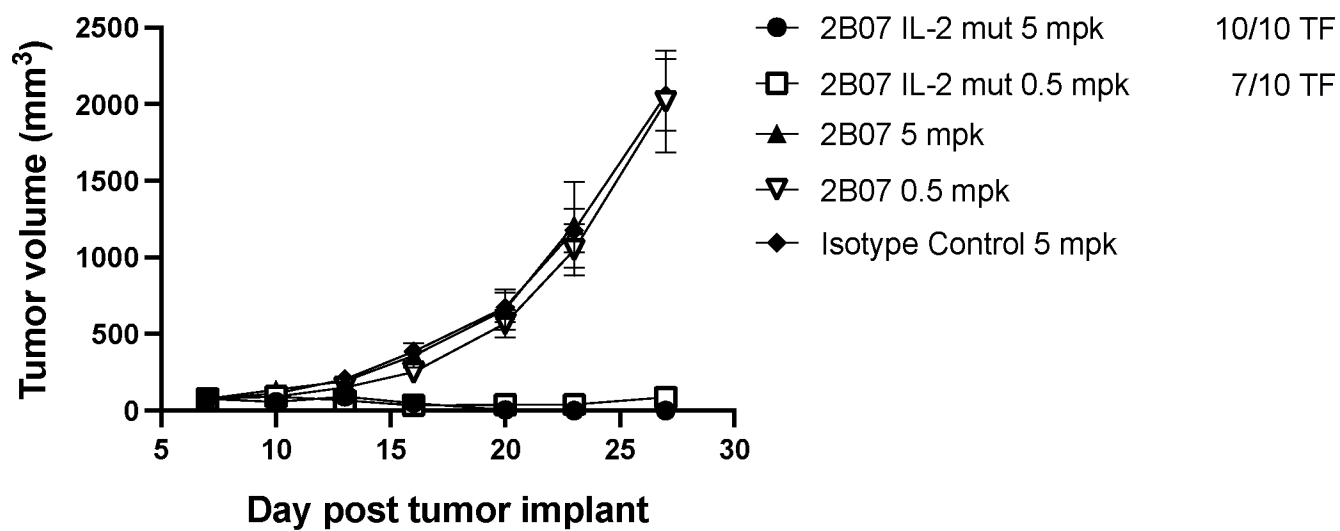


FIG. 8

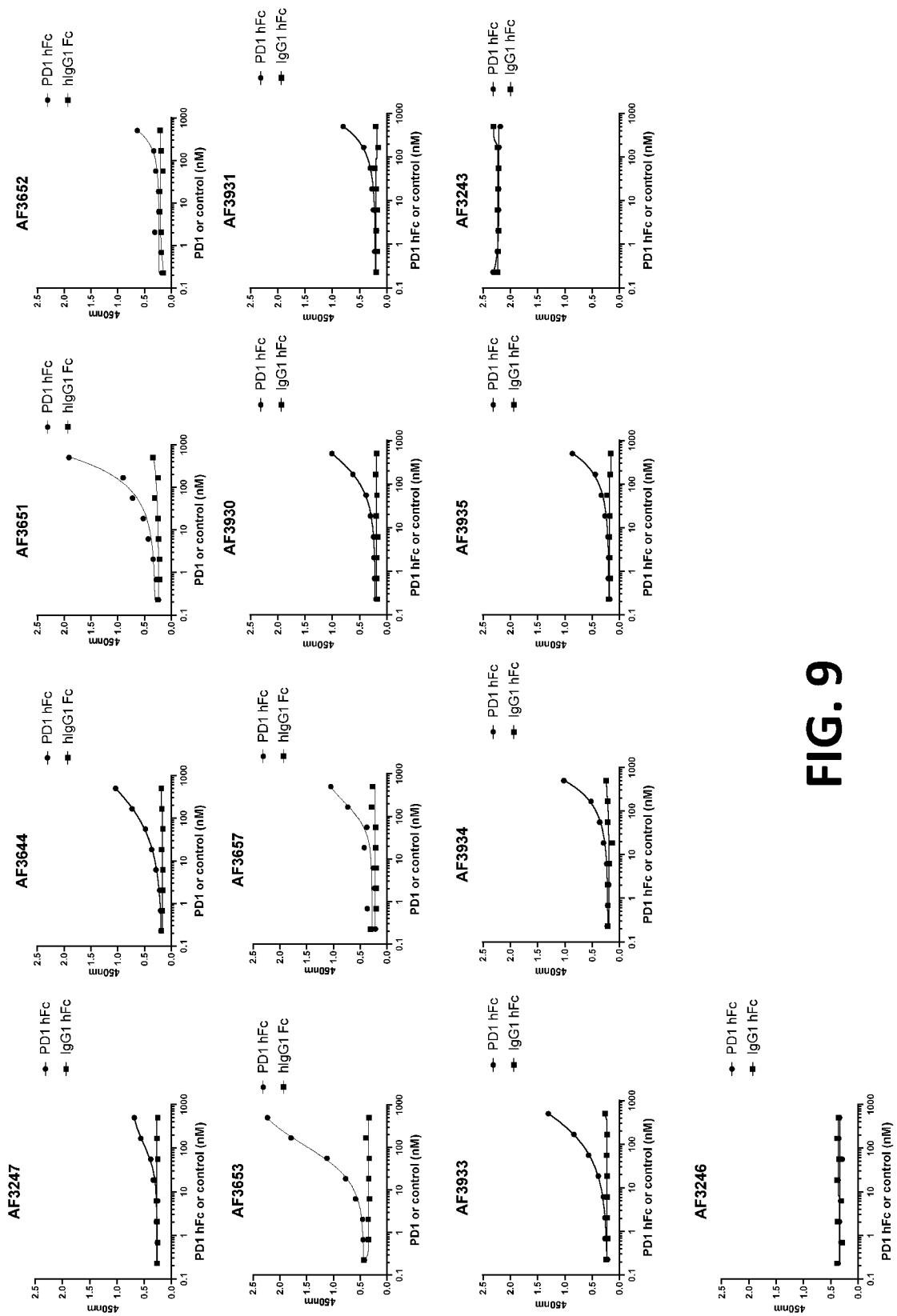


FIG. 9

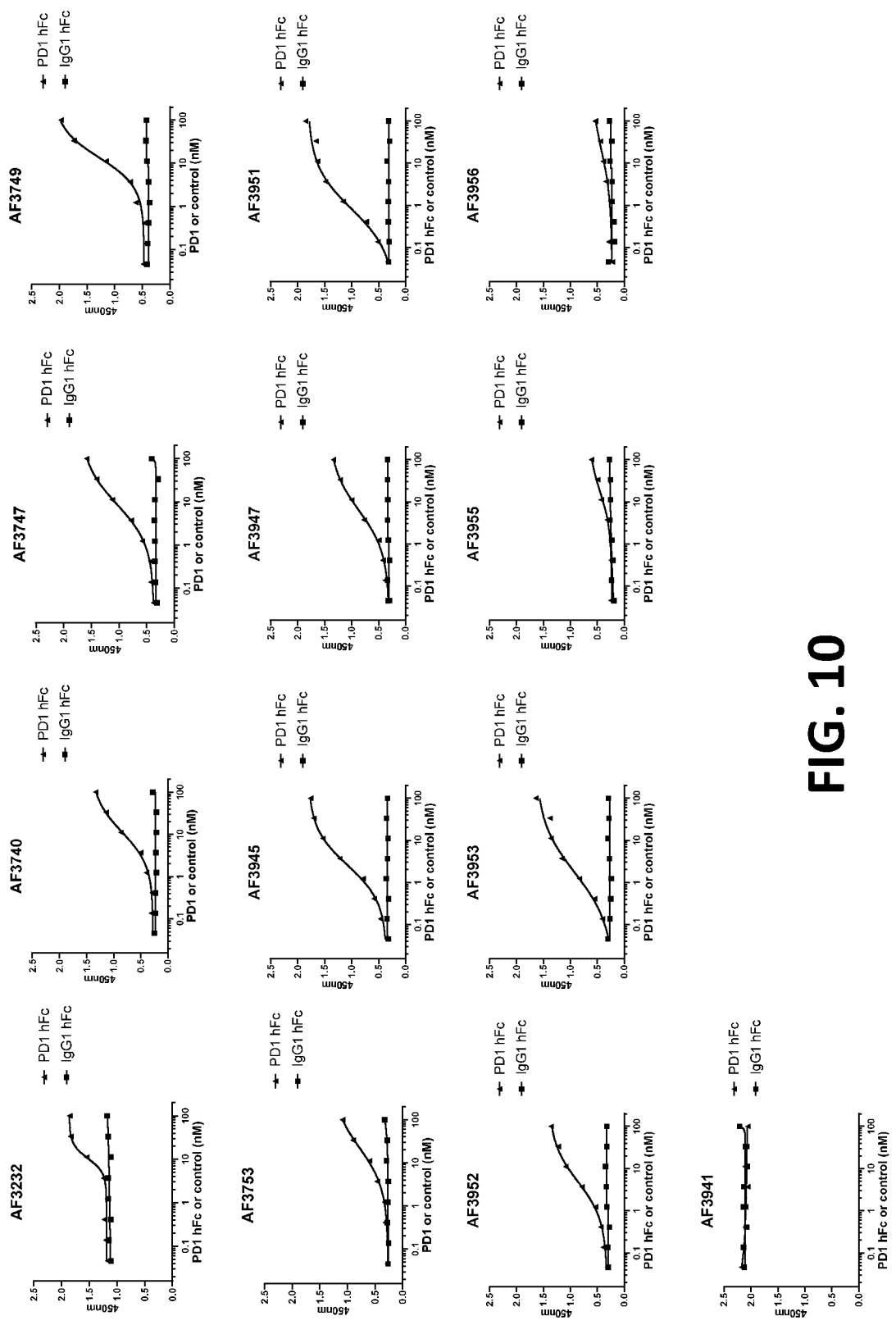


FIG. 10

FIG. 11A

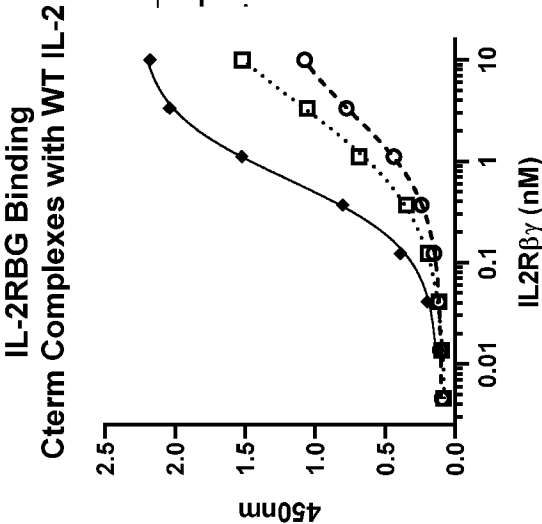


FIG. 11B

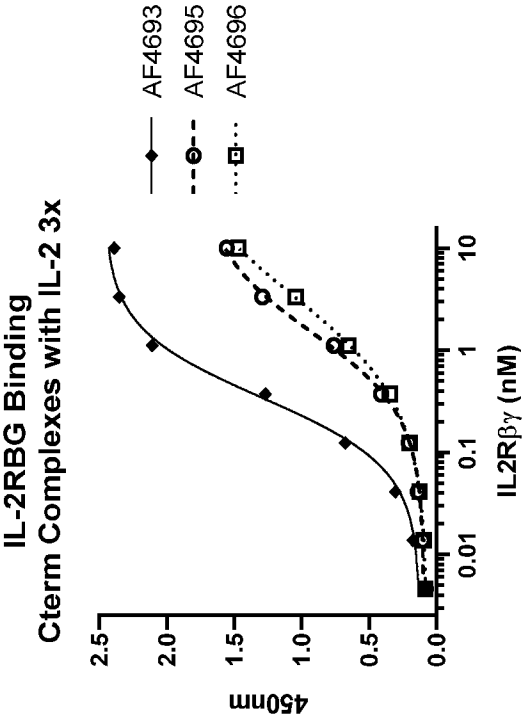


FIG. 11

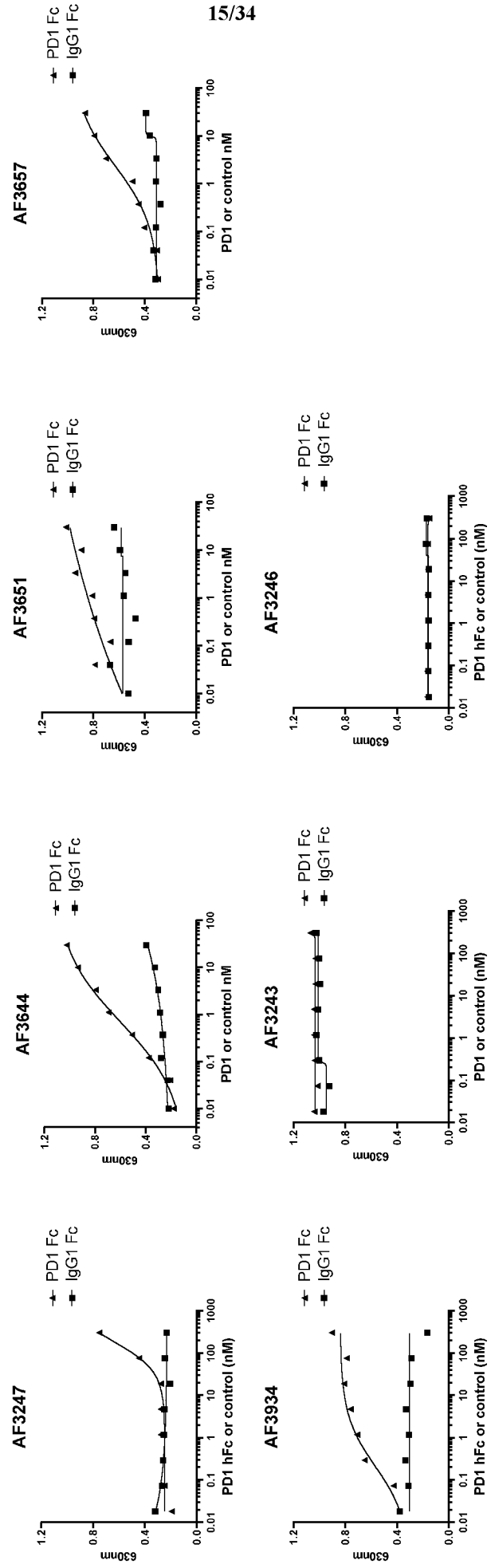


FIG. 12A

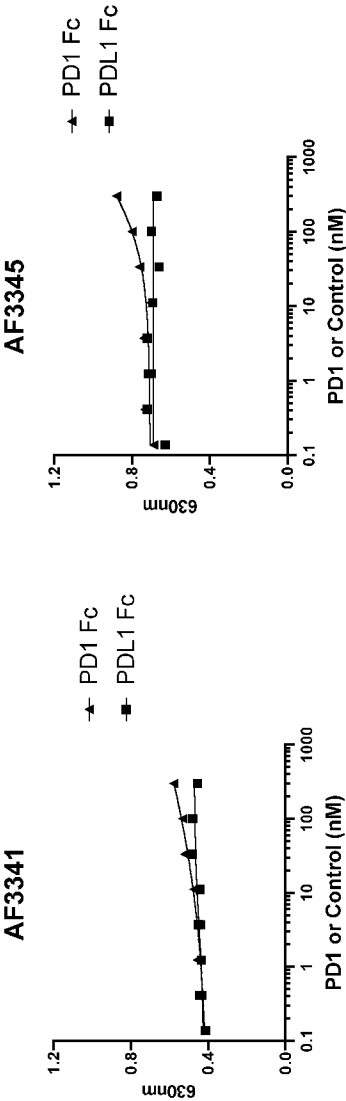


FIG. 12B

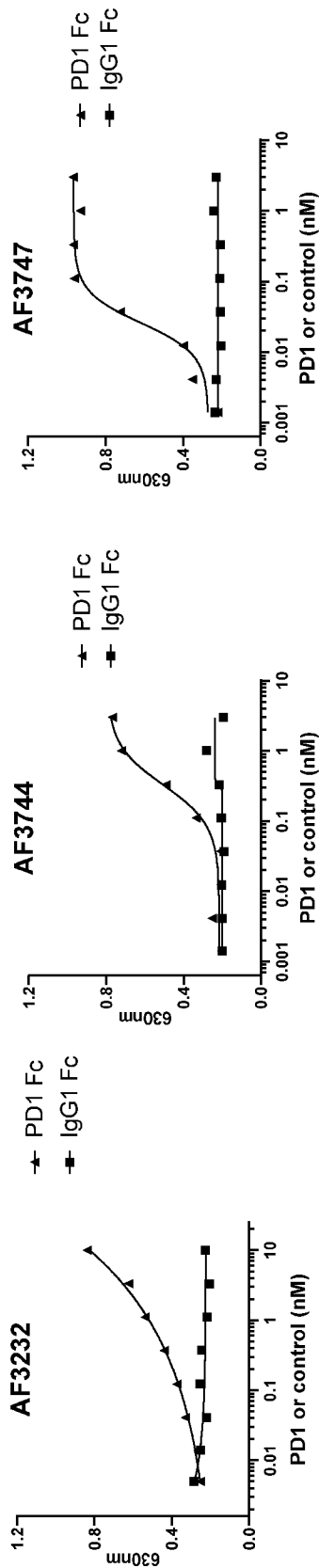


FIG. 13A

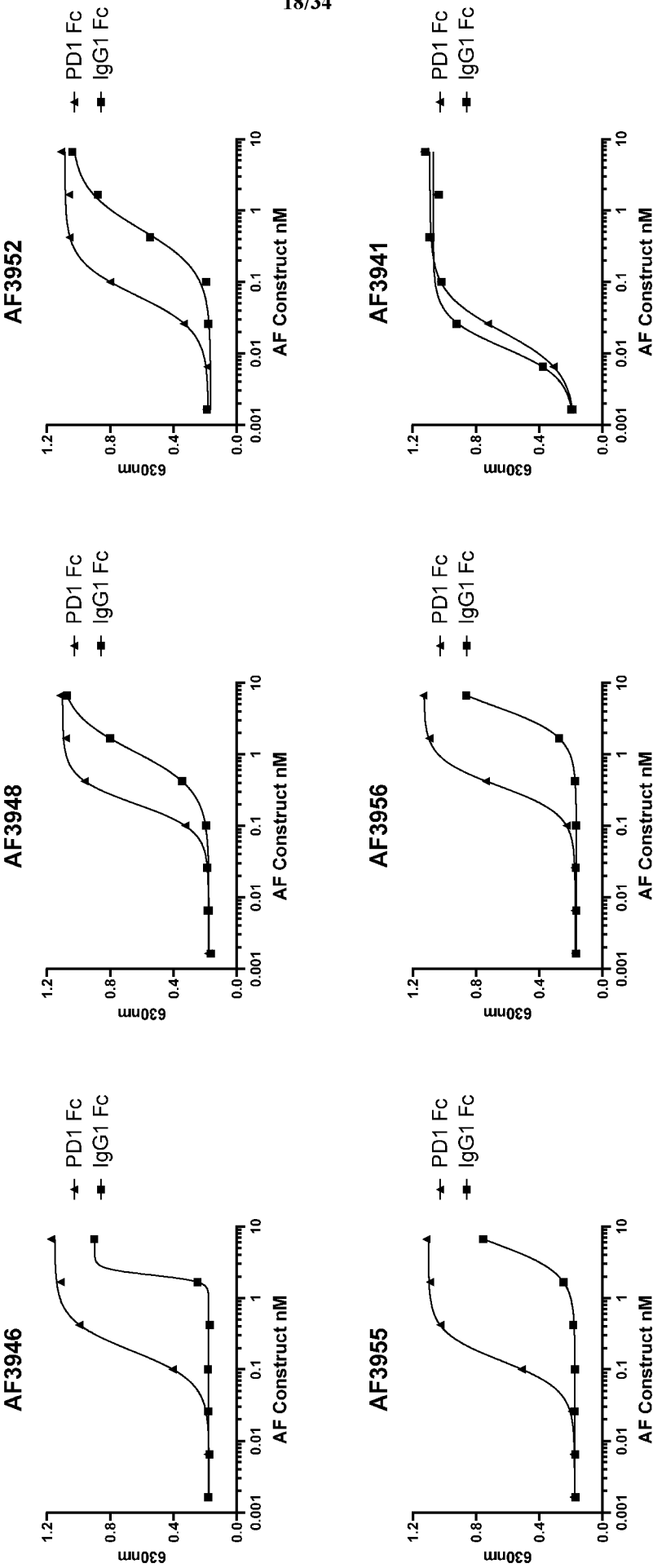


FIG. 13B

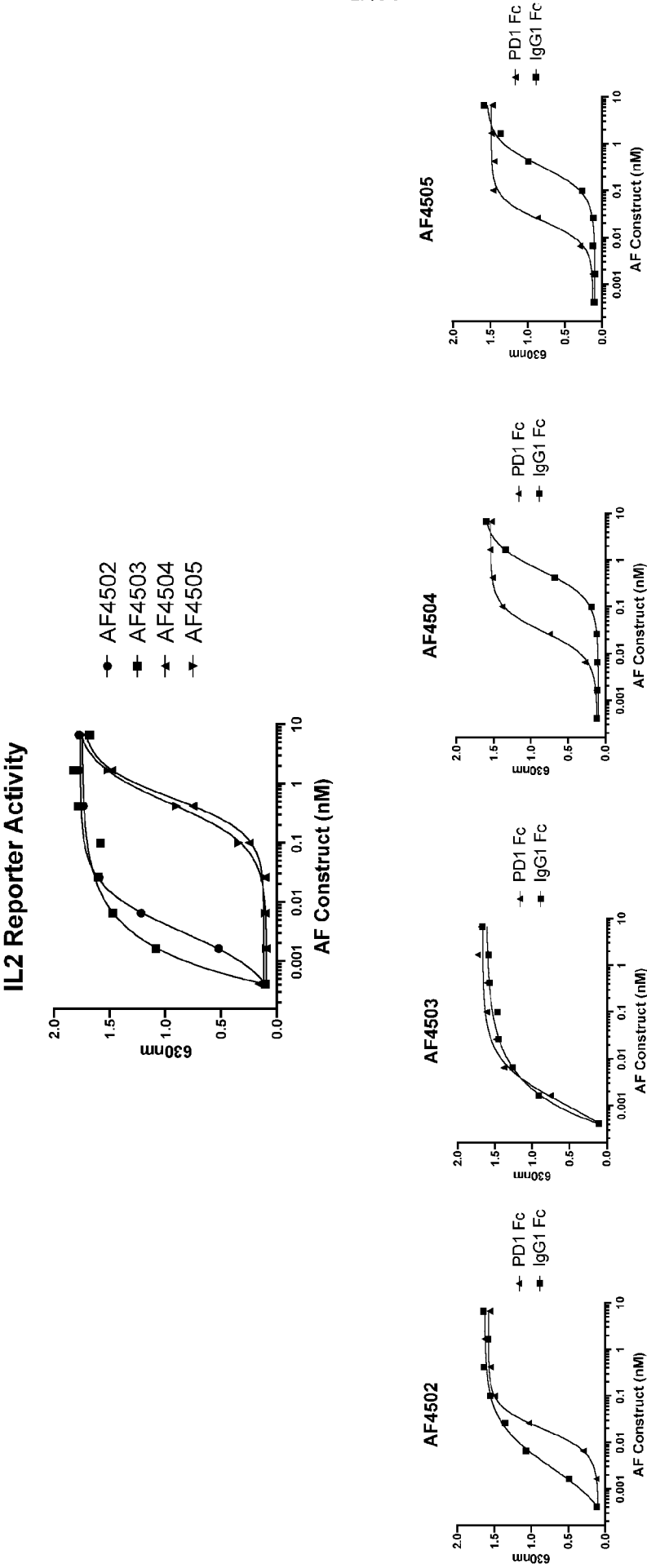


FIG. 14A

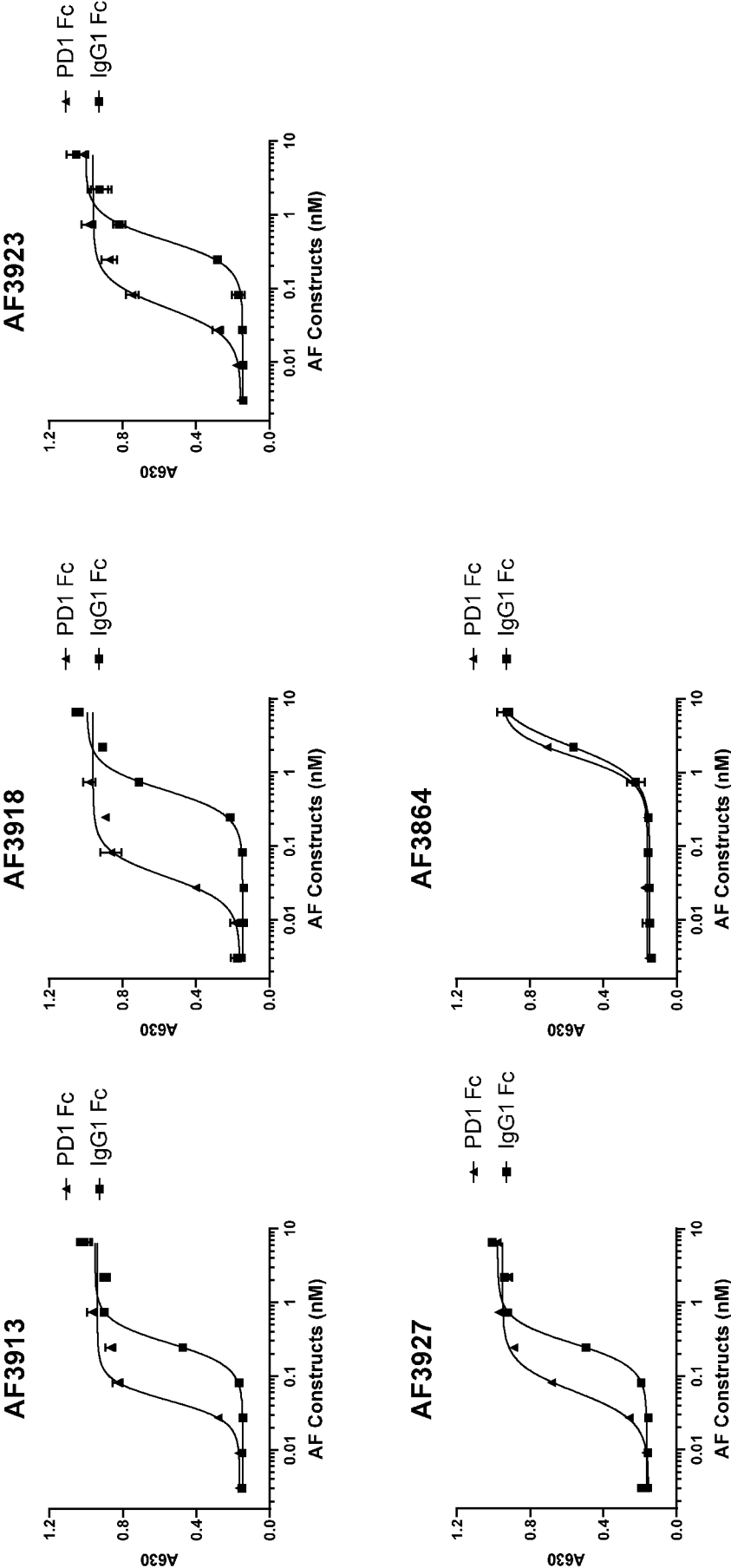


FIG. 14B

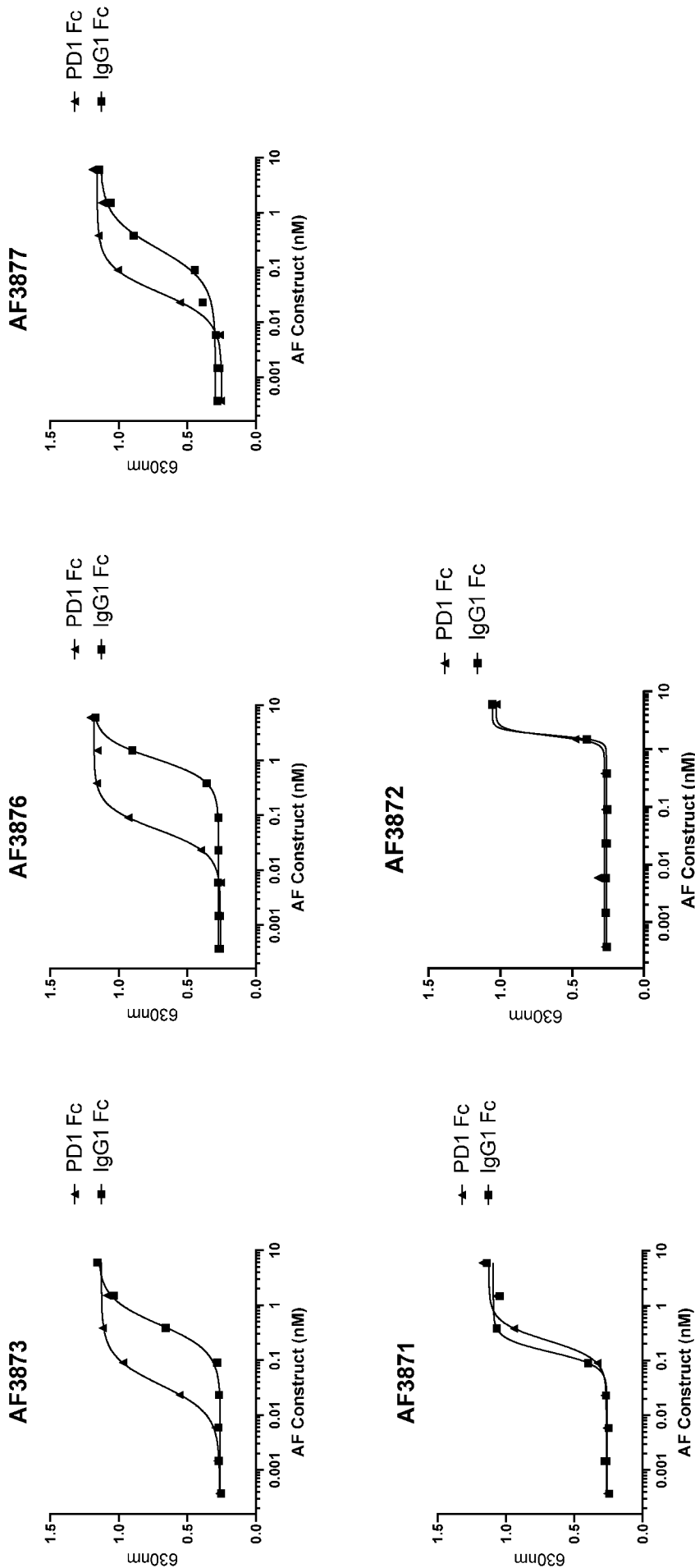


