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(54) Title: BI-FUNCTIONAL MOLECULES

(57) Abstract: Provided are proteins comprising a PD- L1-binding moiety linked to a TGFβ-binding moiety, IL-1-binding moiety, immunostimulatory polypeptides (e.g., soluble LAG3 or soluble CD4) or CD47-binding moiety, isolated polynucleotides encoding the same, pharmaceutical compositions comprising the same and the uses thereof.

BI-FUNCTIONAL MOLECULES

FIELD OF THE INVENTION

[001] The present disclosure generally relates to novel bi-functional molecules targeting an immune checkpoint molecule (e.g., PD-L1) and blocking activity of an anti-tumor immunity suppressing (ATIS) cytokine (e.g., IL-1 or TGFβ) or stimulating immunity.

BACKGROUND

[002] Programmed death 1 (PD-1) and its ligands PD-L1 and PD-L2 are key co-inhibitory molecules in the modulation of T-cell mediated immune responses. PD-1 is a type I membrane protein with a single extracellular immunoglobulin superfamily (IgSF) V-set domain that is expressed on the surface of activated T cells in peripheral tissues (Zhang X, et al, Immunity, 2004, 20(3): 337-347). PD-L1 and PD-L2 are commonly expressed on dendritic cells and macrophages, and their ectodomains are composed of a membrane distal IgSF V-set and a membrane proximal IgSF C-set domain (Latchman Y, et al, Nature immunology, 2001, 2(3): 261-268). Ligation of PD-1 with its two ligands initiates co-inhibitory signaling through the cytoplasmic domain of PD-1, containing an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine-based switch motif, thus leading to activation of SHP phosphatases that downregulates TCR signaling by dephosphorylating effector molecules involved in the signaling (Chemnitz J M, et al, J. Immunol., 2004, 173(2): 945-954). As a result, PD-1 signaling prevents excessive or harmful inflammation and maintains immune tolerance to self-antigens under normal conditions (Collins A V, et al, Immunity, 2002, 17(2): 201-210).

[003] PD-L1 negatively regulates T-cell function also through interaction with another receptor, B7.1 (also known as B7-1 or CD80). Formation of the PD-L1/PD-1 and PD-L1/B7.1 complex negatively regulate T-cell receptor signaling, resulting in

the subsequent downregulation of T cell activation and suppression of anti-tumor immune activity (Butte M J, et al, Immunity, 2007, 27(1): 111-122).

[004] PD-L1 is often overexpressed in different tumors, and its interaction with PD-1 on T cells enables cancer cells to evade T-cell-mediated immune responses (Okazaki T, et al, Nature immunology, 2013, 14(12): 1212-1218). Thus, blocking the PD-1/PD-L1 interaction can restore T-cell activation and antitumor responses (Callahan M K, et al, Immunity, 2016, 44(5): 1069-1078). The success of antibody-based PD-1/PD-L1 blockade therapy, such as atezolizumab (Tecentriq®) (Rittmeyer A, et al, The Lancet, 2017, 389(10066): 255-265), avelumab (Bavencio®) (Hamilton G, et al, Expert Opinion on Biological Therapy, 2017, 17(4): 515-523) and durvalumab (Imfinzi®) (Brower V, The Lancet Oncology, 2016, 17(7): e275), has provided a breakthrough in the fight against human cancers, especially for solid tumors. Although an association between PD-L1 expression by tumor cells and/or infiltrating immune cells and clinical response to PD-1/PD-L1-targeted therapies has been shown, this association is not flawless (Herbst, R., et al, Nature 515, 563–567 (2014); Taube J M, et al, Clinical cancer research, 2014, 20(19): 5064-5074). Only a minority of PD-L1-positive tumors respond to these treatments, and certain PD-L1-negative tumors are nevertheless responsive to treatment. This raises the possibility that additional factors govern patient response to PD-1/PD-L1-targeted therapies, and that additional predictive biomarkers must be identified to improve the clinical use of these agents.

[005] Mariathasan S et al. found that lack of response was associated with a signature of transforming growth factor β (TGF-β) signaling in fibroblasts (Mariathasan S, et al, Nature, 2018, 554(7693): 544-548). David JM et al also found that as a pleiotropic cytokine known to induce epithelial mesenchymal transition (EMT) and suppress antitumor immunity, TGF-β could upregulate tumor PD-L1 expression in several epithelial NSCLC cell lines and the upregulation is associated with phosphorylation of Smad2, which is a key downstream effector of TGF-β signaling (David J M, et al, Oncoimmunology, 2017, 6(10): e1349589). In mouse,

therapeutic administration of a TGF-β blocking antibody together with anti-PD-L1 reduced TGF-β signaling in stromal cells, facilitated T cell penetration into the center of the tumor, and provoked vigorous anti-tumor immunity and tumor regression (Mariathasan S, et al, Nature, 2018, 554(7693): 544-548).

[006] However, low affinity of anti-PD-L1 antibodies still poses a challenge to achieve high treatment efficacy and low toxic side effect.

[007] Therefore, there is a need for therapeutic molecules with high binding affinity to PD-L1, improved therapeutic efficacy and reduced toxic side effect.

SUMMARY OF THE INVENTION

[008] Throughout the present disclosure, the articles "a," "an," and "the" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an antibody" means one antibody or more than one antibody.

[009] In one aspect, the present disclosure provides a bi-functional molecule comprising a first moiety that binds to an immune checkpoint molecule, and a second moiety that blocks activity of Interleukin-1 (IL-1).

[0010] In certain embodiments, the first moiety comprises an agonist of immunostimulatory check point molecule, optionally selected from the group consisting of: CD27, CD70, CD28, CD80 (B7-1), CD86 (B7-2), CD40, CD40L (CD154), CD122, CD137, CD137L, OX40 (CD134), OX40L (CD252), GITR, ICOS (CD278), and ICOSLG (CD275), CD2, ICAM-1, LFA-1 (CD11a/CD18), CD30, BAFFR, HVEM, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, and CD83.

[0011] In certain embodiments, the first moiety comprises an antagonist of immunoinhibitory check point molecule, optionally selected from the group consisting of: A2AR, B7-H3 (CD276), B7-H4 (VTCN1), BTLA (CD272), CTLA-4 (CD152), IDO1, IDO2, TDO, KIR, LAG3, NOX2, PD-1, PD-L1, PD-L2, TIM-3,

VISTA, SIGLEC7 (CD328), TIGIT, PVR(CD155), SIGLEC9 (CD329), CD160, LAIR1, 2B4 (CD244), CD47, and B7-H5.

[0012] In certain embodiments, the immune checkpoint molecule is PD-L1.

[0013] In certain embodiments, the first moiety comprises an antibody against PD-L1 or an antigen-binding fragment thereof, and the second moiety comprises an IL-1-binding moiety or an IL-1 Receptor (IL-1R)-binding moiety.

[0014] In certain embodiments, the IL-1-binding moiety comprises an IL-1R or an IL-1-binding fragment or variant thereof, or an antibody against IL-1 or an antigen-binding fragment thereof.

[0015] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises a heavy chain variable region and/or a light variable region from an anti-IL-1α antibody selected from the group consisting of: XB2001, lutikizumab, LY2189102 and bermekimab, or from an anti-IL-1β antibody selected from the group consisting of: SSGJ-613, CDP484, canakinumab and gevokizumab.

[0016] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 104 or SEQ ID NO: 112, a HCDR2 comprising a sequence of SEQ ID NO: 105 or SEQ ID NO: 113, and a HCDR3 comprising a sequence of SEQ ID NO: 106 or SEQ ID NO: 114, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 107 or SEQ ID NO: 115, a LCDR2 comprising a sequence of SEQ ID NO: 108 or SEQ ID NO: 116, and a LCDR3 comprising a sequence of SEQ ID NO: 109 or SEQ ID NO: 117.

[0017] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 104, a HCDR2 comprising a sequence of SEQ

ID NO: 105, and a HCDR3 comprising a sequence of SEQ ID NO: 106, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 107, a LCDR2 comprising a sequence of SEQ ID NO: 108, and a LCDR3 comprising a sequence of SEQ ID NO: 109.

[0018] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 112, a HCDR2 comprising a sequence of SEQ ID NO: 113, and a HCDR3 comprising a sequence of SEQ ID NO: 114, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 115, a LCDR2 comprising a sequence of SEQ ID NO: 116, and a LCDR3 comprising a sequence of SEQ ID NO: 117.

[0019] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 102, SEQ ID NO: 110, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 103, SEQ ID NO: 111, and a homologous sequence thereof having at least 80% sequence identity thereof.

[0020] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 102, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 103, and a homologous sequence thereof having at least 80% sequence identity thereof.

[0021] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 110, and a homologous sequence

thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 111, and a homologous sequence thereof having at least 80% sequence identity thereof.

[0022] In certain embodiments, the IL-1R-binding moiety comprises Interleukin-1 receptor antagonist or a fragment or variant thereof, or an antibody against IL-1R or an antigen-binding fragment thereof.

[0023] In certain embodiments, the antibody against IL-1R or an antigen-binding fragment thereof comprises a heavy chain variable region and/or a light variable region from an antibody selected from the group consisting of: spesolimab, astegolimab, imsidolimab, AMG 108, melrilimab, nidanilimab, MEDI8968, REGN6490, HB0034 and CSC012.

[0024] In another aspect, a bi-functional molecule comprises a first moiety that binds to PD-L1, and a second moiety that a) blocks activity of an immunosuppressive cytokine or b) stimulates immunity, wherein the first moiety comprises an antibody against PD-L1 or an antigen-binding fragment thereof comprising a heavy chain variable (VH) region and/or a light chain variable (VL) region, wherein the heavy chain variable region comprises:

a) a HCDR1 comprising **DYYMN** (SEQ ID NO: 1) or a homologous sequence of at least 80% sequence identity thereof,
b) a HCDR2 comprising **DINPNNX$_1$X$_2$TX$_3$YNHKFKG** (SEQ ID NO: 19) or a homologous sequence of at least 80% sequence identity thereof, and
c) a HCDR3 comprising **WGDGPFAY** (SEQ ID NO: 3) or a homologous sequence of at least 80% sequence identity thereof, and/or

wherein the light chain variable region comprises:

d) a LCDR1 comprises a sequence selected from the group consisting of **KASQNVX$_4$X$_5$X$_6$VA** (SEQ ID NO: 20) or a homologous sequence of at least 80% sequence identity thereof,

e) a LCDR2 comprises a sequence selected from the group consisting of **SX_7SX_8RYT** (SEQ ID NO: 21) or a homologous sequence of at least 80% sequence identity thereof, and

f) a LCDR3 comprises a sequence selected from the group consisting of **QQYSNYPT** (SEQ ID NO: 6) or a homologous sequence of at least 80% sequence identity thereof;

wherein X_1 is G or A, X_2 is G or D or Q or E or L, X_3 is S or M or Q or L or V, X_4 is G or P or K, X_5 is A or G, X_6 is A or I, X_7 is A or N or R or V, and X_8 is N or H or V or D.

[0025] In certain embodiments, the heavy chain variable region comprises:

a) a HCDR1 comprises a sequence of SEQ ID NO: 1,

b) a HCDR2 comprises a sequence selected from group consisting of SEQ ID NO: 2, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 17, and SEQ ID NO: 18 and

c) a HCDR3 comprises a sequence of SEQ ID NO: 3,

and/or

a light chain variable region comprising:

d) a LCDR1 comprises a sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9,

e) a LCDR2 comprises a sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, and

f) a LCDR3 comprises a sequence of SEQ ID NO: 6.

[0026] In certain embodiments, the heavy chain variable region is selected from the group consisting of:

a) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 2, and a HCDR3 comprising the sequence of SEQ ID NO: 3;

b) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 13, and a HCDR3 comprising the sequence of SEQ ID NO: 3;

c) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 14, and a HCDR3 comprising the sequence of SEQ ID NO: 3;

d) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 15, and a HCDR3 comprising the sequence of SEQ ID NO: 3;

e) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 17, and a HCDR3 comprising the sequence of SEQ ID NO: 3; and

f) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 18, and a HCDR3 comprising the sequence of SEQ ID NO: 3.

[0027] In certain embodiments, the light chain variable region is selected from the group consisting of:

a) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 4, a LCDR2 comprising the sequence of SEQ ID NO: 5, and a LCDR3 comprising the sequence of SEQ ID NO: 6;

b) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 9, a LCDR2 comprising the sequence of SEQ ID NO: 5, and a LCDR3 comprising the sequence of SEQ ID NO: 6;

c) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 8, a LCDR2 comprising the sequence of SEQ ID NO: 5, and a LCDR3 comprising the sequence of SEQ ID NO: 6;

d) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 4, a LCDR2 comprising the sequence of SEQ ID NO: 12, and a LCDR3 comprising the sequence of SEQ ID NO: 6; and

e) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 4, a LCDR2 comprising the sequence of SEQ ID NO: 11, and a LCDR3 comprising the sequence of SEQ ID NO: 6.

[0028] In certain embodiments, the antibody against PD-L1 or the antigen-binding fragment thereof further comprises one or more of heavy chain HFR1, HFR2, HFR3 and HFR4, and/or one or more of light chain LFR1, LFR2, LFR3 and LFR4, wherein:

a) the HFR1 comprises an amino acid sequence of **QVQLVQSGAEVKKPGASVKVSCKASGYX$_9$FT** (SEQ ID NO: 40) or a homologous sequence of at least 80% sequence identity thereof,

b) the HFR2 comprises an amino acid sequence of **WVRQAPGQX$_{10}$LEWMG** (SEQ ID NO: 41) or a homologous sequence of at least 80% sequence identity thereof,

c) the HFR3 sequence comprises an amino acid sequence of **RVTX$_{16}$TVDX$_{11}$SISTAYMELSRLRSDDTAVYYCX$_{12}$X$_{13}$** (SEQ ID NO: 42) or a homologous sequence of at least 80% sequence identity thereof,

d) the HFR4 comprises an amino acid sequence of **WGQGTLVTVSS** (SEQ ID NO: 25) or a homologous sequence of at least 80% sequence identity thereof,

e) the LFR1 comprises an amino acid sequence of **DIQMTQSPSSLSASVGDRVTITC** (SEQ ID NO: 26) or a homologous sequence of at least 80% sequence identity thereof,

f) the LFR2 comprises an amino acid sequence of **WYQQKPGKX$_{14}$PKLLIY** (SEQ ID NO: 43) or a homologous sequence of at least 80% sequence identity thereof,

g) the LFR3 comprises an amino acid sequence of **GVPX$_{15}$RFSGSGSGTDFTX$_{17}$TISSLQPEDIATYYC** (SEQ ID NO: 44) or a homologous sequence of at least 80% sequence identity thereof, and

h) the LFR4 comprises an amino acid sequence of **FGQGTKLEIK** (SEQ ID NO: 29) or a homologous sequence of at least 80% sequence identity thereof,

wherein X_9 is T or V, X_{10} is G or S, X_{11} is T or K, X_{12} is A or V, X_{13} is R or K, X_{14} is A or S, X_{15} is S or D, X_{16} is M or V, and X_{17} is F or L.

[0029] In certain embodiments,

the HFR1 comprises a sequence selected from the group consisting of SEQ ID NOs: 22 and 30,

the HFR2 comprises a sequence selected from the group consisting of SEQ ID NOs: 23 and 31,

the HFR3 comprises the sequence selected from the group consisting of SEQ ID NOs: 24 and 32-35,

the HFR4 comprises a sequence of SEQ ID NOs: 25,

the LFR1 comprises the sequence from the group consisting of SEQ ID NO: 26,

the LFR2 comprises a sequence selected from the group consisting of SEQ ID NOs: 27 and 36,

the LFR3 comprises a sequence selected from the group consisting of SEQ ID NOs: 28, and 37-38, 39, 45, and

the LFR4 comprises a sequence of SEQ ID NO: 29.