FIG. 15

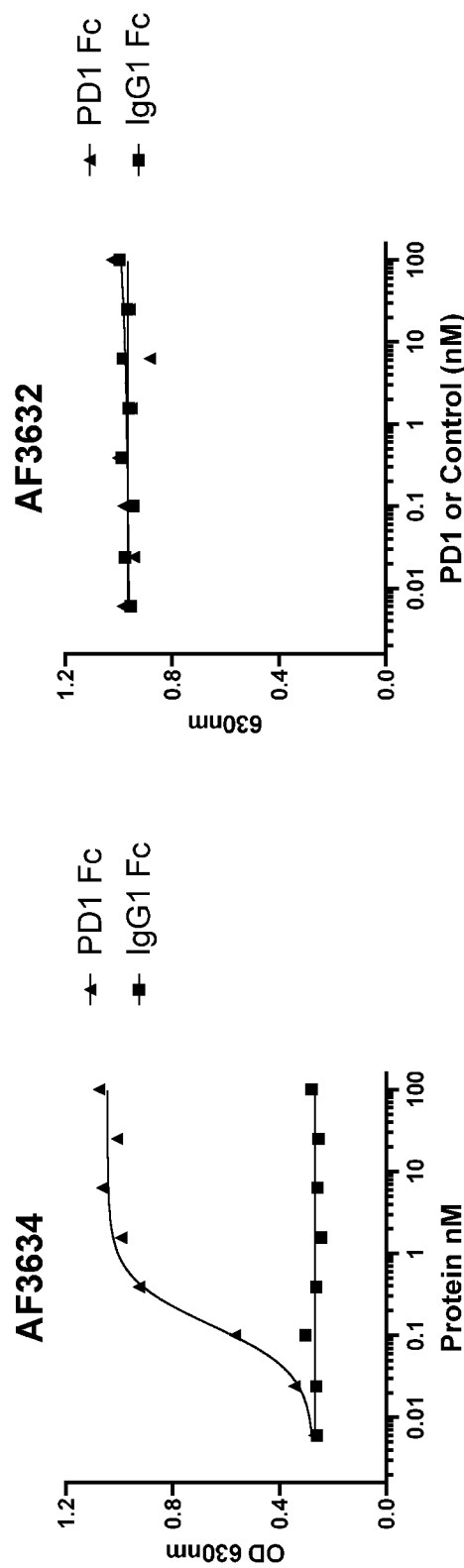


FIG. 16

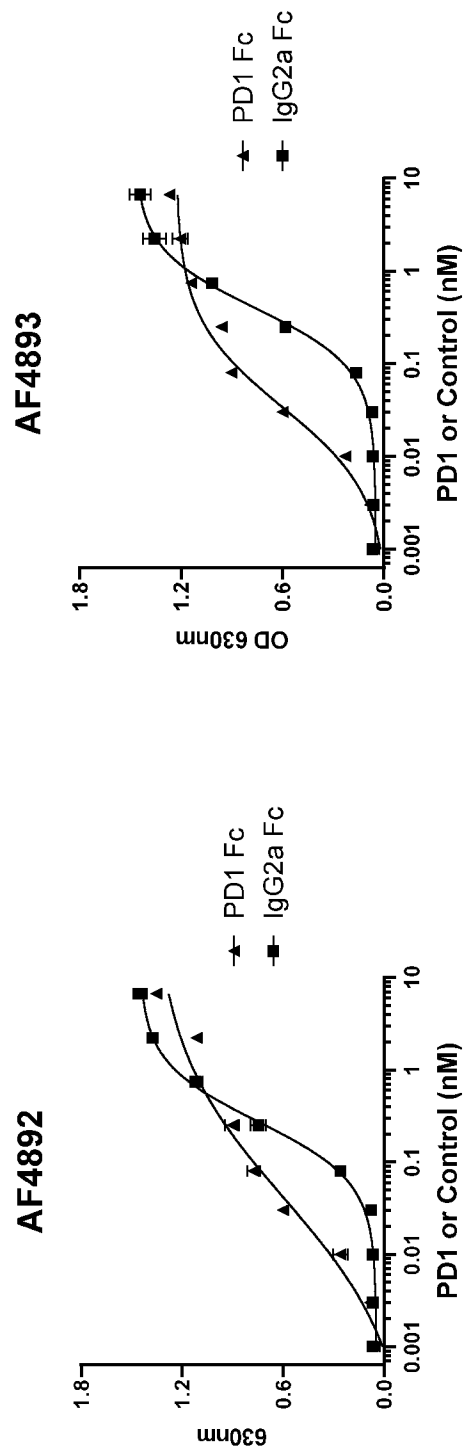


FIG. 17

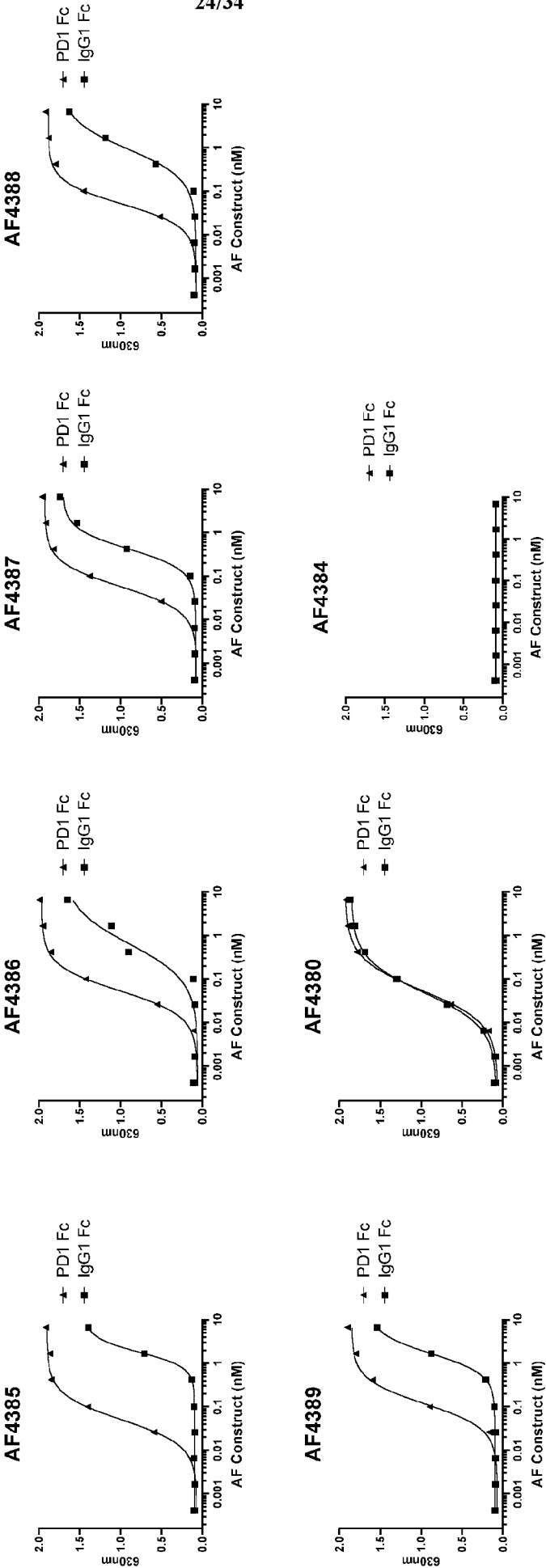


FIG. 18

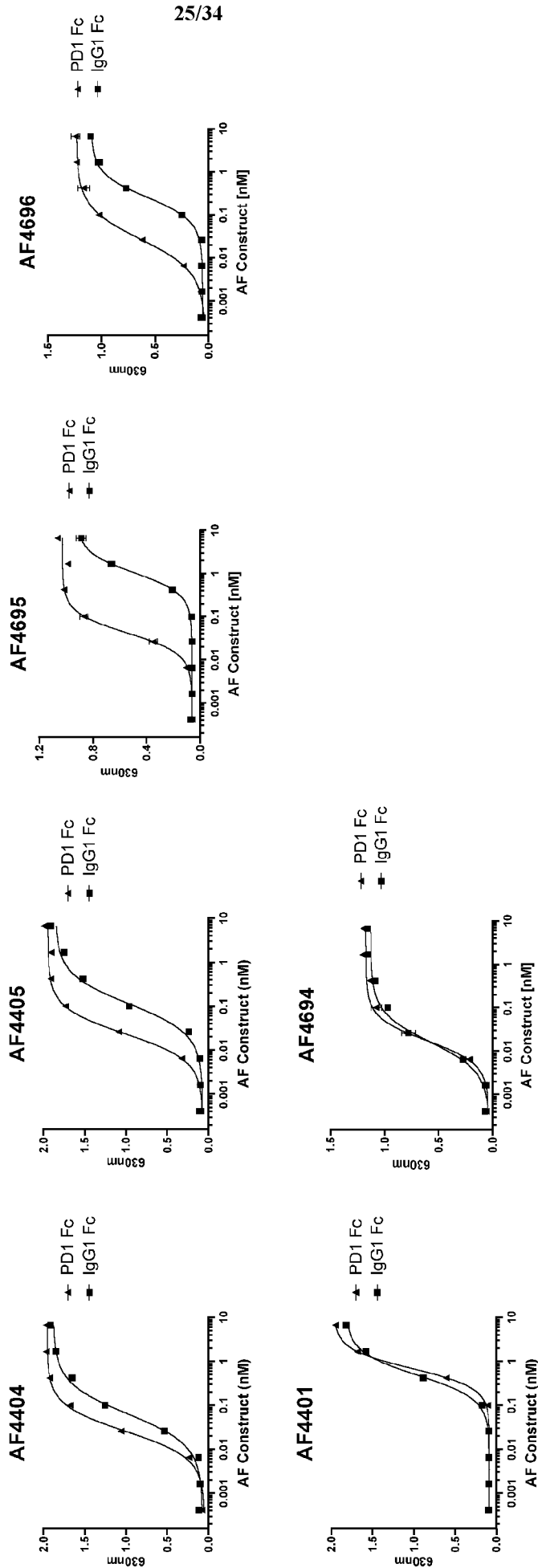


FIG. 19A

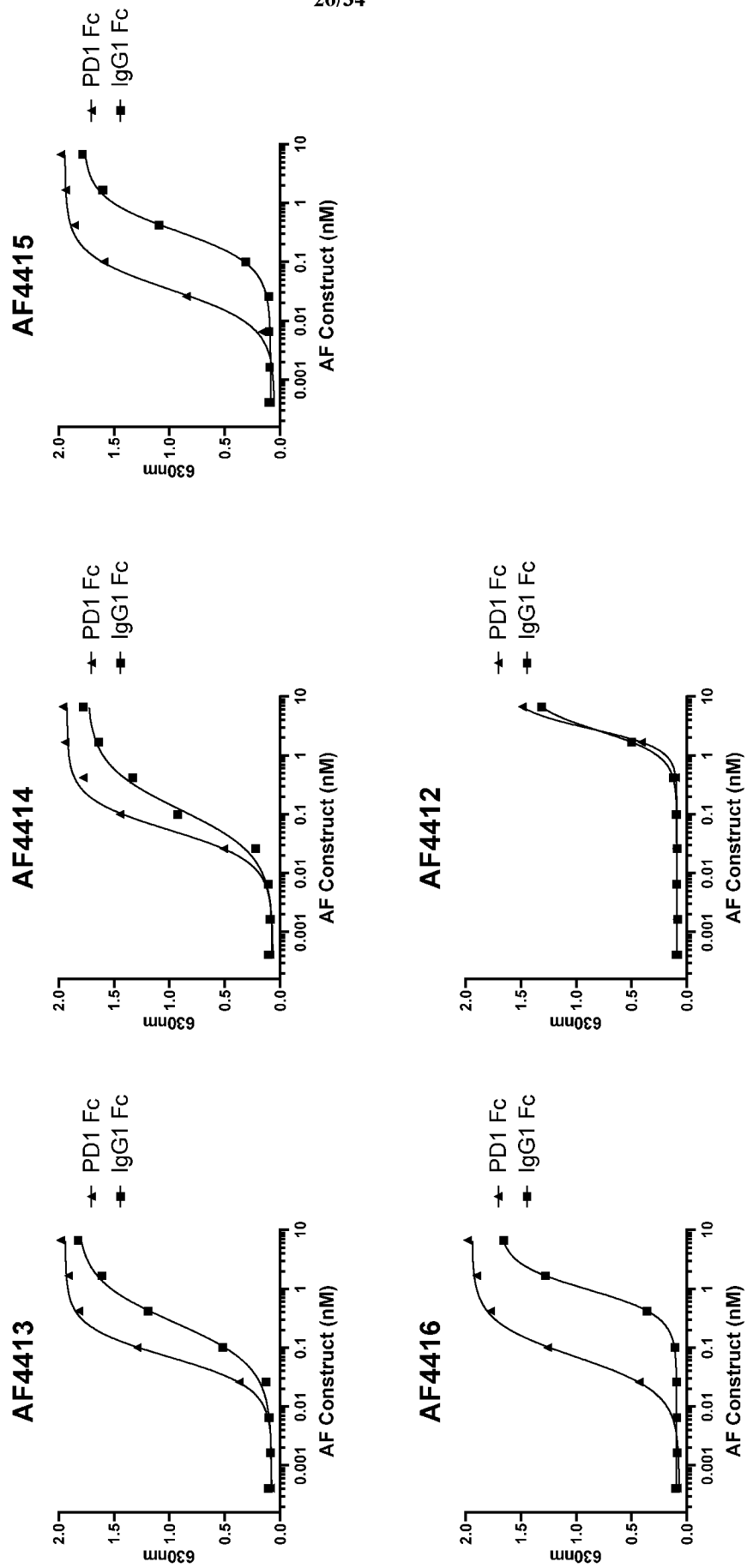


FIG. 19B

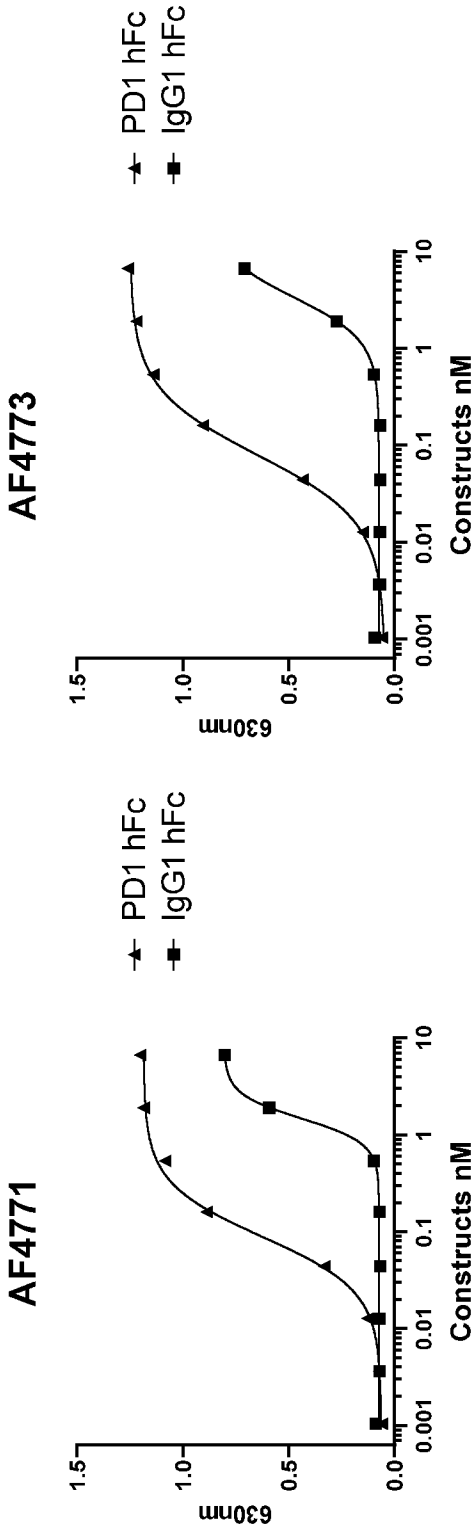


FIG. 19C

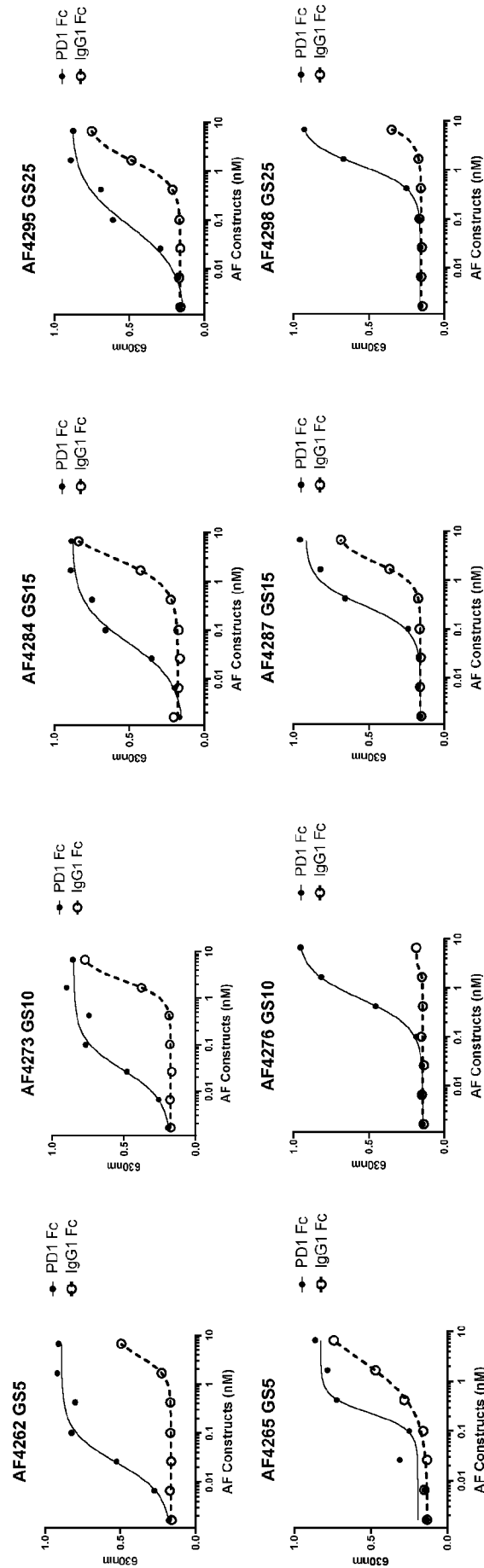


FIG. 20

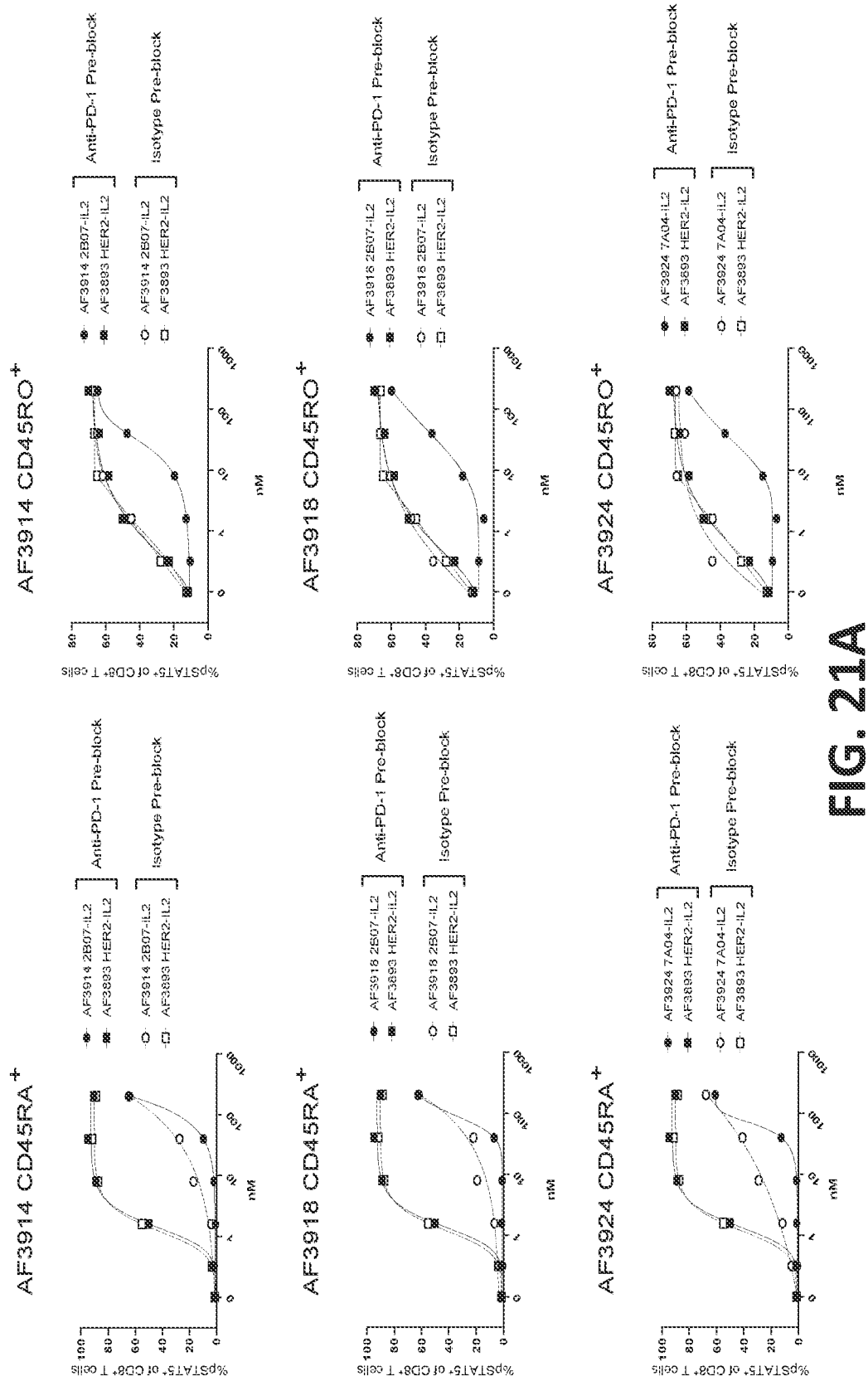


FIG. 21A

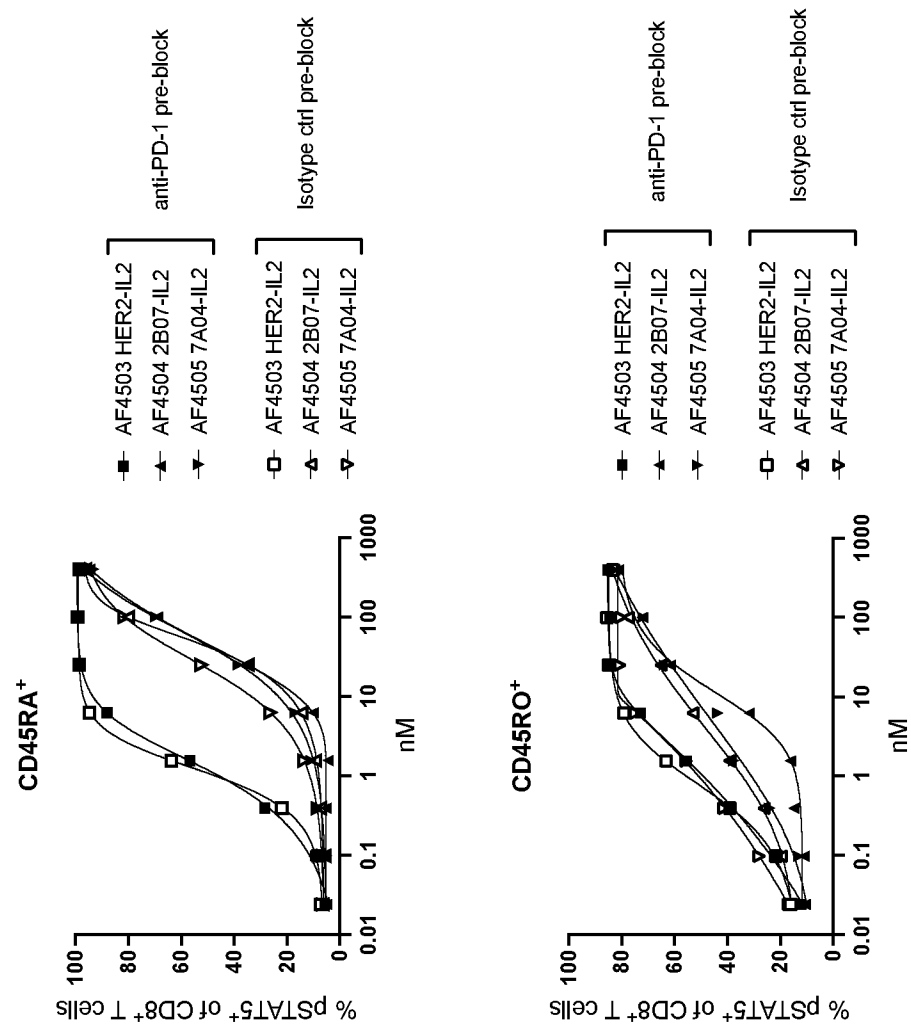


FIG. 21B

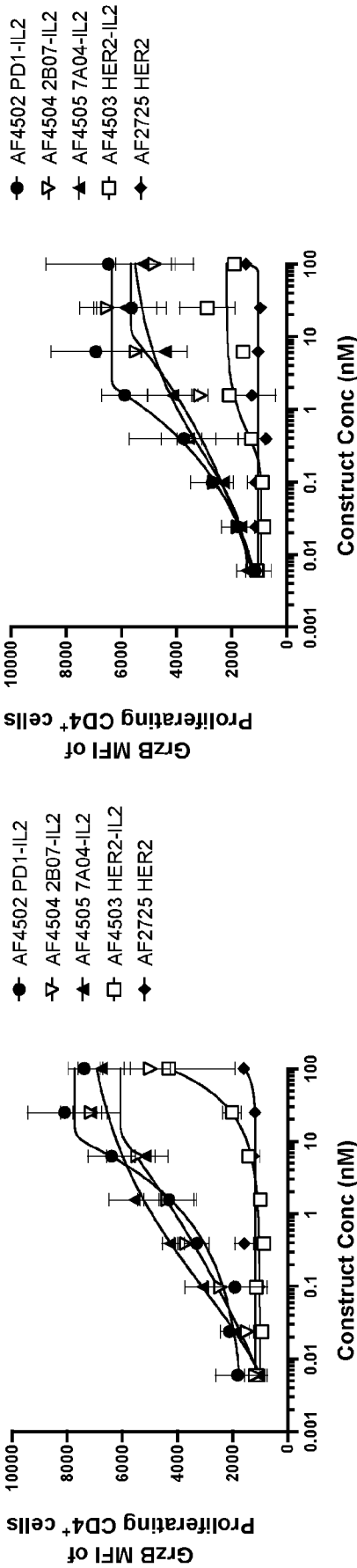


FIG. 22A

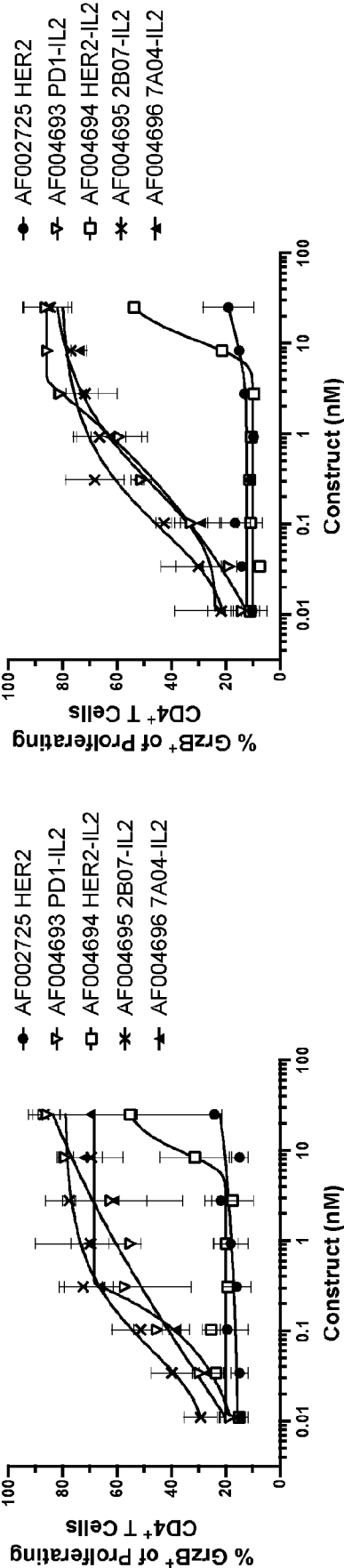


FIG. 22B