[0030] In certain embodiments, the heavy chain variable region comprises a sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, and a homologous sequence thereof having at least 80% sequence identity thereof.

[0031] In certain embodiments, the light chain variable region comprises a sequence selected from the group consisting of SEQ ID NO: 47, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, and a homologous sequence thereof having at least 80% sequence identity thereof.

[0032] In certain embodiments, the antibody against PD-L1 or antigen-binding fragment thereof comprises a pair of heavy chain variable region and light chain

variable region sequences selected from the group consisting of: SEQ ID NOs: 49/54, 50/54, 51/54, 52/54, 49/55, 50/55, 51/55, 52/55, 58/62, 58/63, 58/64, 58/65, 59/62, 59/63, 59/64, 59/65, 60/62, 60/63, 60/64, and 60/65.

[0033] In certain embodiments, the antibody against PD-L1 or antigen-binding fragment thereof further comprises one or more amino acid residue substitutions or modifications yet retains specific binding specificity and/or affinity to PD-L1.

[0034] In certain embodiments, at least one of the substitutions or modifications is in one or more of the CDR sequences, and/or in one or more of the non-CDR regions of the VH or VL sequences.

[0035] In certain embodiments, the antibody against PD-L1 or antigen-binding fragment thereof further comprises an immunoglobulin constant region, optionally a constant region of human Ig, or optionally a constant region of human IgG.

[0036] In certain embodiments, the constant region comprises an Fc region of human IgG1, IgG2, IgG3, or IgG4.

[0037] In certain embodiments, the Fc region of human IgG1 comprises SEQ ID NO: 80, or a variant thereof having at least 80% (e.g. at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity thereof.

[0038] In certain embodiments, the constant region comprises an Fc variant having reduced effector function relative to the corresponding wildtype Fc region. In certain embodiments, the Fc region comprises one or more amino acid residue modifications or substitutions resulting in reduced effector functions relative to SEQ ID NO: 80.

[0039] In certain embodiments, the Fc region comprises one or more amino acid residue substitutions selected from the group consisting of: 220S, 226S, 228P, 229S, 233P, 234V, 234G, 234A, 234F, 234A, 235A, 235G, 235E, 236E, 236R, 237A, 237K, 238S, 267R, 268A, 268Q, 269R, 297A, 297Q, 297G, 309L, 318A, 322A, 325L, 328R,

330S, 331S and any combination thereof, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat.

[0040] In certain embodiments, the Fc region comprises a combination of mutations selected from the group consisting of: a) K322A, L234A, and L235A; b) P331S, L234F, and L235E; c) L234A and L235A; c) N297A; d) N297Q; e) N297G; f) L235E; g) L234A and L235A (IgG1); h) F234A and L235A (IgG4); i) H268Q, V309L, A330S and P331S (IgG2); j) V234A, G237A, P238S, H268A, V309L, A330S and P331S (IgG2), wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat.

[0041] In certain embodiments, the Fc variant comprises an amino acid sequence of SEQ ID NO: 81.

[0042] In certain embodiments, the antibody against PD-L1 or antigen-binding fragment thereof is humanized.

[0043] In certain embodiments, the antigen-binding fragment is a diabody, a Fab, a Fab', a $F(ab')_2$, a Fd, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multispecific antibody, a camelized single domain antibody, a nanobody, a domain antibody, and a bivalent domain antibody.

[0044] In certain embodiments, the antibody or antigen-binding fragment thereof is capable of binding to both human PD-L1 and cyno PD-L1.

[0045] In certain embodiments, the first moiety comprises an antibody or an antigen-binding fragment thereof that competes for binding to PD-L1 with the antibody or antigen-binding fragment thereof provided herein.

[0046] In certain embodiments, the immunosuppressive cytokine comprises a cytokine in transforming growth factor beta (TGF-β) superfamily, IL-1, or Vascular endothelial growth factor (VEGF).

[0047] In certain embodiments, the immunosuppressive cytokine in TGF-β superfamily includes TGF-β, bone morphogenetic proteins (BMPs), activins, NODAL, and growth and differentiation factors (GDFs).

[0048] In certain embodiments, the immunosuppressive cytokine is TGF-β.

[0049] In certain embodiments, the second moiety comprises a TGFβ-binding moiety.

[0050] In certain embodiments, the TGFβ-binding moiety comprises a soluble TGFβ Receptor (TGFβR) or a TGFβ-binding fragment or variant thereof, or an antibody against TGFβ and an antigen-binding fragment thereof.

[0051] In certain embodiments, the soluble TGFβR comprises an extracellular domain (ECD) of the TGFβR, or a TGFβ-binding fragment, or variant thereof.

[0052] In certain embodiments, the TGFβR is selected from the group consisting of TGFβ Receptor I (TGFβRI), TGFβ Receptor II (TGFβRII), TGFβ Receptor III (TGFβRIII), and any combination thereof.

[0053] In certain embodiments, the TGFβR is TGFβRII.

[0054] In certain embodiments, the TGFβRII selectively binds to TGFβ1 over TGFβ2 and TGFβ3.

[0055] In certain embodiments, the TGFβ1 is human TGFβ1 or mouse TGFβ1.

[0056] In certain embodiments, the ECD of TGFβR comprises an amino acid sequence of SEQ ID NO: 66, 79, 78, 77 or a sequence having at least 80% sequence identity thereof yet retains specific binding specificity and/or affinity to TGF-β.

[0057] In certain embodiments, the second moiety comprises an IL-1-binding moiety or an IL-1 Receptor (IL-1R)-binding moiety.

[0058] In certain embodiments, the IL-1-binding moiety comprises a soluble IL-1R, an IL-1-binding fragment or variant of an IL-1R, or an antibody against IL-1 or an antigen-binding fragment thereof.

[0059] In certain embodiments, the IL-1-binding moiety comprises an extracellular domain (ECD) of the IL-1RI, an IL-1-binding fragment or variant of any of IL-1RI, ECD of IL-1RI, IL-1RII, or ECD of IL-1RII, or IL-1RAP, or ECD of IL-1RAP, IL-1sRI or IL-1sRII.

[0060] In certain embodiments, the IL-1R-binding moiety comprises IL-1Ra or an IL-1-binding fragment or variant thereof, or an antibody against IL-1R or an antigen-binding fragment thereof.

[0061] In certain embodiments, the antibody against IL-1R or an antigen-binding fragment thereof comprises a heavy chain variable region and/or a light variable region from an antibody selected from the group consisting of: spesolimab, astegolimab, imsidolimab, AMG 108, melrilimab, nidanilimab, MEDI8968, REGN6490, HB0034 and CSC012.

[0062] In certain embodiments, the IL-1R-binding moiety comprises an amino acid sequence of SEQ ID NO: 67 or 76, or an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 67 or 76, or an IL-1 binding fragment or variant thereof.

[0063] In certain embodiments, the IL-1 is IL-1α or IL-1β.

[0064] In certain embodiments, the IL-1β is human IL-1β.

[0065] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises a heavy chain variable region and/or a light variable

region from an anti-IL-1α antibody selected from the group consisting of: XB2001, lutikizumab, LY2189102 and bermekimab, or from an anti-IL-1β antibody selected from the group consisting of: SSGJ-613, CDP484, canakinumab and gevokizumab.

[0066] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 104 or SEQ ID NO: 112, a HCDR2 comprising a sequence of SEQ ID NO: 105 or SEQ ID NO: 113, and a HCDR3 comprising a sequence of SEQ ID NO: 106 or SEQ ID NO: 114, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 107 or SEQ ID NO: 115, a LCDR2 comprising a sequence of SEQ ID NO: 108 or SEQ ID NO: 116, and a LCDR3 comprising a sequence of SEQ ID NO: 109 or SEQ ID NO: 117.

[0067] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 104, a HCDR2 comprising a sequence of SEQ ID NO: 105, and a HCDR3 comprising a sequence of SEQ ID NO: 106, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 107, a LCDR2 comprising a sequence of SEQ ID NO: 108, and a LCDR3 comprising a sequence of SEQ ID NO: 109.

[0068] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 112, a HCDR2 comprising a sequence of SEQ ID NO: 113, and a HCDR3 comprising a sequence of SEQ ID NO: 114, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 115, a LCDR2 comprising a sequence of SEQ ID NO: 116, and a LCDR3 comprising a sequence of SEQ ID NO: 117.

[0069] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 102, SEQ ID NO: 110, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 103, SEQ ID NO: 111, and a homologous sequence thereof having at least 80% sequence identity thereof.

[0070] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 102, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 103, and a homologous sequence thereof having at least 80% sequence identity thereof.

[0071] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 110, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 111, and a homologous sequence thereof having at least 80% sequence identity thereof.

[0072] In certain embodiments, the bi-functional molecule comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 118 or SEQ ID NO: 120, and/or a light chain comprising an amino acid sequence of SEQ ID NO: 119 or SEQ ID NO: 121.

[0073] In certain embodiments, the second moiety stimulates anti-tumor immunity and comprises an immunostimulatory polypeptide.

[0074] In certain embodiments, the immunostimulatory polypeptide comprises Interleukin (IL)-2 (IL-2), IL-15, IL-21, IL-10, IL-12, IL-23, IL-27, IL-35,

granulocyte-macrophage colony-stimulating factor (GM-CSF), soluble CD4, soluble LAG-3, or IFN-α, or a functional equivalent thereof.

[0075] In certain embodiments, the soluble LAG-3 comprises an extracellular domain (ECD) of the LAG-3 or a MHCII-binding fragment or variant thereof.

[0076] In certain embodiments, the second moiety stimulates anti-tumor immunity and comprises an antagonist of an immunoinhibitory receptor signaling.

[0077] In certain embodiments, the immunoinhibitory receptor is Signal-regulatory protein alpha (SIRPα).

[0078] In certain embodiments, the second moiety blocks interaction between CD47 and SIRPα.

[0079] In certain embodiments, the second moiety comprises a CD47 binding domain or a SIRPα binding domain.

[0080] In certain embodiments, the CD47 binding domain comprises a soluble SIRPα or a CD47 binding fragment or variant thereof, or an anti-CD47 antibody or an antigen-binding fragment thereof.

[0081] In certain embodiments, the soluble SIRPα comprises an extracellular domain (ECD) of the SIRPα, or a CD47-binding fragment or variant thereof.

[0082] In certain embodiments, the soluble SIRPα comprises an amino acid sequence of SEQ ID NO: 84 or an amino acid sequence having at least 80% sequence identity thereof yet retaining binding specificity to CD47.

[0083] In certain embodiments, the SIRPα binding domain comprises a soluble CD47 or a SIRPα binding fragment or variant thereof, or an anti-SIRPα antibody or an antigen-binding fragment thereof.

[0084] In certain embodiments, the soluble CD47 comprises an extracellular domain (ECD) of the CD47 or a SIRPα binding fragment or a variant thereof, an anti-SIRPα antibody or an antigen-binding fragment thereof.

[0085] In certain embodiments, the bi-functional molecule further comprises a linker connecting the first moiety and the second moiety.

[0086] In certain embodiments, the linker is selected from the group consisting of a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, and a non-helical linker.

[0087] In certain embodiments, the linker comprises an amino acid sequence of ((G)nS)m, wherein m and n are independently an integer selected from 0 to 30 (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10). In certain embodiments, n is 2, 3, 4 or 5, and m is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In certain embodiments, the linker comprises an amino acid sequence of SEQ ID NO: 68.

[0088] In certain embodiments, the bi-functional molecule comprises one or more of the second moieties.

[0089] In certain embodiments, at least one of the second moieties is linked to an N terminus or a C terminus of a polypeptide chain of the first moiety.

[0090] In certain embodiments, at least one of the second moieties is linked to: a) an N terminus or a C terminus of a heavy chain of the first moiety, or b) an N terminus or a C terminus of a light chain of the first moiety.

[0091] In certain embodiments, at least one of the second moieties is linked to a C terminus of a heavy chain constant region of the first moiety.

[0092] In certain embodiments, each of the second moieties is linked respectively to the C terminus of each heavy chain constant region of the first moiety.

[0093] In certain embodiments, the bi-functional molecule comprises more than one of the second moieties that are linked respectively to: an N terminus of a heavy chain of the first moiety, a C terminus of a heavy chain of the first moiety, an N terminus of a light chain of the first moiety, a C terminus of a light chain of the first moiety, or any combination thereof.

[0094] In certain embodiments, the bi-functional molecule comprises homodimeric or heterodimeric heavy chains.

[0095] In certain embodiments, the heavy chains are heterodimeric with respect to presence or position of the second moiety.

[0096] In certain embodiments, the heterodimeric heavy chains comprise one heavy chain having the second moiety but the other heavy chain having not.

[0097] In certain embodiments, the heterodimeric heavy chains further comprise heterodimeric Fc regions that associate in a way that discourages homodimerization and/or favors heterodimerization.

[0098] In certain embodiments, the first and the heterodimeric Fc regions are capable of associating into heterodimers via knobs-into-holes, hydrophobic interaction, electrostatic interaction, hydrophilic interaction, or increased flexibility.

[0099] In certain embodiments, the heterodimeric Fc regions comprises Y349C, T366S, L368A or Y407V or any combination thereof in one Fc region, and S354C, or T366W or combination thereof in another Fc region, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat.

[00100] In certain embodiments, the bi-functional molecule is further linked to one or more conjugate moieties.

[00101] In certain embodiments, the conjugate moiety comprises a clearance-modifying agent, a chemotherapeutic agent, a toxin, a radioactive isotope, a

lanthanide, a luminescent label, a fluorescent label, an enzyme-substrate label, a DNA-alkylator, a topoisomerase inhibitor, a tubulin-binders, or other anticancer drugs such as androgen receptor inhibitor.

[00102] In another aspect, the present disclosure further provides a pharmaceutical composition or kit comprising the bi-functional molecule provided herein and a pharmaceutically acceptable carrier.

[00103] In another aspect, the present disclosure further provides an isolated polynucleotide encoding the bi-functional molecule provided herein.

[00104] In another aspect, the present disclosure further provides a vector comprising the isolated polynucleotide provided herein.

[00105] In another aspect, the present disclosure further provides a host cell comprising the vector provided herein.

[00106] In another aspect, the present disclosure further provides a method of expressing the bi-functional molecule provided herein, comprising culturing the host cell provided herein under the condition at which the vector is expressed.

[00107] In another aspect, the present disclosure further provides a method of treating, preventing or alleviating a PD-L1 related disease in a subject, comprising administering to the subject a therapeutically effective amount of the bi-functional molecule provided herein and/or the pharmaceutical composition or kit provided herein.

[00108] In certain embodiments, the disease is immune related disease or disorder, cancers, autoimmune diseases, or infectious disease.

[00109] In certain embodiments, the cancer is selected from the group consisting of: lung cancer (e.g., non-small cell lung cancer), liver cancer, pancreatic cancer, breast cancer, bronchial cancer, bone cancer, liver and bile duct cancer, ovarian cancer,

testicle cancer, kidney cancer, bladder cancer, head and neck cancer, spine cancer, brain cancer, cervix cancer, uterine cancer, endometrial cancer, colon cancer, colorectal cancer, prostate cancer, gastric-esophageal cancer, rectal cancer, anal cancer, gastrointestinal cancer, skin cancer, pituitary cancer, stomach cancer, vagina cancer, thyroid cancer, glioblastoma, astrocytoma, melanoma, myelodysplastic syndrome, sarcoma, teratoma, glioma, and adenocarcinoma.

[00110] In certain embodiments, the subject has been identified as having a PD-L1-expressing cancer cell.

[00111] In certain embodiments, the subject is human.

[00112] In certain embodiments, the method further comprises administering a therapeutically effective amount of a second therapeutic agent.

[00113] In certain embodiments, the second therapeutic agent is selected from a chemotherapeutic agent, an anti-cancer drug, radiation therapy, an immunotherapy agent, anti-angiogenesis agent, a targeted therapy agent, a cellular therapy agent, a gene therapy agent, a hormonal therapy agent, or cytokines.

[00114] In another aspect, the present disclosure provides use of the bi-functional molecule provided herein in the manufacture of a medicament for treating a PD-L1 related disease or condition in a subject.

[00115] In another aspect, the present disclosure provides a method of treating, preventing or alleviating in a subject a disease or condition that would benefit from suppression of an immunosuppressive cytokine, from induction of sustained immune responses, or from stimulation of anti-tumor immunity, comprising administering an effective amount of the bi-functional molecule provided herein.

[00116] In certain embodiments, the immunosuppressive cytokine is TGFβ.

[00117] In certain embodiments, the disease or condition is a TGFβ-related disease or condition.

[00118] In certain embodiments, the TGFβ-related disease is cancer, fibrotic disease, or kidney disease.

[00119] In certain embodiments, the immunosuppressive cytokine is IL-1.

[00120] In certain embodiments, the disease or condition is an IL-1-related disease or condition.

[00121] In certain embodiments, the disease or condition would benefit from stimulation of anti-tumor immunity by inhibiting an immunoinhibitory receptor signaling, e.g., SIRPα signaling.

BRIEF DESCFRIPTION OF THE DRAWINGS

[00122] Figure 1 shows humanized 4B6 antibodies binding to human PD-L1 by ELISA.

[00123] Figure 2 shows Hu4B6_HgLa binding to human PD-L1 by ELISA.

[00124] Figure 3A - Figure 3C show AM-4B6-IgG1-TGFβRII variants binding to PD-L1 by ELISA.

[00125] Figure 4 shows affinity ranking of AM-4B6-IgG1-TGFβRII variants using flow cytometry.

[00126] Figure 5A and 5B show blockade of PD-L1/PD-1 or PD-L1/B7-1 by AM-4B6-IgG1-TGFβRII variants.

[00127] Figure 6 shows blockade of PD-L1/PD-1 by AM-4B6-IgG1-TGFβRII variants using cell based assay.

[00128] Figure 7 shows SDS-PAGE of AM4B6_hIgG1_TBRII (20-136) expressed with stable cell line.

[00129] Figure 8A and Figure 8B show binding to human PD-L1 or cyno PD-L1 by ELISA analysis.

[00130] Figure 9A - Figure 9C show binding to human PD-L1 and B7 family other members and other members of TGFβ superfamily by ELISA analysis.

[00131] Figure 10A - Figure 10F show binding to PD-L1 expressing cells by FACS analysis.

[00132] Figure 11 shows binding to human PD-L1 on activated human T cells by FACS analysis.

[00133] Figure 12A - Figure 12B show blockade of human PD-L1 binding to human PD-1 or cyno PD-L1 binding to cyno PD-1 by ELISA analysis.

[00134] Figure 13 shows simultaneously binding to hPD-L1 and TGFb1 by ELISA analysis.

[00135] Figure 14 shows blocking hPD-L1/hPD-1 using a reporter assay.

[00136] Figure 15 shows blocking TGFβ1 signaling using a TGF-β reporter HEK-293 cell line.

[00137] Figure 16 shows effect of AM4B6-hIgG1-TGFβRII' on IFNγ release of PBMC stimulated by tuberculin (TB).

[00138] Figure 17A - Figure 17B show anti-tumor activity in MC38-hPD-L1 tumor model.

[00139] Figure 18A - Figure 18B show anti-tumor activity in H460 tumor model.

[00140] Figure 19A - Figure 19B show anti-tumor activity in EMT6-hPD-L1 tumor model.

[00141] Figure 20A - Figure 20C show pharmacokinetics and pharmacodynamics study of AM4B6-hIgG1-TGFβRII in vivo.

[00142] Figure 21 shows binding activity of AM4B6-hIgG1-IL-1RA to human PD-L1 by ELISA.

[00143] Figure 22 shows binding activity of AM4B6-hIgG1-IL-1RA to human PD-L1 by FACS analysis.

[00144] Figure 23 shows blockade of PD-L1/PD-1 by AM4B6-hIgG1-IL-1RA using cell based assay.

[00145] Figure 24 shows blocking activity of AM4B6-IgG1-IL-1RA to human IL-1β by ELISA.

[00146] Figure 25 shows blocking activity of AM4B6-hIgG1-IL-1RA to human IL-1β on reporter cells.

[00147] Figure 26 shows SEC-HPLC purity of asymmetric bifunctional antibodies.

[00148] Figure 27 shows binding of the bi-functional molecule to human PD-L1 as measured by ELISA.

[00149] Figure 28 shows binding of the bi-functional molecule to human CD47 as measured by ELISA.

[00150] Figure 29 shows ELISA binding activities of IgG-scFv-ACZ885-AM4B6 and IgG-scFv-XOMA052-AM4B6 bsAbs to hIL-1β protein.

[00151] Figure 30 shows ELISA binding activities of IgG-scFv-ACZ885-AM4B6 and IgG-scFv-XOMA052-AM4B6 bsAbs to hPD-L1 protein.

[00152] Figure 31 shows binding of IgG-scFv-ACZ885-AM4B6 and IgG-scFv-XOMA052-AM4B6 to PD-L1 expressing 293T cells by FACS method.

[00153] Figure 32 shows cell based PD1/PD-L1 blockade activity of IgG-scFv-ACZ885-AM4B6 and IgG-scFv-XOMA052-AM4B6.

[00154] Figure 33 shows blocking activity of IgG-scFv-XOMA052-AM4B6 to human IL-1β on HDF cells.

[00155] Figure 34 shows blocking activity of IgG-scFv-ACZ885-AM4B6 to hIL-1β on reporter cell.

DETAILED DESCRIPTION OF THE INVENTION

[00156] The following description of the disclosure is merely intended to illustrate various embodiments of the disclosure. As such, the specific modifications discussed are not to be construed as limitations on the scope of the disclosure. It will be apparent to a person skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the disclosure, and it is understood that such equivalent embodiments are to be included herein. All references cited herein, including publications, patents and patent applications are incorporated herein by reference in their entirety.

Definitions

[00157] The term "antibody" as used herein includes any immunoglobulin, monoclonal antibody, polyclonal antibody, multivalent antibody, bivalent antibody, monovalent antibody, multispecific antibody, or bispecific antibody that binds to a specific antigen. A native intact antibody comprises two heavy (H) chains and two light (L) chains. Mammalian heavy chains are classified as alpha, delta, epsilon, gamma, and mu, each heavy chain consists of a variable region (VH) and a first, second, third, and optionally fourth constant region (CH1, CH2, CH3, CH4 respectively); mammalian light chains are classified as λ or κ, while each light chain consists of a variable region (VL) and a constant region. The antibody has a "Y"

shape, with the stem of the Y consisting of the second and third constant regions of two heavy chains bound together via disulfide bonding. Each arm of the Y includes the variable region and first constant region of a single heavy chain bound to the variable and constant regions of a single light chain. The variable regions of the light and heavy chains are responsible for antigen binding. The variable regions in both chains generally contain three highly variable loops called the complementarity determining regions (CDRs) (light chain CDRs including LCDR1, LCDR2, and LCDR3, heavy chain CDRs including HCDR1, HCDR2, HCDR3). CDR boundaries for the antibodies and antigen-binding fragments disclosed herein may be defined or identified by the conventions of Kabat, IMGT, Chothia, or Al-Lazikani (Al-Lazikani, B., Chothia, C., Lesk, A. M., *J. Mol. Biol.*, 273(4), 927 (1997); Chothia, C. *et al.*, *J Mol Biol.* Dec 5;186(3):651-63 (1985); Chothia, C. and Lesk, A.M., *J.Mol.Biol.*, 196,901 (1987); Chothia, C. *et al.*, *Nature*. Dec 21-28;342(6252):877-83 (1989) ; Kabat E.A. *et al.*, Sequences of Proteins of immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991); Marie-Paule Lefranc *et al.*, *Developmental and Comparative Immunology*, 27: 55-77 (2003); Marie-Paule Lefranc *et al.*, *Immunome Research*, 1(3), (2005); Marie-Paule Lefranc, Molecular Biology of B cells (second edition), chapter 26, 481-514, (2015)). The three CDRs are interposed between flanking stretches known as framework regions (FRs) (light chain FRs including LFR1, LFR2, LFR3, and LFR4, heavy chain FRs including HFR1, HFR2, HFR3, and HFR4), which are more highly conserved than the CDRs and form a scaffold to support the highly variable loops. The constant regions of the heavy and light chains are not involved in antigen-binding, but exhibit various effector functions. The term "effector function" as used herein refers to cell-mediated or complement-mediated cytotoxic effects brought about by interactions between the Fc region of an antibody and C1q complement protein or Fc receptors (FcRs) on immune cells. Exemplary effector functions include, without limitation, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) effects. Antibodies are assigned to classes based on the amino acid sequences of the constant

regions of their heavy chains. The five major classes or isotypes of antibodies are IgA, IgD, IgE, IgG, and IgM, which are characterized by the presence of alpha, delta, epsilon, gamma, and mu heavy chains, respectively. Several of the major antibody classes are divided into subclasses such as IgG1 (gamma1 heavy chain), IgG2 (gamma2 heavy chain), IgG3 (gamma3 heavy chain), IgG4 (gamma4 heavy chain), IgA1 (alpha1 heavy chain), or IgA2 (alpha2 heavy chain).

[00158] In certain embodiments, the antibody provided herein encompasses any antigen-binding fragments thereof. The term "antigen-binding fragment" as used herein refers to an antibody fragment formed from a portion of an antibody comprising one or more CDRs, or any other antibody fragment that binds to an antigen but does not comprise an intact native antibody structure. Examples of antigen-binding fragments include, without limitation, a diabody, a Fab, a Fab', a $F(ab')_2$, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a bispecific antibody, a multispecific antibody, a camelized single domain antibody, a nanobody, a domain antibody, and a bivalent domain antibody. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody binds.

[00159] "Fab" with regard to an antibody refers to that portion of the antibody consisting of a single light chain (both variable and constant regions) bound to the variable region and first constant region of a single heavy chain by a disulfide bond.

[00160] "Fab'" refers to a Fab fragment that includes a portion of the hinge region.

[00161] "$F(ab')_2$" refers to a dimer of Fab'.

[00162] "Fc" with regard to an antibody (e.g. of IgG, IgA, or IgD isotype) refers to that portion of the antibody consisting of the second and third constant domains of a first heavy chain bound to the second and third constant domains of a second heavy chain via disulfide bonding. Fc with regard to antibody of IgM and IgE isotype

further comprises a fourth constant domain. The Fc portion of the antibody is responsible for various effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC), and complement dependent cytotoxicity (CDC), but does not function in antigen binding.

[00163] "Fv" with regard to an antibody refers to the smallest fragment of the antibody to bear the complete antigen binding site. An Fv fragment consists of the variable region of a single light chain bound to the variable region of a single heavy chain.

[00164] "Single-chain Fv antibody" or "scFv" refers to an engineered antibody consisting of a light chain variable region and a heavy chain variable region connected to one another directly or via a peptide linker sequence (Huston JS *et al. Proc Natl Acad Sci USA*, 85:5879(1988)).

[00165] "Single-chain Fv-Fc antibody" or "scFv-Fc" refers to an engineered antibody consisting of a scFv connected to the Fc region of an antibody.

[00166] "Camelized single domain antibody," "heavy chain antibody," or "HCAb" refers to an antibody that contains two V_H domains and no light chains (Riechmann L. and Muyldermans S., *J Immunol Methods.* Dec 10; 231(1-2):25-38 (1999); Muyldermans S., *J Biotechnol.* Jun; 74(4):277-302 (2001); WO94/04678; WO94/25591; U.S. Patent No. 6,005,079). Heavy chain antibodies were originally derived from *Camelidae* (camels, dromedaries, and llamas). Although devoid of light chains, camelized antibodies have an authentic antigen-binding repertoire (Hamers-Casterman C. *et al.*, *Nature*. Jun 3; 363(6428):446-8 (1993); Nguyen VK. *et al. Immunogenetics*. Apr; 54(1):39-47 (2002); Nguyen VK. *et al. Immunology*. May; 109(1): 93-101 (2003)). The variable domain of a heavy chain antibody (VHH domain) represents the smallest known antigen-binding unit generated by adaptive immune responses (Koch-Nolte F. *et al.*, *FASEB J.* Nov; 21(13): 3490-8. Epub 2007 Jun 15 (2007)).

[00167] A "nanobody" refers to an antibody fragment that consists of a VHH domain from a heavy chain antibody and two constant domains, CH2 and CH3.

[00168] A "diabody" or "dAb" includes small antibody fragments with two antigen-binding sites, wherein the fragments comprise a VH domain connected to a VL domain in the same polypeptide chain (VH-VL or VL-VH) (see, e.g. Holliger P. et al., Proc Natl Acad Sci USA. Jul 15;90(14):6444-8 (1993); EP404097; WO93/11161). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain, thereby creating two antigen-binding sites. The antigen-binding sites may target the same or different antigens (or epitopes). In certain embodiments, a "bispecific ds diabody" is a diabody target two different antigens (or epitopes).

[00169] A "domain antibody" refers to an antibody fragment containing only the variable region of a heavy chain or the variable region of a light chain. In certain instances, two or more VH domains are covalently joined with a peptide linker to create a bivalent or multivalent domain antibody. The two VH domains of a bivalent domain antibody may target the same or different antigens.

[00170] The term "valent" as used herein refers to the presence of a specified number of antigen binding sites in a given molecule. The term "monovalent" refers to an antibody or an antigen-binding fragment having only one single antigen-binding site; and the term "multivalent" refers to an antibody or antigen-binding fragment having multiple antigen-binding sites. As such, the terms "bivalent", "tetravalent", and "hexavalent" denote the presence of two binding sites, four binding sites, and six binding sites, respectively, in an antigen-binding molecule. In some embodiments, the antibody or antigen-binding fragment thereof is bivalent.

[00171] As used herein, a "bispecific" antibody refers to an artificial antibody which has fragments derived from two different monoclonal antibodies and is capable of

binding to two different epitopes. The two epitopes may present on the same antigen, or they may present on two different antigens.

[00172] In certain embodiments, an "scFv dimer" is a bivalent diabody or bispecific scFv (BsFv) comprising VH-VL (linked by a peptide linker) dimerized with another VH-VL moiety such that VH's of one moiety coordinate with the VL's of the other moiety and form two binding sites which can target the same antigens (or epitopes) or different antigens (or epitopes). In other embodiments, an "scFv dimer" is a bispecific diabody comprising VH1-VL2 (linked by a peptide linker) associated with VL1-VH2 (also linked by a peptide linker) such that VH1 and VL1 coordinate and VH2 and VL2 coordinate and each coordinated pair has a different antigen specificity.