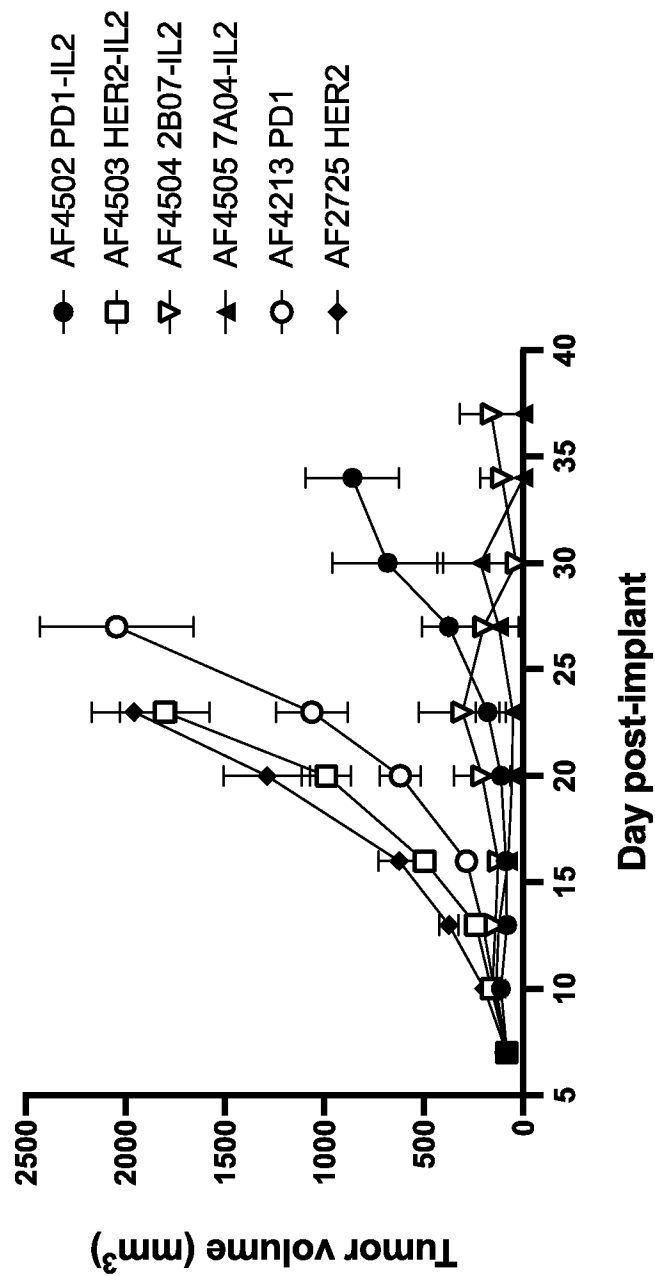


FIG. 23A

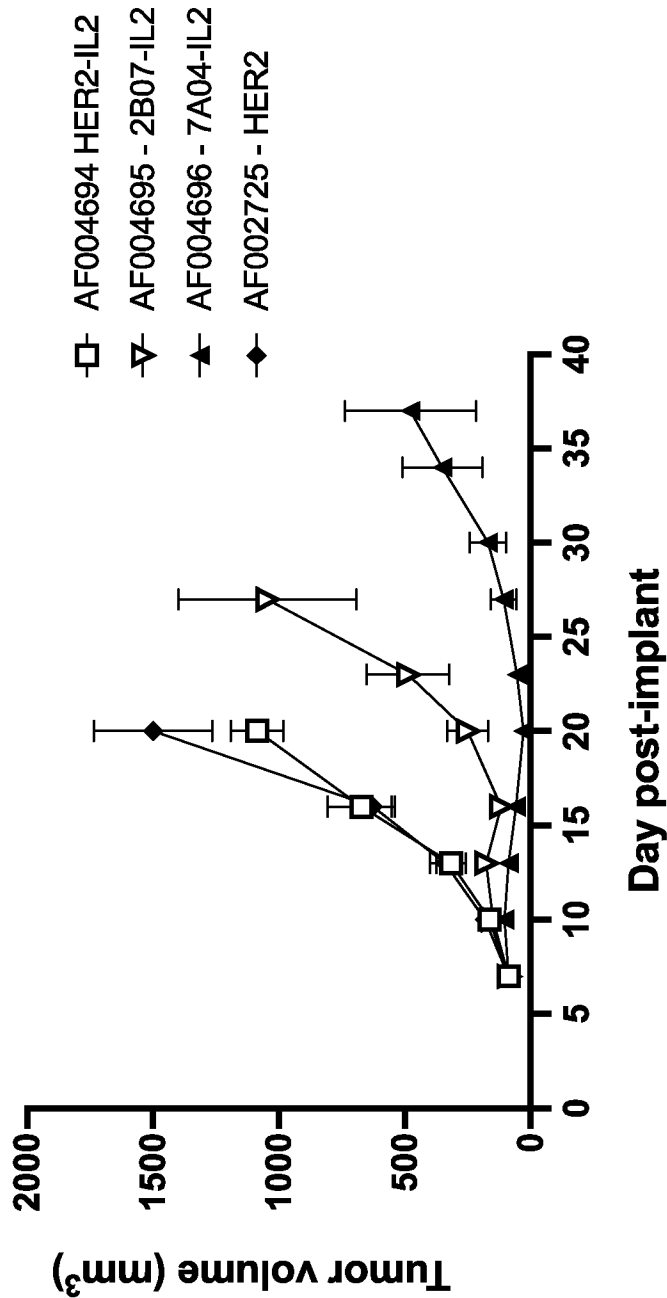


FIG. 23B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/080043

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K47/68 A61P35/00
ADD.

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)

A61K A61P

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2018/326011 A1 (CODARRI DEAK LAURA [CH] ET AL) 15 November 2018 (2018-11-15) claims 1, 21 -----	1-42
A	WO 2018/184964 A1 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 11 October 2018 (2018-10-11) claims 1, 11, 12 -----	1-42