[00173] A "dsFv" refers to a disulfide-stabilized Fv fragment that the linkage between the variable region of a single light chain and the variable region of a single heavy chain is a disulfide bond. In some embodiments, a "(dsFv)2" or "(dsFv-dsFv')" comprises three peptide chains: two VH moieties linked by a peptide linker (e.g. a long flexible linker) and bound to two VL moieties, respectively, via disulfide bridges. In some embodiments, dsFv-dsFv' is bispecific in which each disulfide paired heavy and light chain has a different antigen specificity.

[00174] The term "chimeric" as used herein, means an antibody or antigen-binding fragment, having a portion of heavy and/or light chain derived from one species, and the rest of the heavy and/or light chain derived from a different species. In an illustrative example, a chimeric antibody may comprise a constant region derived from human and a variable region from a non-human animal, such as from mouse. In some embodiments, the non-human animal is a mammal, for example, a mouse, a rat, a rabbit, a goat, a sheep, a guinea pig, or a hamster.

[00175] The term "humanized" as used herein means that the antibody or antigen-binding fragment comprises CDRs derived from non-human animals, FR

regions derived from human, and when applicable, the constant regions derived from human.

[00176] The term "affinity" as used herein refers to the strength of non-covalent interaction between an immunoglobulin molecule (i.e. antibody) or fragment thereof and an antigen.

[00177] The term "specific binding" or "specifically binds" as used herein refers to a non-random binding reaction between two molecules, such as for example between an antibody and an antigen. Specific binding can be characterized in binding affinity, for example, represented by K_D value, i.e., the ratio of dissociation rate to association rate (k_{off}/k_{on}) when the binding between the antigen and antigen-binding molecule reaches equilibrium. K_D may be determined by using any conventional method known in the art, including but are not limited to surface plasmon resonance method, Octet method, microscale thermophoresis method, HPLC-MS method and FACS assay method. A K_D value of $\leqslant 10^{-6}$ M (e.g. $\leqslant 5x10^{-7}$ M, $\leqslant 2x10^{-7}$ M, $\leqslant 10^{-7}$ M, $\leqslant 5x10^{-8}$ M, $\leqslant 2x10^{-8}$ M, $\leqslant 10^{-8}$ M, $\leqslant 5x10^{-9}$ M, $\leqslant 4x10^{-9}$ M, $\leqslant 3x10^{-9}$ M, $\leqslant 2x10^{-9}$ M, or $\leqslant 10^{-9}$ M) can indicate specific binding between an antibody or antigen binding fragments thereof and PD-L1 (e.g. human PD-L1 or cynomolgus PD-L1).

[00178] The ability to "compete for binding to PD-L1" as used herein refers to the ability of a first antibody or antigen-binding fragment to inhibit the binding interaction between PD-L1 and a second anti-PD-L1 antibody to any detectable degree. In certain embodiments, an antibody or antigen-binding fragment that compete for binding to PD-L1 inhibits the binding interaction between PD-L1 and a second anti- PD-L1 antibody by at least 85%, or at least 90%. In certain embodiments, this inhibition may be greater than 95%, or greater than 99%.

[00179] The term "amino acid" as used herein refers to an organic compound containing amine ($-NH_2$) and carboxyl (-COOH) functional groups, along with a side chain specific to each amino acid. The names of amino acids are also represented as

standard single letter or three-letter codes in the present disclosure, which are summarized as follows.

Names	Three-letter Code	Single-letter Code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[00180] The terms “polypeptide”, “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms also apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers.

[00181] A “conservative substitution” with reference to amino acid sequence refers to replacing an amino acid residue with a different amino acid residue having a side chain with similar physiochemical properties. For example, conservative substitutions can be made among amino acid residues with hydrophobic side chains (e.g. Met, Ala, Val, Leu, and Ile), among amino acid residues with neutral hydrophilic side chains (e.g. Cys, Ser, Thr, Asn and Gln), among amino acid residues with acidic

side chains (e.g. Asp, Glu), among amino acid residues with basic side chains (e.g. His, Lys, and Arg), or among amino acid residues with aromatic side chains (e.g. Trp, Tyr, and Phe). As known in the art, conservative substitution usually does not cause significant change in the protein conformational structure, and therefore could retain the biological activity of a protein.

[00182] "Percent (%) sequence identity" with respect to amino acid sequence (or nucleic acid sequence) is defined as the percentage of amino acid (or nucleic acid) residues in a candidate sequence that are identical to the amino acid (or nucleic acid) residues in a reference sequence, after aligning the sequences and, if necessary, introducing gaps, to achieve the maximum correspondence. Alignment for purposes of determining percent amino acid (or nucleic acid) sequence identity can be achieved, for example, using publicly available tools such as BLASTN, BLASTp (available on the website of U.S. National Center for Biotechnology Information (NCBI), see also, Altschul S.F. et al, J. Mol. Biol., 215:403–410 (1990); Stephen F. et al, Nucleic Acids Res., 25:3389–3402 (1997)), ClustalW2 (available on the website of European Bioinformatics Institute, see also, Higgins D.G. et al, Methods in Enzymology, 266:383-402 (1996); Larkin M.A. et al, Bioinformatics (Oxford, England), 23(21): 2947-8 (2007)), and ALIGN or Megalign (DNASTAR) software. Those skilled in the art may use the default parameters provided by the tool, or may customize the parameters as appropriate for the alignment, such as for example, by selecting a suitable algorithm. In certain embodiments, the non-identical residue positions may differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well

known to those of skill in the art. See, e.g., Pearson (1994) Methods Mol. Biol. 24: 307-331, which is herein incorporated by reference.

[00183] As used herein, a "homologous sequence" refers to a polynucleotide sequence (or its complementary strand) or an amino acid sequence that has sequence identity of at least 80% (e.g. at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) to another sequence when optionally aligned.

[00184] An "isolated" substance has been altered by the hand of man from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide is "isolated" if it has been sufficiently separated from the coexisting materials of its natural state so as to exist in a substantially pure state. An isolated "nucleic acid" or "polynucleotide" are used interchangeably and refer to the sequence of an isolated nucleic acid molecule. In certain embodiments, an "isolated antibody or antigen-binding fragment thereof" refers to the antibody or antigen-binding fragments having a purity of at least 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% as determined by electrophoretic methods (such as SDS-PAGE, isoelectric focusing, capillary electrophoresis), or chromatographic methods (such as ion exchange chromatography or reverse phase HPLC).

[00185] The term "subject" includes human and non-human animals. Non-human animals include all vertebrates, e.g., mammals and non-mammals, such as non-human primates, mouse, rat, cat, rabbit, sheep, dog, cow, chickens, amphibians, and reptiles. Except when noted, the terms "patient" or "subject" are used herein interchangeably.

[00186] "Treating" or "treatment" of a condition as used herein includes preventing or alleviating a condition, slowing the onset or rate of development of a condition, reducing the risk of developing a condition, preventing or delaying the development

of symptoms associated with a condition, reducing or ending symptoms associated with a condition, generating a complete or partial regression of a condition, curing a condition, or some combination thereof.

[00187] The term "vector" as used herein refers to a vehicle into which a genetic element may be operably inserted so as to bring about the expression of that genetic element, such as to produce the protein, RNA or DNA encoded by the genetic element, or to replicate the genetic element. A vector may be used to transform, transduce, or transfect a host cell so as to bring about expression of the genetic element it carries within the host cell. Examples of vectors include plasmids, phagemids, cosmids, artificial chromosomes such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or P1-derived artificial chromosome (PAC), bacteriophages such as lambda phage or M13 phage, and animal viruses. A vector may contain a variety of elements for controlling expression, including promoter sequences, transcription initiation sequences, enhancer sequences, selectable elements, and reporter genes. In addition, the vector may contain an origin of replication. A vector may also include materials to aid in its entry into the cell, including but not limited to a viral particle, a liposome, or a protein coating. A vector can be an expression vector or a cloning vector. The present disclosure provides vectors (e.g. expression vectors) containing the nucleic acid sequence provided herein encoding the antibody or antigen-binding fragment thereof, at least one promoter (e.g. SV40, CMV, EF-1α) operably linked to the nucleic acid sequence, and at least one selection marker.

[00188] The "host cell" as used herein refers to a cell into which an exogenous polynucleotide and/or a vector has been introduced.

[00189] The term "soluble" as used herein refers to the capability of a molecule (e.g., protein) of being dissolved in a solvent, such as a liquid and an aqueous environment.

[00190] The terms "transforming growth factor beta" and "TGFβ" as used herein refer to any of the TGFβ family proteins that have either the full-length, native amino

acid sequence of any of the TGF-betas from subjects (e.g. human), including the latent forms and associated or unassociated complex of precursor and mature TGFβ ("latent TGFβ"). Reference to such TGFβ herein will be understood to be a reference to any one of the currently identified forms, including TGFβ1, TGFβ2, TGFβ3 isoforms and latent versions thereof, as well as to human TGFβ species identified in the future, including polypeptides derived from the sequence of any known TGFβ and being at least about 75%, preferably at least about 80%, more preferably at least about 85%, still more preferably at least about 90%, and even more preferably at least about 95% homologous with the sequence. The specific terms "TGFβ1," "TGFβ2," and "TGFβ3" refer to the TGF-betas defined in the literature, e.g., Derynck *et al.*, *Nature*, *Cancer Res.*, 47: 707 (1987); Seyedin *et al.*, *J. Biol. Chem.*, 261: 5693-5695 (1986); deMartin *et al.*, *EMBO J.*, 6: 3673 (1987); Kuppner *et al.*, *Int. J. Cancer*, 42: 562 (1988). The terms "transforming growth factor beta", "TGFβ", "TGFbeta", "TGF-β", and "TGF-beta" are used interchangeably in the present disclosure.

[00191] As used herein, the term "human TGFβ1" refers to a TGFβ1 protein encoded by a human TGFB1 gene (e.g., a wild-type human TGFB1 gene). An exemplary wild-type human TGFβ1 protein is provided by GenBank Accession No. NP_000651.3. As used herein, the term "human TGFβ2" refers to a TGFβ2 protein encoded by a human TGFB2 gene (e.g., a wild-type human TGFB2 gene). Exemplary wild-type human TGFβ2 proteins are provided by GenBank Accession Nos. NP_001129071.1 and NP_003229.1. As used herein, the term "human TGFβ3" refers to a TGFβ3 protein encoded by a human TGFB3 gene (e.g., a wild-type human TGFB3 gene). Exemplary wild-type human TGFβ3 proteins are provided by GenBank Accession Nos. NP_003230.1, NP_001316868.1, and NP_001316867.1.

[00192] As used herein, the terms "mouse TGFβ1", "mouse TGFβ2", and "mouse TGFβ3" refer to a TGFβ1 protein, TGFβ2 protein, and TGFβ3 protein encoded by a mouse TGFB1 gene (e.g., a wild-type mouse TGFB1 gene), mouse TGFB2 gene (e.g., a wild-type mouse TGFB2 gene), and mouse TGFB3 gene (e.g., a

wild-type mouse TGFB3 gene), respectively. Exemplary wild-type mouse (*Mus musculus*) TGFβ1 protein are provided by GenBank Accession Nos. NP_035707.1 and CAA08900.1. An exemplary wild-type mouse TGFβ2 protein is provided by GenBank Accession No. NP_033393.2. An exemplary wild-type mouse TGFβ3 protein is provided by GenBank Accession No. AAA40422.1.

[00193] The term "TGFβ receptor" as used herein refers to any receptor that binds at least one TGFβ isoform. Generally, the TGFβ receptor includes TGFβ Receptor I (TGFβRI), TGFβ Receptor II (TGFβRII), or TGFβ Receptor III (TGFβRIII).

[00194] With regard to human, the term "TGFβ Receptor I" or "TGFβRI" refers to human TGFβ Receptor Type 1 sequence, including the wild type TGFβRI as well as all isoforms and variants thereof known to be capable of binding to at least one TGFβ isoform. Exemplary amino acid sequence of wild type TGFβRI is available under GenBank Accession No. ABD46753.1 or under UniProtKB-P36897, also included herein as SEQ ID NO: 69. A variant TGFβRI may have a sequence of at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to the amino acid sequence of SEQ ID NO: 69 and retain at least 25%, 35%, 50%, 75%, 90%, 95%, or 99% of the TGFβ-binding activity of the wild-type sequence (e.g. SEQ ID NO: 69).

[00195] With regard to human, the term "TGFβ Receptor II" or "TGFβRII" refers to human TGFβ Receptor Type 2 Isoform A sequence, including the wild type TGFβRII as well as all isoforms and variants thereof known to be capable of binding to at least one TGFβ isoform. Exemplary amino acid sequence of wild type TGFβRII isoform A or isoform 1 is available under GenBank Accession No. NP_001020018.1 or under UniProtKB - P37173-1, also included herein as SEQ ID NO: 70, and wild type TGFβRII isoform B is available under GenBank Accession No. NP_003233.4 or UniProtKB - P37173-2, also included herein as SEQ ID NO: 71. A variant TGFβRII may have a sequence of at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 70 or 71, and retains at least 25%,

35%, 50%, 75%, 90%, 95%, or 99% of the TGFβ-binding activity of the wild-type sequence (e.g. SEQ ID NO: 70 or 71).

[00196] With regard to human, the term "TGFβ Receptor III" or "TGFβRIII" refers to human TGFβ Receptor Type 3 sequence, including the wild type TGFβRII as well as all isoforms and variants. Exemplary amino acid sequence of wild type TGFβRIII is available under GenBank Accession No. NP_003234.2 or under UniProtKB - Q03167, also included herein as SEQ ID NO: 72.

[00197] As used herein, the term "variant" with respect to a certain reference protein or peptide means a modified version of the reference protein or peptide, e.g., functional equivalents, fragments, fusions, derivatives, mimetics, or any combination thereof, that has an amino acid sequence of at least 70% (e.g. 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity to the reference sequence, and retains at least 25% (e.g. 35%, 50%, 75%, 90%, 95%, or 99%) of the biological activity or binding activity of the reference sequence (e.g. the wild-type sequence). The variant can be a fragment, mutant, a fusion, a truncation, or any combination thereof, of the reference protein or peptide.

[00198] The term "Interleukin-1" or "IL-1" as used herein include IL-1α and IL-1β, their precursors (e.g. pro-IL-1α and pro-IL-1β), isoforms, and variants.

[00199] As used herein, the term "human IL-1α" refers to an IL-1α protein encoded by a human IL1A gene (e.g., a wild-type human IL1A gene), and the isoforms, and variants. An exemplary wild-type human IL1α protein is provided by UniProtKB - P01583.

[00200] As used herein, the term "human IL-1β" refers to an IL-1β protein encoded by a human IL1B gene (e.g., a wild-type human IL1B gene). An exemplary wild-type human IL1β protein is provided by GenBank Accession No. NP_000567.1, or under UniProtKB - C9JVK0.

[00201] The term "IL-1 receptor" or "IL-1R" as used herein refers to a receptor that can bind to IL-1, including all wild type receptors, isoforms, and variants thereof

capable of binding to IL-1. Generally, there are two types of IL-1 receptors, i.e., IL-1 Receptor I (IL-1RI), and IL-1 Receptor II (IL-1RII). IL-1RII acts as a decoy receptor that binds to ligand without transducing a signal. Proteolytical cleavage of IL-1RII results in formation of soluble receptors, e.g., IL-1sRI and IL-1sRII, which bind to ligand without transducing signal (see, details in Thomas G. Kennedy, Chapter V.B.2., in Encyclopedia of Hormones, 2003). IL-1sRI and IL-1sRII are proteolytic cleavage products of IL-1RII and can be a group of extracellular domain fragments of IL-1RII. The term IL-1R is also intended to encompass the coreceptor IL-1RAP, which can associate with IL-1RI bound to IL-1β to form the high affinity interleukin-1 receptor complex which mediates interleukin-1-dependent activation of NF-kappa-B and other pathways.

[00202] As used herein, the term "IL-1RI" includes the wild type IL-1RI as well as all isoforms and variants thereof capable of binding to IL-1α and/or IL-1β. Exemplary amino acid sequence of wild type IL-1RI is available under UniProtKB - P14778, also included herein as SEQ ID NO: 73.

[00203] As used herein, the term "IL-1RII" includes the wild type IL-1RII as well as all isoforms and variants thereof capable of binding to IL-1α and/or IL-1β. Exemplary amino acid sequence of wild type IL-1RII is available under UniProtKB - P27930, also included herein as SEQ ID NO: 75.

[00204] As used herein, the term "IL-1RAP" includes the wild type IL-1RAP as well as all isoforms and variants thereof capable of binding to IL-1R bound to IL-1β. Exemplary amino acid sequence of wild type IL-1RAP is available under UniProtKB - Q9NPH3, also included herein as SEQ ID NO: 74.

[00205] As used herein, the term "IL-1sRI" includes all soluble forms of IL-1RI that may be produced by proteolytic cleavage involving metalloproteinase. Naturally occurring IL-1sRI may have a molecular weight ranging from about 45 kDa to 60 Kda. This term also encompasses all isoforms and variants of IL-1sRI, capable of binding to IL-1α and/or IL-1β.

[00206] As used herein, the term "IL-1sRII" includes all soluble forms of IL-1RII that may be produced by proteolytic cleavage involving metalloproteinase. Naturally occurring IL-1sRII may have a molecular weight ranging from about 45 kDa to 60 Kda. This term also encompasses all isoforms and variants of IL-1sRII, capable of binding to IL-1α and/or IL-1β.

[00207] The term "IL-1 receptor antagonist" as used herein generally include any protein that can compete with IL-1α or IL-1β for binding to IL-1 receptor, and inhibits activity of IL-1α or IL-1β. IL-1 receptor antagonist can include naturally-occurring antagonists such as IL-1Ra, IL-1sRI and IL-1sRII, as well as other artificial antagonists that can block binding of IL-1α or IL-1β for binding to IL-1 receptor, in particular IL-1RI.

[00208] The term "IL-1Ra" as used herein include the wild type IL-1Ra as well as all isoforms and variants thereof capable of binding to IL-1α and/or IL-1β. Exemplary amino acid sequence of wild type IL-1Ra is available under UniProtKB - P18510, also included herein as SEQ ID NO: 76.

[00209] "Cancer" as used herein refers to any medical condition characterized by malignant cell growth or neoplasm, abnormal proliferation, infiltration or metastasis, and can be benign or malignant, and includes both solid tumors and non-solid cancers (e.g. hematologic malignancies) such as leukemia. As used herein "solid tumor" refers to a solid mass of neoplastic and/or malignant cells.

[00210] The term "pharmaceutically acceptable" indicates that the designated carrier, vehicle, diluent, excipient(s), and/or salt is generally chemically and/or physically compatible with the other ingredients comprising the formulation, and physiologically compatible with the recipient thereof.

[00211] Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X." Numeric ranges are inclusive of the numbers defining the range. Generally speaking, the term

"about" refers to the indicated value of the variable and to all values of the variable that are within the experimental error of the indicated value (e.g. within the 95% confidence interval for the mean) or within 10 percent of the indicated value, whichever is greater.

[00212] The term "fusion" or "fused" when used with respect to amino acid sequences (e.g. peptide, polypeptide or protein) refers to combination of two or more amino acid sequences, for example by chemical bonding or recombinant means, into a single amino acid sequence which does not exist naturally. A fusion amino acid sequence may be produced by genetic recombination of two encoding polynucleotide sequences, and can be expressed by a method of introducing a construct containing the recombinant polynucleotides into a host cell.

[00213] The term "pharmaceutically acceptable" indicates that the designated carrier, vehicle, diluent, excipient(s), and/or salt is generally chemically and/or physically compatible with the other ingredients comprising the formulation, and physiologically compatible with the recipient thereof.

I. Bi-functional Molecules Targeting an Immune Checkpoint Molecule and Blocking IL-1 Activity

[00214] The present disclosure provides a bi-functional molecule comprising a first moiety that binds to an immune checkpoint molecule, and a second moiety that blocks activity of Interleukin-1 (IL-1). The bi-functional molecule provided herein allows blockade and/or reduction in IL-1 activity in a tumor microenvironment by blocking the interaction between IL-1 and the IL-1 Receptor with either an IL-1-binding moiety or an IL-1 Receptor (IL-1R)-binding moiety (i.e., the second moiety of the bi-functional molecule). The IL-1-binding moiety and/or the IL-1R-binding moiety can be linked to a moiety targeting an immune checkpoint molecule which can be found on the surface of certain tumor cells or immune cells (i.e., the first moiety of the bi-functional molecule).

[00215] IL-1 is an inflammatory cytokine. Inflammation is an important component of the tumor microenvironment, and IL-1 plays a key role in carcinogenesis and tumor progression (A. Mantovani et al, Immunol Rev. 2018 Jan; 281(1): 57–61.). IL-1 acts at different levels in tumor initiation and progression, including driving chronic non-resolving inflammation, tumor angiogenesis, activation of the IL-17 pathway, induction of myeloid-derived suppressor cells (MDSC) and macrophage recruitment, invasion and metastasis (*Id.*).

[00216] Immune checkpoint molecules are expressed on certain immune cells such as T cells, Natural Killer cells, and so on. Some cancer cells may also express certain immune checkpoint molecules, which can block activation of the immune check point, thereby enabling the cancer cells to evade surveillance of the immune system.

[00217] By reducing IL-1 in the tumor microenvironment, and reducing check point blockade, the present disclosure provides a novel bi-functional molecule that could be useful for treating immune check point related diseases such as cancer, autoimmune diseases, infectious diseases, and so on.

[00218] In certain embodiments, the first moiety comprises an agonist of a check point molecule that has immunostimulatory or costimulatory activity. Such immunostimulatory check point molecules can include, without limitation, CD27, CD70, CD28, CD80 (B7-1), CD86 (B7-2), CD40, CD40L (CD154), CD122, CD137, CD137L, OX40 (CD134), OX40L (CD252), GITR, ICOS (CD278), and ICOSLG (CD275), CD2, , ICAM-1, LFA-1 (CD11a/CD18), CD30, BAFFR, HVEM, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, and CD83.

[00219] In certain embodiments, the first moiety comprises an inhibitor of a check point molecule that has immunoinhibitory or co-inhibitory activity. Such immune inhibitory check point molecules can include, without limitation, A2AR, B7-H3 (CD276), B7-H4 (VTCN1), BTLA (CD272), CTLA-4 (CD152), IDO1, IDO2,

TDO, KIR, LAG3, NOX2, PD-1, PD-L1, PD-L2, TIM-3, VISTA, SIGLEC7 (CD328), TIGIT, PVR(CD155), SIGLEC9 (CD329), CD160, LAIR1, 2B4 (CD244), CD47, and B7-H5.

[00220] In certain embodiments, the immune checkpoint molecule is PD-L1. In certain embodiments, the first moiety comprises an antibody moiety against PD-L1 or an antigen-binding fragment thereof. In certain embodiments, the first moiety comprises an antagonist antibody moiety against PD-L1 or an antigen-binding fragment thereof.

[00221] In certain embodiments, the second moiety comprises an IL-1-binding moiety or an IL-1 Receptor (IL-1R)-binding moiety.

[00222] Both IL-1α and IL-1β are proinflammatory and bind to IL-1R. Upon binding to IL-1α or IL-1β, IL-1R can recruit both the IL-1R accessory protein and the adaptor protein MyD88 to the receptor complex, resulting in activation of the downstream signaling cascade and ultimately in the activation of a myriad of immune and inflammatory genes. It is found by the present inventors that, blocking the activity of IL-1 or its binding to IL-1R would be useful in combination with modulation of immune check point molecules.

[00223] In certain embodiments, the IL-1 is IL-1α or IL-1β. In certain embodiments, the IL-1β is human IL-1β.

[00224] In certain embodiments, the second moiety comprises an IL-1-binding moiety. In certain embodiments, the IL-1-binding moiety specifically binds to IL-1α or IL-1β. In certain embodiments, the IL-1-binding moiety comprises a soluble IL-1R, an IL-1-binding fragment or variant of an IL-1R, or an antibody against IL-1 or an antigen-binding fragment thereof.

[00225] A soluble IL-1R can be a domain or fragment of the IL-1R, for example, the extracellular domain (ECD) of the IL-1R. Alternatively, a soluble IL-1R can

also be IL-1sRI or IL-1sRII, which are isoforms that are naturally soluble and capable of binding to IL-1.

[00226] A skilled person would understand that it could be sufficient to for a shortened fragment of IL-1RI, or ECD of IL-1RI, or IL-1RII, or ECD of IL-1RII, or IL-1RAP, or ECD of IL-1RAP, or IL-1sRI or IL-1sRII, to bind to IL-1 (e.g. IL-1α or IL-1β), as long as such a fragment contains the IL-1 binding domain. Therefore, the present disclosure also encompasses all the IL-1-binding fragments and variants of any of IL-1RI, ECD of IL-1RI, or IL-1RII, or ECD of IL-1RII, or IL-1RAP, or ECD of IL-1RAP IL-1sRI and IL-1sRII. In certain embodiments, the IL-1-binding moiety comprises an amino acid sequence of SEQ ID NOs: 73, 74, or 75, or an IL-1 binding fragment or variant thereof. In certain embodiments, the IL-1-binding moiety comprises an amino acid sequence having at least 80% sequence identity to any of SEQ ID NOs: 73, 74, and 75, or an IL-1 binding fragment or variant thereof.

[00227] In certain embodiments, the IL-1-binding moiety comprises an antibody against IL-1 or an antigen-binding fragment thereof. Antibodies against IL-1 or its antigen-binding fragment may also be used, as long as such antibodies or antigen-binding fragment can interfere with the binding of IL-1 (e.g., IL-1α or IL-1β) to IL-1R.

[00228] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises a heavy chain variable region and/or a light variable region from an anti-IL-1α antibody selected from the group consisting of: XB2001, lutikizumab, LY2189102 and bermekimab, or from an anti-IL-1β antibody selected from the group consisting of: SSGJ-613, CDP484, canakinumab and gevokizumab.

[00229] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 104 or SEQ ID NO: 112, a HCDR2 comprising a sequence of SEQ ID NO: 105 or SEQ ID NO: 113, and a HCDR3

comprising a sequence of SEQ ID NO: 106 or SEQ ID NO: 114, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 107 or SEQ ID NO: 115, a LCDR2 comprising a sequence of SEQ ID NO: 108 or SEQ ID NO: 116, and a LCDR3 comprising a sequence of SEQ ID NO: 109 or SEQ ID NO: 117.

[00230] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 104, a HCDR2 comprising a sequence of SEQ ID NO: 105, and a HCDR3 comprising a sequence of SEQ ID NO: 106, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 107, a LCDR2 comprising a sequence of SEQ ID NO: 108, and a LCDR3 comprising a sequence of SEQ ID NO: 109.

[00231] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 112, a HCDR2 comprising a sequence of SEQ ID NO: 113, and a HCDR3 comprising a sequence of SEQ ID NO: 114, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 115, a LCDR2 comprising a sequence of SEQ ID NO: 116, and a LCDR3 comprising a sequence of SEQ ID NO: 117.

[00232] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 102, SEQ ID NO: 110, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 103, SEQ ID NO: 111, and a homologous sequence thereof having at least 80% sequence identity thereof.

[00233] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 102, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 103, and a homologous sequence thereof having at least 80% sequence identity thereof.

[00234] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 110, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 111, and a homologous sequence thereof having at least 80% sequence identity thereof.In certain embodiments, the second moiety comprises an IL-1R-binding moiety.

[00235] In certain embodiments, the IL-1R-binding moiety comprises IL-1Ra or an IL-1R-binding fragment or variant thereof. IL-1Ra is an antagonist of IL-1R and can compete with IL-1α or IL-1β for binding to IL-1R. Similarly, a skilled person would understand that it could be sufficient for a shortened fragment of IL-1Ra to be useful in binding to IL-1R and/or compete with IL-1α or IL-1β. In certain embodiments, the IL-1R-binding moiety comprises a truncated form of IL-1Ra. In certain embodiments, the IL-1R-binding moiety comprises an amino acid sequence of SEQ ID NO: 67 or 76, or any IL-1 binding fragment or variant thereof. In certain embodiments, the IL-1R-binding moiety comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 67 or 76, or any IL-1 binding fragment or variant thereof. A skilled person would understand that, a variant of a wild-type IL-1Ra could also be useful in the present disclosure, as long as such a variant is capable of compete with IL-1α or IL-1β for binding with IL-1R.

[00236] In certain embodiments, the IL-1R-binding moiety comprises an antibody against IL-1R or an antigen-binding fragment thereof. Antibodies against

IL-1R or its antigen-binding fragment may also be used, as long as such antibodies or antigen-binding fragment can compete with IL-1α or IL-1β for binding with IL-1R.

[00237] In certain embodiments, the antibody against IL-1R or an antigen-binding fragment thereof comprises a heavy chain variable region and/or a light variable region from an antibody selected from the group consisting of: spesolimab, astegolimab, imsidolimab, AMG 108, melrilimab, nidanilimab, MEDI8968, REGN6490, HB0034 and CSC012.

II. Bi-functional Molecules Targeting PD-L1 and a second moiety

[00238] Therapeutic efficacy of PD-1/PD-L1 axis checkpoint inhibitors (e.g. PD-L1 antibodies) could be limited when a tumor microenvironment ("TME") is enriched with immunosuppressive cytokines. Signaling of such immunosuppressive cytokines in the localized microenvironment can reduce tumor-infiltrating T cells, and skew them toward Tregs and attenuate the activation of immune effector cells.

[00239] In one aspect, the present disclosure provides a novel bi-functional molecule, comprising a first moiety that binds to PD-L1, and a second moiety that a) blocks activity of an immunosuppressive cytokine or b) stimulates anti-tumor immunity. The molecule may be a compound, a peptide, a polypeptide, a protein, or any combination thereof. The second moiety can restore the immune response in the tumor microenvironment, by either blocking an immunosuppressive activity or cytokine, or increasing or stimulating immunity.

[00240] In certain embodiments, the bi-functional molecule provided herein comprises first moiety that binds to PD-L1 (i.e., a PD-L1-binding moiety), and a second moiety that blocks activity of an immunosuppressive cytokine.

[00241] In certain embodiments, an immunosuppressive cytokine comprises a cytokine in transforming growth factor beta (TGF-β) superfamily, IL-1, or Vascular endothelial growth factor (VEGF). In certain embodiments, the immunosuppressive

cytokine in TGF-β superfamily includes bone morphogenetic proteins (BMPs), activins, NODAL, and growth and differentiation factors (GDFs).

[00242] In certain embodiments, the immunosuppressive cytokine is TGF-β. In certain embodiments, the immunosuppressive cytokine is IL-1.