A	US 2020/317787 A1 (LI ZHONGDAO [CN] ET AL) 8 October 2020 (2020-10-08) -----	1-42
A	WO 2018/234862 A1 (MEDICENNA THERAPEUTICS INC [CA]) 27 December 2018 (2018-12-27) -----	1-42
A	WO 2020/247843 A2 (ASHER BIOTHERAPEUTICS INC [US]) 10 December 2020 (2020-12-10) ----- -/-	1-42



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

24 March 2023

Date of mailing of the international search report

30/03/2023

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Langer, Miren

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2022/080043

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2021/231773 A1 (GOOD THERAPEUTICS INC [US]) 18 November 2021 (2021-11-18) claims 1, 35, 36 -----	1-42

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/080043

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2022/080043

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 2018326011 A1	15-11-2018	AR	111203 A1	12-06-2019
		CN	110382525 A	25-10-2019
		EP	3606947 A1	12-02-2020
		JP	7148539 B2	05-10-2022
		JP	2020512814 A	30-04-2020
		TW	201842937 A	16-12-2018
		US	2018326011 A1	15-11-2018
		US	2023071733 A1	09-03-2023
		WO	2018184965 A1	11-10-2018

WO 2018184964 A1	11-10-2018	AR	111400 A1	10-07-2019
		AU	2018247765 A1	22-08-2019
		BR	112019017329 A2	14-04-2020