[00243] In certain embodiments, the second moiety comprises a TGFβ-binding moiety. In certain embodiments, the second moiety comprises an IL-1-binding moiety. As used herein, the term "binding moiety", "binding fragment" refers to a moiety or fragment that has an ability to specifically bind to a target molecule or complex. The term "TGFβ-binding moiety" refers to a moiety that has an ability to specifically bind to one or more family members or isoforms of the TGFβ family (e.g. TGFβ1, TGFβ2, or TGFβ3). Similarly, the term "IL-1-binding moiety" refers to a moiety that has an ability to specifically bind to one or more family members of the IL-1 family (e.g., IL-1α, IL-1β).

[00244] In certain embodiments, the bi-functional molecule provided herein comprises first moiety that binds to PD-L1 (i.e., a PD-L1-binding moiety), and a second moiety that stimulates anti-tumor immunity. In certain embodiments, the second moiety comprises an immunostimulatory polypeptide or a functional equivalent thereof or a variant thereof. In certain embodiments, the immunostimulatory polypeptide is Interleukin(IL)-2 (IL-2), IL-15, IL-21, IL-10, IL-12, IL-23, IL-27, IL-35, granulocyte-macrophage colony-stimulating factor (GM-CSF), soluble CD4, soluble LAG-3, or IFN-α, or a functional equivalent thereof.

[00245] In certain embodiments, the second moiety comprises an antagonist of an immunoinhibitory receptor signaling. In certain embodiments, the immunoinhibitory receptor is SIRPα.

[00246] In certain embodiments, the bi-functional molecule comprises one or more of the second moieties. In certain embodiments, the one or more of the second moieties may be of the same type, for example, each of them may block activity of an

immunosuppressive cytokine, or each of them may stimulate anti-tumor immunity. In certain embodiments, the one or more of the second moieties may be of different types. In certain embodiments, each of the second moieties may have the same sequence, or may have different in amino acid sequences.

i. TGFβ-Binding Moiety

[00247] In certain embodiments, the TGFβ-binding moiety comprises a soluble TGFβ Receptor (TGFβR) or a TGFβ-binding fragment or variant thereof, or an antibody against TGFβ and an antigen-binding fragment thereof.

[00248] The "TGFβ-binding moiety" may also be referred to as "TGFβ Trap" in the present disclosure. Accordingly, a protein targeting both PD-L1 and TGFβ may also be referred to as "anti-PD-L1/TGFβ Trap" in the present disclosure.

[00249] In certain embodiments, the TGFβ-binding moiety binds to human and/or mouse TGFβ. In certain embodiments, the TGFβ-binding moiety is capable of antagonizing and/or inhibiting TGFβ signaling pathway. In certain embodiments, the TGFβ-binding moiety is capable of antagonizing and/or inhibiting TGFβ.

[00250] In the present disclosure, the TGFβ-binding moiety can comprise any moiety that specifically binds to one or more family members or isoforms of TGFβ family. In certain embodiments, the TGFβ-binding moiety comprises a moiety that binds to TGFβ1 (e.g. human TGFβ1), TGFβ2 (e.g. human TGFβ2), and/or TGFβ3 (e.g. human TGFβ3), or a variant thereof that has similar or improved TGFβ binding affinity. In certain embodiments, the TGFβ-binding moiety comprises a moiety that binds to TGFβ1 (e.g. human TGFβ1). In certain embodiments, the TGFβ-binding moiety comprises a moiety that binds to TGFβ2 (e.g. human TGFβ2). In certain embodiments, the TGFβ-binding moiety comprises a moiety that binds to TGFβ3 (e.g. human TGFβ3). In certain embodiments, the TGFβ-binding moiety comprises a moiety that specifically binds to both TGFβ1 (e.g. human TGFβ1) and TGFβ2 (e.g. human TGFβ2). In certain embodiments, the TGFβ-binding moiety comprises a moiety that specifically binds to both TGFβ1 (e.g. human TGFβ1) and TGFβ3 (e.g.

human TGFβ3). In certain embodiments, the TGFβ-binding moiety comprises a moiety that specifically binds to both TGFβ2 (e.g. human TGFβ2) and TGFβ3 (e.g. human TGFβ3). In certain embodiments, the TGFβ-binding moiety comprises a moiety that specifically binds to each of TGFβ1 (e.g. human TGFβ1), TGFβ2 (e.g. human TGFβ2), and TGFβ3 (e.g. human TGFβ3). A person skilled in the art would appreciate that a TGFβ-binding moiety that binds to one family member or isoform of TGFβ family may be capable of binding to one or more other family members or isoforms of TGFβ family with similar or higher affinity.

[00251] In certain embodiments, the TGFβ-binding moiety comprises a moiety that selectively binds to TGFβ1 over TGFβ2, and/or over TGFβ3.

[00252] In certain embodiments, the TGFβ-binding moiety comprises a moiety that specifically binds to human TGFβ1 and mouse TGFβ1 with similar affinity.

[00253] In certain embodiments, the TGFβ-binding moiety of the present disclosure comprises a soluble TGFβ Receptor (TGFβR) or a TGFβ-binding fragment or a variant thereof.

[00254] Exemplary TGFβ Receptors include TGFβRI, TGFβRII and TGFβRIII. In certain embodiments, the TGFβ Receptor is selected from the group consisting of TGFβ Receptor I (TGFβRI), TGFβ Receptor II (TGFβRII), TGFβ Receptor III (TGFβRIII), and any combination thereof. In certain embodiments, the TGFβ receptor is TGFβRI (e.g. human TGFβRI). In certain embodiments, the TGFβ receptor is TGFβRII (e.g. human TGFβRII). In certain embodiments, the TGFβ receptor is TGFβRIII (e.g. human TGFβRIII).

[00255] In certain embodiments, the TGFβ-binding moiety comprises an extracellular domain (ECD) of a TGFβ receptor (e.g. a human TGFβ receptor), or a TGFβ-binding fragment or a variant thereof. In certain embodiments, the ECD of a TGFβ receptor comprises an ECD of TGFβRI (e.g. human TGFβRI), an ECD of TGFβRII (e.g. human TGFβRII), an ECD of TGFβRIII (e.g. human TGFβRIII), or

any combination thereof. In certain embodiments, the ECD of the TGFβRII comprises an amino acid sequence of SEQ ID NO: 66, 79, or an amino acid sequence having at least 80% (e.g. at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity thereof yet retaining binding specificity to TGFβ. In certain embodiments, the ECD of the TGFβRI comprises an amino acid sequence of SEQ ID NO: 77, or an amino acid sequence having at least 80% (e.g. at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity thereof yet retaining binding specificity to TGFβ. In certain embodiments, the ECD of the TGFβRIII comprises an amino acid sequence of SEQ ID NO: 78, or an amino acid sequence having at least 80% (e.g. at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity thereof yet retaining binding specificity to TGFβ.

[00256] In certain embodiments, the TGFβ-binding moiety comprises an antibody against TGFβ and an antigen-binding fragment thereof. Exemplary anti-TGFβ antibodies include fresolimumab and metelimumab, as well as the anti-TGFβ antibodies or antigen-binding fragments thereof described in, for example, US7494651B2, US8383780B2, US8012482B2, WO2017141208A1, each of which is incorporated herein by reference in its entirety.

[00257] In certain embodiments, the TGFβ-binding moiety comprises a combination of one or more ECDs of one or more TGFβ receptors and/or one or more anti-TGFβ antibodies or antigen-binding fragments thereof.

[00258] The one or more ECDs may be the same or different. For example, the TGFβ-binding moiety may comprise identical repeats of an ECD of a TGFβ receptor, or alternatively may comprise a combination of different ECD sequences of the same TGFβ receptor, or alternatively may comprise a combination of different ECDs from different TGFβ receptors. Similarly, the one or more anti-TGFβ antibodies may be the same of different.

[00259] In certain embodiments, the TGFβ-binding moiety comprises a combination (or fusion) of ECDs selected from the group consisting of: ECD of TGFβRI (e.g. human TGFβRI), ECD of TGFβRII (e.g. human TGFβRII), ECD of TGFβRIII (e.g. human TGFβRIII), or any combination thereof.

[00260] In certain embodiments, the TGFβ-binding moiety comprises a combination (or fusion) of one or more anti-TGFβ antibodies or antigen-binding fragments thereof.

[00261] In certain embodiments, the TGFβ-binding moiety comprises a combination (or fusion) of ECDs selected from the group consisting of: ECD of TGFβRI (e.g. human TGFβRI), ECD of TGFβRII (e.g. human TGFβRII), ECD of TGFβRIII (e.g. human TGFβRIII), one or more anti-TGFβ antibodies or antigen-binding fragments thereof, or any combination thereof.

ii. <u>IL-1-Binding Moiety</u>

[00262] In certain embodiments, the second moiety comprises an IL-1-binding moiety. In certain embodiments, the IL-1 is IL-1α or IL-1β. In certain embodiments, the IL-1β is human IL-1β.

[00263] In certain embodiments, the IL-1-binding moiety specifically binds to IL-1α or IL-1β. In certain embodiments, the IL-1-binding moiety comprises a moiety that selectively binds to IL-1β over IL-1α, or selectively binds to IL-1α over IL-1β.

[00264] In certain embodiments, the IL-1-binding moiety comprises a soluble IL-1R, an IL-1-binding fragment or variant of an IL-1R, or an antibody against IL-1 or an antigen-binding fragment thereof.

[00265] A soluble IL-1R can comprise a domain or fragment or variant of the IL-1R, for example, the extracellular domain (ECD) of the IL-1R. Alternatively, a soluble IL-1R can also comprise IL-1sRI or IL-1sRII, which are isoforms that are naturally soluble and capable of binding to IL-1.

[00266] A skilled person would understand that it could be sufficient to for a shortened fragment of IL-1R, or ECD of IL-1R, or IL-1sRI or IL-1sRII, to bind to IL-1 (e.g. IL-1α or IL-1β), as long as such a fragment contains the IL-1 binding domain. Therefore, the IL-1-binding moiety provided herein can also comprise an IL-1-binding fragment of any of IL-1R, ECD of IL-1R, IL-1sRI and IL-1sRII. In certain embodiments, the IL-1-binding moiety comprises an amino acid sequence of SEQ ID NOs: 73, 74, or 75, or an IL-1 binding fragment or variant thereof. In certain embodiments, the IL-1-binding moiety comprises an amino acid sequence having at least 80% sequence identity to any of SEQ ID NOs: 73, 74, and 75, or an IL-1 binding fragment or variant thereof.

[00267] In certain embodiments, the IL-1-binding moiety comprises an antibody against IL-1 or an antigen-binding fragment thereof. Antibodies against IL-1 or its antigen-binding fragment may also be used, as long as such antibodies or antigen-binding fragment can interfere with the binding of IL-1 (e.g., IL-1α or IL-1β) to IL-1R.

[00268] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises a heavy chain variable region and/or a light variable region from an anti-IL-1α antibody selected from the group consisting of: XB2001, lutikizumab, LY2189102 and bermekimab, or from an anti-IL-1β antibody selected from the group consisting of: SSGJ-613, CDP484, canakinumab and gevokizumab.

[00269] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 104 or SEQ ID NO: 112, a HCDR2 comprising a sequence of SEQ ID NO: 105 or SEQ ID NO: 113, and a HCDR3 comprising a sequence of SEQ ID NO: 106 or SEQ ID NO: 114, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 107 or SEQ ID NO: 115, a LCDR2 comprising a sequence of SEQ ID NO: 108 or SEQ ID

NO: 116, and a LCDR3 comprising a sequence of SEQ ID NO: 109 or SEQ ID NO: 117.

[00270] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 104, a HCDR2 comprising a sequence of SEQ ID NO: 105, and a HCDR3 comprising a sequence of SEQ ID NO: 106, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 107, a LCDR2 comprising a sequence of SEQ ID NO: 108, and a LCDR3 comprising a sequence of SEQ ID NO: 109.

[00271] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 112, a HCDR2 comprising a sequence of SEQ ID NO: 113, and a HCDR3 comprising a sequence of SEQ ID NO: 114, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 115, a LCDR2 comprising a sequence of SEQ ID NO: 116, and a LCDR3 comprising a sequence of SEQ ID NO: 117.

[00272] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 102, SEQ ID NO: 110, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 103, SEQ ID NO: 111, and a homologous sequence thereof having at least 80% sequence identity thereof.

[00273] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 102, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable

region comprising a sequence selected from the group consisting of SEQ ID NO: 103, and a homologous sequence thereof having at least 80% sequence identity thereof.

[00274] In certain embodiments, the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 110, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 111, and a homologous sequence thereof having at least 80% sequence identity thereof.

[00275] In certain embodiments, the IL-1-binding moiety comprises a combination of one or more moieties selected from the group consisting of IL-1R, ECD of IL-1R, IL-1sRI, IL-1sRII, antibody against IL-1, any IL-1-binding fragments thereof, and any combination thereof. Such one or more moieties can be linked by direct bond or can be linked by a suitable linker.

[00276] In certain embodiments, the IL-1R-binding moiety comprises IL-1Ra or an IL-1R-binding fragment or variant thereof. IL-1Ra is an antagonist of IL-1R and can compete with IL-1α or IL-1β for binding to IL-1R. Similarly, a skilled person would understand that it could be sufficient for a shortened fragment of IL-1Ra to be useful in binding to IL-1R and/or compete with IL-1α or IL-1β. In certain embodiments, the IL-1R-binding moiety comprises a truncated form of IL-1Ra. In certain embodiments, the IL-1R-binding moiety comprises an amino acid sequence of SEQ ID NO: 67, or any IL-1 binding fragment or variant thereof. In certain embodiments, the IL-1R-binding moiety comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 67, or any IL-1 binding fragment or variant thereof. A skilled person would understand that, a variant of a wild-type IL-1Ra could also be useful in the present disclosure, as long as such a variant is capable of compete with IL-1α or IL-1β for binding with IL-1R.

[00277] In certain embodiments, the IL-1R-binding moiety comprises an antibody against IL-1R or an antigen-binding fragment thereof. Antibodies against IL-1R or its antigen-binding fragment may also be used, as long as such antibodies or antigen-binding fragment can compete with IL-1α or IL-1β for binding with IL-1R.

[00278] In certain embodiments, the IL-1R-binding moiety comprises a combination of one or more moieties selected from the group consisting of IL-1Ra, an antibody against IL-1R, any IL-1R-binding fragment or variant thereof and any combination thereof. Such one or more moieties can be linked by direct bond or can be linked by a suitable linker.

[00279] In certain embodiments, the antibody against IL-1R or an antigen-binding fragment thereof comprises a heavy chain variable region and/or a light variable region from an antibody selected from the group consisting of: spesolimab, astegolimab, imsidolimab, AMG 108, melrilimab, nidanilimab, MEDI8968, REGN6490, HB0034 and CSC012.

iii. Immunostimulatory polypeptide

[00280] In certain embodiments, the second moiety comprises an immunostimulatory polypeptide or a functional equivalent thereof or a variant thereof. In certain embodiments, the immunostimulatory polypeptide is soluble CD4, soluble LAG-3, or a functional equivalent thereof.

[00281] In certain embodiments, the soluble LAG-3 comprises an extracellular domain (ECD) of the LAG-3 or a MHC class II (MHCII)-binding fragment or variant thereof.

[00282] LAG-3 (Uniprot number: Q61790) belongs to immunoglobulin (Ig) superfamily, which is a type I transmembrane protein comprising 503 amino acid. Lag-3 comprises an intracellular domain (ICD), a transmembrane domain (TMD), and an extracellular domain (ECD). The ECD comprises four Ig-like domains, i.e., D1 to

D4, wherein D1 comprises 9 β-chains: A, B, C, C', C'', D, E, F and G chains. Between the C and C' chains, there is an additional sequence having about 30 amino acids that forms an "extra loop". Such "extra loop" has been reported to be involved in the interaction between LAG-3 and MHCII. In certain embodiments, the soluble LAG-3 comprises the amino acid sequence of the extra loop, the D1 domain, D1 plus D2 domains, or any MHC II-binding fragment or variant thereof. In certain embodiments, the soluble LAG-3 comprises the amino acid sequence of SEQ ID NO: 99, SEQ ID NO:100, SEQ ID NO: 101, or any MHC II-binding fragment or variant thereof.