		CA	3053357 A1	11-10-2018
		CL	2019002346 A1	10-01-2020
		CN	110392692 A	29-10-2019
		CO	2019009354 A2	09-09-2019
		CR	20190426 A	01-11-2019
		DK	3606946 T3	24-10-2022
		EP	3606946 A1	12-02-2020
		ES	2928718 T3	22-11-2022
		HR	P20221254 T1	23-12-2022
		HU	E059885 T2	28-01-2023
		IL	269395 A	28-11-2019
		JP	2020515275 A	28-05-2020
		JP	2022062001 A	19-04-2022
		KR	20190121816 A	28-10-2019
		KR	20210124518 A	14-10-2021
		LT	3606946 T	25-10-2022
		MA	49033 A	12-02-2020
		PE	20191494 A1	21-10-2019
		PH	12019502273 A1	07-12-2020
		PL	3606946 T3	28-11-2022
		PT	3606946 T	17-10-2022
		RS	63663 B1	30-11-2022
		RU	2019134338 A	05-05-2021
		SG	11201909205Y A	28-11-2019
		TW	201900220 A	01-01-2019
		UA	125700 C2	18-05-2022
		US	2018326010 A1	15-11-2018
		WO	2018184964 A1	11-10-2018
		ZA	201905517 B	26-05-2021

US 2020317787 A1	08-10-2020	CN	111527109 A	11-08-2020
		EP	3733716 A1	04-11-2020
		JP	2021511069 A	06-05-2021
		US	2020317787 A1	08-10-2020
		WO	2019129053 A1	04-07-2019

WO 2018234862 A1	27-12-2018	AU	2018287317 A1	06-02-2020
		CA	3067909 A1	27-12-2018
		CN	111201035 A	26-05-2020
		EP	3641814 A1	29-04-2020
		JP	2020524174 A	13-08-2020
		US	2019062395 A1	28-02-2019
		US	2023080403 A1	16-03-2023
		WO	2018234862 A1	27-12-2018

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2022/080043

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2020247843 A2	10-12-2020	AU 2020287373 A1 CA 3142738 A1 CN 114786708 A EP 3980051 A2 JP 2022535130 A KR 20220083660 A US 2022251202 A1 WO 2020247843 A2	06-01-2022 10-12-2020 22-07-2022 13-04-2022 04-08-2022 20-06-2022 11-08-2022 10-12-2020
WO 2021231773 A1	18-11-2021	AU 2021273009 A1 CA 3177843 A1 EP 4149554 A1 GB 2602612 A IL 298136 A KR 20230010251 A US 2022047714 A1 WO 2021231773 A1	15-12-2022 18-11-2021 22-03-2023 06-07-2022 01-01-2023 18-01-2023 17-02-2022 18-11-2021