[00283] LAG-3 is expressed on activated T cells, natural killer cells, B cells and plasmacytoid dendritic cells. Its principal ligand is MHC class II, to which it binds with higher affinity than CD4. A connecting peptide (CP) exists between D4 and the TMD of LAG-3, where cleavage occurs in presence of metalloproteinase ADAM10 and/or ADAM17 to produce cleaved soluble LAG-3. See, e.g., Huard et al., Proc Natl Acad Sci U S A 1997;94:5744 – 9.; Workman et al., J Immunol 2002;169:5392 – 5.doi:10.4049/jimmunol.169.10.5392; and Lawrence et al., J Immunother Cancer. 2015; 3(Suppl 2): P216, which have herein incorporated by reference.

[00284] LAG-3 also encodes an alternative splice variant that is translated to a soluble form of LAG-3. Soluble LAG-3 activates antigen-presenting cells (APCs) through MHCII signaling, leading to increased antigen-specific T-cell responses in vivo. For example, soluble LAG-3 activates dendritic cells (DC) and has been reported to be involved in the proinflammatory activity of cytokine-activated (such as TNF-α and/or IL-12-activated) bystander T cells and it may directly activate DC. See, e.g., Triebel, Trends Immunol., 2003, 24: 619-622, which is herein incorporated by reference.

[00285] In certain embodiments, the soluble LAG-3 comprises Eftilagimod alpha (IMP321) or a MHC II-binding fragment or variant thereof. IMP321 is a soluble dimeric recombinant form of LAG-3. IMP321 induces sustained immune

responses by stimulating dendritic cells through MHCII molecules. Combinatory therapy of MP321 and an anti-PD-1 antibody or an anti-PD-L1 antibody has been shown to synergistically activate T cells (in particular, CD8+ T cells). See, e.g., Luc et al., Future Oncol Actions Search in PubMed Search in NLM Catalog Add to Search. 2019 Jun;15(17):1963-1973. doi: 10.2217/fon-2018-0807. Epub 2019 Apr 12.; Julio et al., Journal of Clinical Oncology, Volume 37, Issue 15; and US10874713 B, which is herein incorporated by reference.

iv. Antagonist of an Immunoinhibitory Receptor Signaling

[00286] In certain embodiments, the second moiety comprises an antagonist of an immunoinhibitory receptor signaling. In certain embodiments, the immunoinhibitory receptor is SIRPα.

[00287] As used herein, the term "SIRPα", interchangeably with the term "Signal-regulatory protein alpha" refers to an inhibitory receptor expressed primarily on myeloid cells and dendritic cells. SIRPα belongs to the SIRPs family that also includes several other transmembrane glycoproteins, including, SIRPβ and SIRPγ. Each member of the SIRPs family contains 3 similar extracellular Ig-like domains with distinct transmembrane and cytoplasmic domains.

[00288] SIRPα can bind to CD47, which delivers a "don't eat me" signal to suppress phagocytosis, and blocking the CD47 mediated engagement of SIRPα on a phagocyte can cause removal of live cells bearing "eat me" signals. CD47 is a broadly expressed transmembrane glycoprotein with an extracellular N-terminal IgV domain, five transmembrane domains, and a short C-terminal intracellular tail. CD47 functions as a cellular ligand for SIRPα. Tumor cells frequently overexpress CD47 to evade macrophage-mediated destruction. The interaction of CD47 and SIRPα has been shown to be involved in the regulation of macrophage-mediated phagocytosis (Takenaka *et al.*, *Nature Immunol.*, 8(12): 1313-1323, 2007).

[00289] In certain embodiments, the second moiety blocks interaction between CD47 and SIRPα. In a diverse range of preclinical models, therapies that block the

interaction of CD47 and SIRPα stimulate phagocytosis of cancer cells *in vitro* and anti-tumor immune responses *in vivo*.

[00290] The second moiety can comprise a CD47 binding domain or a SIRPα binding domain. In certain embodiments, the immunoinhibitory receptor is signal-regulatory protein alpha (SIRPα). In certain embodiments, the second moiety blocks interaction between CD47 and SIRPα. In certain embodiments, the second moiety comprises a CD47 binding domain or a SIRPα binding domain. In certain embodiments, the CD47 binding domain comprises a soluble SIRPα or a CD47 binding fragment thereof, or an anti-CD47 antibody or an antigen-binding fragment thereof.

[00291] In certain embodiments, the soluble SIRPα comprises an extracellular domain (ECD) of the SIRPα, or a CD47-binding fragment or a variant thereof. In certain embodiments, the soluble SIRP comprises an amino acid sequence of SEQ ID NO: 84 or an amino acid sequence having at least 80% (e.g. at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity thereof yet retaining binding specificity to CD47. Optionally, the soluble SIRP is an engineered high-affinity SIRP variant, which potently antagonized CD47 on cancer cells but does not induce macrophage phagocytosis on its own. In certain embodiments, the SIRP variant comprises one or more mutations selected from the group consisting of: L4V, L4I, V6I, V6L, A21V, V27I, V27L, I31T, I31S, I31F, E47V, E47L, K53R, E54Q, H56P, H56R, V63I, S66T, S66G, K68R, V92I, F94L, F94V and F103V, relative to SEQ ID NO: 98. In certain embodiments, the SIRPα variant comprises a combination of mutations selected from the group consisting of: 1) V27I, K53R, S66T, K68R, F103V; 2) L4V, V27L, E47V, K53R, E54Q, S66G, K68R, V92I; 3) L4V, V6I, A21V, V27I, I31T, E47L, K53R, H56P, S66T, K68R, F94L; 4) V6I, V27I, I31S, E47V, K53R, E54Q, H56P, S66G, V92I, F94L; 5) L4I, A21V, V27I, I31F, E47V, K53R, E54Q, H56R, S66G, F94V, F103V; 6) L4V, V6I, V27I, I31F, E47V, K53R, H56R, S66G, K68R, V92I, F94L; 7) L4V, V6L, I31F, E47V, K53R, H56P, S66G, V92I, F103V; 8) V6I, V27I, I31F, E47L, K53R,

E54Q, H56P, S66T; 9) L4V, V6I, V27I, I31F, E47V, K53R, E54Q, H56P, V63, S66T, K68R, V92I; 10) V6I, V27I, I31T, E47V, K53R, E54Q, H56P, S66G, K68R, V92I, F103V; and 11) V6I, V27I, I31F, E47V, K53R, E54Q, H56P, S66T,V92I. See, e.g., Kipp Weiskopf et al. Science 341, 88 (2013), which is herein incorporated by reference.

[00292] In certain embodiments, the SIRPα binding domain comprises a soluble CD47 or a SIRPα binding fragment thereof, or an anti-SIRPα antibody or an antigen-binding fragment thereof. In certain embodiments, the soluble CD47 comprises an extracellular domain (ECD) of the CD47 or a SIRPα binding fragment thereof, an anti-SIRPα antibody or an antigen-binding fragment thereof.

[00293] In certain embodiments, the CD47-binding domain comprises an anti-CD47 antibody and an antigen-binding fragment thereof. Exemplary anti-CD47 antibodies include, without limitation, humanized 5F9 antibody, B6H12 antibody and ZF1 antibody. See, Lu et al., OncoTargets and Therapy, Volume 13, DOI https://doi.org/10.2147/OTT.S249822, which is herein incorporated by reference.. In certain embodiments, the SIRPα binding domain comprises an anti-SIRPα antibody or an antigen-binding fragment thereof. Exemplary anti-SIRPα antibodies include, without limitation, BI765064 and AL008. See, e.g., WO2019073080A1, WO2019175218A1 and WO2018107058A1, which are herein incorporated by reference.

[00294] In certain embodiments, the CD47-binding domain comprises a combination of one or more ECDs of one or more SIRPα, SIRPβ or SIRPγ, and/or one or more anti-CD47 antibodies or antigen-binding fragments thereof.

[00295] The one or more ECDs may be the same or different. For example, the CD47-binding domain may comprise identical repeats of an ECD of a SIRPα, SIRPβ or SIRPγ, or alternatively may comprise a combination of different ECD sequences of the same SIRPα, SIRPβ or SIRPγ, or alternatively may comprise a

combination of different ECDs from different SIRPα, SIRPβ or SIRPγ. Similarly, the one or more anti-CD47 antibodies may be the same of different.

[00296] In certain embodiments, the CD47-binding domain comprises a combination (or fusion) of ECDs selected from the group consisting of: ECD of SIRPα, ECD of SIRPβ, ECD of SIRPγ, or any combination thereof.

[00297] In certain embodiments, the CD47-binding domain comprises a combination (or fusion) of one or more anti-CD47 antibodies or antigen-binding fragments thereof.

[00298] In certain embodiments, the CD47-binding domain comprises a combination (or fusion) of ECDs selected from the group consisting of: ECD of SIRPα, ECD of SIRPβ, ECD of SIRPγ, one or more anti-CD47 antibodies or antigen-binding fragments thereof, or any combination thereof.

v. **PD-L1-Binding Moiety**

[00299] In certain embodiments, the bi-functional molecule provided herein comprises a first moiety which is a PD-L1-binding moiety.

[00300] In certain embodiments, the PD-L1-binding moiety of the present disclosure binds to PD-L1 (e.g. human PD-L1, or cynomolgus PD-L1). In certain embodiments, the PD-L1-binding moiety of the present disclosure binds to human PD-L1. In certain embodiments, the PD-L1-binding moiety of the present disclosure binds to cynomolgus PD-L1.

[00301] In certain embodiments, the PD-L1-binding moiety of the present disclosure comprises an anti-PD-L1 antibody moiety. In certain embodiments, exemplary anti-PD-L1 antibodies are disclosed in Section Anti-PD-L1 Antibodies and Section Illustrative Anti-PD-L1 Antibodies of the present disclosure.

[00302] In certain embodiments, the anti-PD-L1 antibody moiety comprises one or more CDRs. In certain embodiments, the anti-PD-L1 antibody moiety comprises

one or more CDRs described in Section Illustrative Anti-PD-L1 Antibodies of the present disclosure. In certain embodiments, the anti-PD-L1 antibody moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL). In certain embodiments, the anti-PD-L1 antibody moiety comprises a VH and a VL of an anti-PD-L1 antibody as disclosed in Section Illustrative Anti-PD-L1 Antibodies of the present disclosure.

[00303] In certain embodiments, the anti-PD-L1 antibody moiety further comprises a heavy chain constant domain appended to a carboxyl terminus of the heavy chain variable region. In certain embodiments, the heavy chain constant region is derived from the group consisting of IgA, IgG, and IgM. In certain embodiments, the heavy chain constant region is derived from human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2 or IgM. In certain embodiments, the anti-PD-L1 antibody moiety further comprises a light chain constant domain appended to a carboxyl terminus of the light chain variable region. In certain embodiments, the light chain constant region is derived from Kappa light chain or Lamda light chain. In certain embodiments, the heavy chain constant region comprises an amino acid sequence of SEQ ID NO: 80 or 81. In certain embodiments, the light chain constant region comprises an amino acid sequence of SEQ ID NO: 82.

vi. Linkage Between the First moiety and the Second Moiety

[00304] In the present disclosure, the second moiety can be linked to any portion of the first moiety. For example, the second moiety such as the TGFβ-binding moiety or the IL-1 -binding moiety can be linked to any suitable portion of the first moiety such as the PD-L1-binding moiety (e.g. the anti-PD-L1 antibody moiety).

[00305] In certain embodiments, the PD-L1-binding moiety comprises one or more polypeptide chains, such as antibody heavy chain and light chain.

[00306] In certain embodiments, the bi-functional molecule comprises one or more of the second moieties. In certain embodiments, at least one of the second

moieties is linked to an amino terminus (N terminus) or a carboxyl (C terminus) of a polypeptide chain of the first moiety. In certain embodiments, the at least one of the second moieties is linked to an N terminus or a C terminus of a heavy chain of the first moiety, or linked to an N terminus or a C terminus of a light chain of the first moiety.

[00307] In certain embodiments, the at least one of the second moieties is linked to a C terminus of a heavy chain constant region of the first moiety. In certain embodiments, each of the second moieties is linked respectively to the C terminus of each heavy chain constant region of the first moiety.

[00308] In certain embodiments, the bi-functional molecule comprises at least two of the second moieties, each of which is linked respectively to the C terminus of each heavy chain of the first moiety, or each of which is linked respectively to the C terminus of each light chain of the first moiety. In certain embodiments, the bi-functional molecule comprises at least two of the second moieties, each of which is linked respectively to the N terminus of each heavy chain of the first moiety, or each of which is linked respectively to the N terminus of each light chain of the first moiety.

[00309] In certain embodiments, the bi-functional molecule comprises more than one of the second moieties that are linked respectively to: an N terminus of a heavy chain of the first moiety, a C terminus of a heavy chain of the first moiety, an N terminus of a light chain of the first moiety, a C terminus of a light chain of the first moiety, or any combination thereof. For example, the bi-functional molecule can comprise at least two of the second moieties, one of which is linked to C terminus of a heavy chain of the first moiety and the other is linked to C terminus of a light chain of the first moiety. For example, the bi-functional molecule can comprise at least two of the second moieties, one of which is linked to N terminus of a heavy chain of the first moiety and the other is linked to N terminus of a light chain of the first moiety.

[00310] In certain embodiments, the one or more TGFβ-binding moiety, the one or more IL-1 -binding moiety, the one or more immunostimulatory polypeptide (e.g., soluble LAG3 or soluble CD4) or the one or more CD47-binding moiety is linked to the anti-PD-L1 antibody moiety at one or more positions selected from the group consisting of: 1) N terminus of the heavy chain variable region, 2) N terminus of the light chain variable region, 3) C terminus of the heavy chain variable region; 4) C terminus of the light chain variable region; 5) C terminus of the heavy chain constant region; 6) C terminus of the light chain constant region, and 7) any combination thereof, of the anti-PD-L1 antibody moiety.

[00311] In certain embodiments, the bi-functional molecule comprises homodimeric heavy chains. In certain embodiments, the bi-functional molecule comprises heterodimeric heavy chains. The heavy chains are heterodimeric with respect to presence or position of the second moiety. In certain embodiments, the heterodimeric heavy chains comprise one heavy chain having the second moiety but the other heavy chain having not.

vii. Linker

[00312] The second moiety can be linked to the first moiety directly or via a linker. The direct linkage can be a chemical linkage (such as a covalent bond).

[00313] In certain embodiments, the bi-functional molecule further comprises a linker connecting the first moiety and the second moiety. The term "linker" as used herein can be any suitable bifunctional moiety capable of reacting with at least two entities to be linked, thereby bonding the entities to form one molecule or maintaining association of the entities in sufficiently close proximity. The linker can be integrated in the resulting linked molecule or structure, with or without its reacted functional groups.

[00314] In certain embodiments, the linker is selected from the group consisting of a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, and a non-helical linker.

[00315] In certain embodiments, the linker comprises a peptide linker. The peptide linker can be made up of amino acid residues linked together by peptide bonds. In certain embodiments, the peptide linker can further comprise one or more non-natural amino acids. In certain embodiments, the peptide linker comprises an amino acid sequence having at least 1, 2, 3, 4, 5, 8, 10, 15, 20, 30, 50 or more amino acid residues, joined by peptide bonds and capable of linking two or more polypeptides. A peptide linker may or may not have a secondary structure.

[00316] Any suitable peptide linkers can be used. Many peptide linker sequences are known in the art, see, for example, Holliger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993); Poljak *et al.*, *Structure* 2:1121-1123 (1994). In certain embodiments, the peptide linker may comprise or consist of amino acid residues selected from the amino acids glycine, serine, alanine, methionine, asparagine, and glutamine. In some embodiments, the peptide linker can be made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. In some embodiments, linkers are polyglycines, polyalanines, combinations of glycine and alanine (such as poly(Gly-Ala)), or combinations of glycine and serine (such as poly(Gly-Ser)).

[00317] In certain embodiments, the linker comprises an amino acid sequence of ((G)nS)m, wherein m and n are independently an integer selected from 0 to 30, 1 to 29, 2 to 28, 3 to 27, 4 to 26, 5 to 25, 6 to 24, 7 to 23, 8 to 22, 9 to 21, 10 to 20, 11 to 19, 12 to 18, 13 to 17, 14 to 16 or 5. In certain embodiments, m is 4 and n is 4.

[00318] In certain embodiments, the linker comprises an amino acid sequence of SEQ ID NO: 68. In certain embodiments, the linker comprises an amino acid sequence having at least 80%, at least 85%, 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% sequence identity to SEQ ID NO: 68.

viii. Anti-PD-L1 Antibodies and Antigen-Binding Fragments Thereof

[00319] In certain embodiments, the PD-L1-binding moiety of the bi-functional molecules provided herein comprises a moiety comprising an anti-PD-L1 antibody or antigen-binding fragments thereof. In certain embodiments, the anti-PD-L1 antibody and antigen-binding fragments thereof are capable of specifically binding to PD-L1.

[00320] In certain embodiments, the anti-PD-L1 antibodies and the antigen-binding fragments thereof provided herein specifically bind to human PD-L1 at an K_D value of no more than 0.8 nM, no more than 0.7 nM, no more than 0.6 nM, no more than 0.5 nM, or no more than 0.4 nM as measured by Biacore assay. Biacore assay is based on surface plasmon resonance technology, see, for example, Murphy, M. *et al*., *Current protocols in protein science*, Chapter 19, unit 19.14, 2006. In certain embodiments, the K_D value is measured by the methods as described in Example 6 of the present disclosure.

[00321] Binding of the antibodies or the antigen-binding fragments thereof provided herein to human PD-L1 can also be represented by "half maximal effective concentration" (EC_{50}) value, which refers to the concentration of an antibody where 50% of its maximal binding is observed. The EC_{50} value can be measured by binding assays known in the art, for example, direct or indirect binding assay such as enzyme-linked immunosorbent assay (ELISA), Fluorescence Activated Cell Sorting (FACS) assay, and other binding assay. In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein specifically bind to PD-L1 at an EC_{50} (i.e. 50% binding concentration) of no more than 0.3 nM, no more than 0.2 nM, no more than 0.1 nM, or no more than 0.09 nM as measured by ELISA. In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein specifically bind to PD-L1 at an EC_{50} (i.e. 50% binding concentration) of no more than 1.4 nM, no more than 1.3 nM, no more than 1.2 nM, no more than 1.1 nM, no more than 1.0 nM, no more than 0.3 nM, no more than 0.25 nM, or no more than 0.21 nM as measured by FACS assay.

[00322] In some embodiments, the anti-PD-L1 antibody or an antigen-binding fragment thereof provided herein specifically binds to PD-L1. In some embodiments, the anti-PD-L1 antibody or an antigen-binding fragment thereof provided herein does not bind to other members of B7 family.

[00323] In certain embodiments, the anti-PD-L1 antibodies and antigen-binding fragments thereof provided herein are capable of blocking the interaction between the PD-L1 with its binding partner (e.g., PD-1 and B7-1) having an IC50 of no more than 2.2, 2.1, 2.0, 1.9, 1.8 or 1.2 ug/ml as measured by ELISA.

[00324] In certain embodiments, the anti-PD-L1 antibodies and antigen-binding fragments thereof provided herein are capable of blocking the interaction between the PD-L1 with its binding partner (e.g., PD-1,) having an EC50 of no more than 1.3, 1.2, 1.1, 1.0, 0.9, or 0.8 nM as measured by cell-based assay.

ix. Illustrative Anti-PD-L1 Antibodies and Antigen-Binding Fragments Thereof

[00325] In certain embodiments, the anti-PD-L1 antibodies (i.e., an antibody against PD-L1) and antigen-binding fragments thereof of the present disclosure comprise one or more (e.g. 1, 2, 3, 4, 5, or 6) CDRs comprising the sequences selected from the group consisting of **DYYMN** (SEQ ID NO: 1), **DINPNNX$_1$X$_2$TX$_3$YNHKFKG** (SEQ ID NO: 19), **WGDGPFAY** (SEQ ID NO: 3), **KASQNVX$_4$X$_5$X$_6$VA** (SEQ ID NO: 20), **SX$_7$SX$_8$RYT** (SEQ ID NO: 21), **QQYSNYPT** (SEQ ID NO: 6), wherein X_1 is G or A, X_2 is G or D or Q or E or L, X_3 is S or M or Q or L or V, X_4 is G or P or K, X_5 is A or G, X_6 is A or I, X_7 is A or N or R or V, X_8 is N or H or V or D.

[00326] In certain embodiments, the heavy chain variable region comprises:

a) a HCDR1 comprises a sequence of SEQ ID NO: 1,

b) a HCDR2 comprises a sequence selected from group consisting of SEQ ID NO: 2, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 17, and SEQ ID NO: 18 and

c) a HCDR3 comprises a sequence of SEQ ID NO: 3,

and/or

a light chain variable region comprising:

d) a LCDR1 comprises a sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9,

e) a LCDR2 comprises a sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, and

f) a LCDR3 comprises a sequence of SEQ ID NO: 6.

[00327] In certain embodiments, the heavy chain variable region is selected from the group consisting of:

g) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 2, and a HCDR3 comprising the sequence of SEQ ID NO: 3;

h) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 13, and a HCDR3 comprising the sequence of SEQ ID NO: 3;

i) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 14, and a HCDR3 comprising the sequence of SEQ ID NO: 3;

j) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 15, and a HCDR3 comprising the sequence of SEQ ID NO: 3; and

k) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 17, and a HCDR3 comprising the sequence of SEQ ID NO: 3; and

l) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 18 and a HCDR3 comprising the sequence of SEQ ID NO: 3.

[00328] In certain embodiments, the light chain variable region is selected from the group consisting of:

a) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 4, a LCDR2 comprising the sequence of SEQ ID NO: 5, and a LCDR3 comprising the sequence of SEQ ID NO: 6;

b) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 9, a LCDR2 comprising the sequence of SEQ ID NO: 5, and a LCDR3 comprising the sequence of SEQ ID NO: 6;

c) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 8, a LCDR2 comprising the sequence of SEQ ID NO: 5, and a LCDR3 comprising the sequence of SEQ ID NO: 6;

d) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 4, a LCDR2 comprising the sequence of SEQ ID NO: 12, and a LCDR3 comprising the sequence of SEQ ID NO: 6; and

e) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 4, a LCDR2 comprising the sequence of SEQ ID NO: 11, and a LCDR3 comprising the sequence of SEQ ID NO: 6.

[00329] Antibody "4B6" as used herein refers to a monoclonal antibody comprising a heavy chain variable region having the sequence of SEQ ID NO: 46, and a light chain variable region having the sequence of SEQ ID NO: 47.

[00330] In certain embodiments, the present disclosure provides anti-PD-L1 antibodies and antigen-binding fragments thereof comprising one or more (e.g. 1, 2, 3, 4, 5, or 6) CDR sequences of Antibody 4B6, or variants of Antibody 4B6. The CDR boundaries were defined or identified by the convention of Kabat.

[00331] In certain embodiments, the present disclosure provides anti-PD-L1 antibodies and antigen-binding fragments thereof comprising HCDR1 comprising an amino acid sequence of SEQ ID NO: 1, HCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 13, 14, 15, 17, and 18, and

HCDR3 comprising an amino acid sequence of SEQ ID NO: 3, and/or LCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 7, 8-9, LCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 10, 11-12, and LCDR3 comprising an amino acid sequence of SEQ ID NO: 6.

[00332] Table 1. CDR amino acid sequences of the antibody 4B6.

Antibody	Region	CDR1	CDR2	CDR3
4B6	HCDR	**SEQ ID NO: 1** DYYMN	**SEQ ID NO: 2** DINPNNGGTSYNHKFKG	**SEQ ID NO: 3** WGDGPFAY
	LCDR	**SEQ ID NO: 4** KASQNVGAAVA	**SEQ ID NO: 5** SASNRYT	**SEQ ID NO: 6** QQYSNYPT

[00333] Table 2. Variable region amino acid sequences of the antibody 4B6.

Antibody	VH	VL
4B6	SEQ ID NO: 46 EVQLQQSGPELVKPGASVKISCKASGYVFTDYYMNWVKQSHGKSLEWIGDINPNNGGTSYNHKFKGKATVTVDKSSRTAYMELLSLTSEDSAVYYCVKWGDGPFAYWGQGTLVTVSA	SEQ ID NO: 47 DIVMTQSQKFMSTSVGDRVSITCKASQNVGAAVAWYQQKPGQSPKLLIYSASNRYTGVPDRFTGSGSGTDFTLTISNMQSEDLADYFCQQYSNYPTFGSGTKLGIK

[00334] CDRs are known to be responsible for antigen binding. However, it has been found that not all of the 6 CDRs are indispensable or unchangeable. In other words, it is possible to replace or change or modify one or more CDRs in anti-PD-L1 antibody 4B6, yet substantially retain the specific binding affinity to PD-L1.

[00335] In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein comprise suitable framework region (FR) sequences, as long as the antibodies and antigen-binding fragments thereof can specifically bind to

PD-L1. The CDR sequences provided in Table 1 above are obtained from mouse antibodies, but they can be grafted to any suitable FR sequences of any suitable species such as mouse, human, rat, rabbit, among others, using suitable methods known in the art such as recombinant techniques.

[00336] In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein are humanized. A humanized antibody or antigen-binding fragment thereof is desirable in its reduced immunogenicity in human. A humanized antibody is chimeric in its variable regions, as non-human CDR sequences are grafted to human or substantially human FR sequences. Humanization of an antibody or antigen-binding fragment can be essentially performed by substituting the non-human (such as murine) CDR genes for the corresponding human CDR genes in a human immunoglobulin gene (see, for example, Jones *et al.* (1986) *Nature* 321:522-525; Riechmann *et al.* (1988) *Nature* 332:323-327; Verhoeyen *et al.* (1988) *Science* 239:1534-1536).

[00337] Suitable human heavy chain and light chain variable domains can be selected to achieve this purpose using methods known in the art. In an illustrative example, "best-fit" approach can be used, where a non-human (e.g. rodent) antibody variable domain sequence is screened or BLASTed against a database of known human variable domain sequences, and the human sequence closest to the non-human query sequence is identified and used as the human scaffold for grafting the non-human CDR sequences (see, for example, Sims *et al.*, (1993) *J. Immunol.* 151:2296; Chothia *et al.* (1987) *J. Mot. Biol.* 196:901). Alternatively, a framework derived from the consensus sequence of all human antibodies may be used for the grafting of the non-human CDRs (see, for example, Carter *et al.* (1992) *Proc. Natl. Acad. Sci. USA*, 89:4285; Presta *et al.* (1993) *J. Immunol.*,151:2623).

[00338] In some embodiments, the present disclosure provides 12 humanized antibodies of 4B6, which are designated as Hu4B6_Hg.2La.1, Hu4B6_Hg.2La.2, Hu4B6_Hg.2La.4, Hu4B6_Hg.2La.6, Hu4B6_Hg.3La.1, Hu4B6_Hg.3La.2,

Hu4B6_Hg.3La.4, Hu4B6_Hg.3La.6, Hu4B6_Hg.5La.1, Hu4B6_Hg.5La.2, Hu4B6_Hg.5La.4 and Hu4B6_Hg.5La.6, respectively. The SEQ ID NOs of the heavy and light chain variable regions of each humanized antibody of 4B6 are shown in Table 5. CDRs of each of the 12 humanized antibodies of 4B6 are shown in Table 5 (underlined sequences). The CDR boundaries were defined or identified by the convention of Kabat.

[00339] Table 3a below shows the amino acid sequences of the variant CDR for humanized 4B6, Table 3b below shows the FR for the humanized 4B6 heavy chain and light chain variable regions. Table 4 below shows the FR amino acid sequences for each heavy and light chains of 12 humanized antibodies for chimeric antibody 4B6, which are designated as Hu4B6_Hg.2La.1, Hu4B6_Hg.2La.2, Hu4B6_Hg.2La.4, Hu4B6_Hg.2La.6, Hu4B6_Hg.3La.1, Hu4B6_Hg.3La.2, Hu4B6_Hg.3La.4, Hu4B6_Hg.3La.6, Hu4B6_Hg.5La.1, Hu4B6_Hg.5La.2, Hu4B6_Hg.5La.4 and Hu4B6_Hg.5La.6, respectively. The heavy chain variable regions and light chain variable regions of these 12 humanized antibodies are shown in Table 5.

[00340] Table 3a. Amino acid sequences of the CDR variants for humanized antibody of 4B6.

SEQ ID NO.	Sequence	Annotation
7	KASQNVGAIVA	4B6-L-CDR1-1
8	KASQNVPAAVA	4B6-L-CDR1-2
9	KASQNVKGAVA	4B6-L-CDR1-3
10	SNSHRYT	4B6-L-CDR2-1
11	SRSVRYT	4B6-L-CDR2-2
12	SVSDRYT	4B6-L-CDR2-3
13	DINPNNADTMYNHKFKG	4B6-H-CDR2-1
14	DINPNNAQTQYNHKFKG	4B6-H-CDR2-2
15	DINPNNAETLYNHKFKG	4B6-H-CDR2-3
16	DINPNNGLTSYNHKFKG	4B6-H-CDR2-4
17	DINPNNAQTVYNHKFKG	4B6-H-CDR2-5
18	DINPNNAGTSYNHKFKG	H-CDR2-WT (G55A)

[00341] Table 3b. Amino acid sequences of the FR sequences for 4B6 and humanized antibody of 4B6.

SEQ ID NO.	Sequence	Annotation
22	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	HFR1 of human germline sequence
23	WVRQAPGQGLEWMG	HFR2 of human germline sequence
24	RVTMTRDTSISTAYMELSRLRSDDTAVYYCAR	HFR3 of human germline sequence
25	WGQGTLVTVSS	HFR4 of human germline sequence
26	DIQMTQSPSSLSASVGDRVTITC	LFR1 of human germline sequence
27	WYQQKPGKAPKLLIY	LFR2 of human germline sequence
28	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	LFR3 of human germline sequence
29	FGQGTKLEIK	LFR4 of human germline sequence
30	QVQLVQSGAEVKKPGASVKVSCKASGYVFT	HFR1 variant
31	WVRQAPGQSLEWMG	HFR2 variant
32	RVTVTVDTSISTAYMELSRLRSDDTAVYYCAR	HFR3 variant 1
33	RVTVTVDTSISTAYMELSRLRSDDTAVYYCVK	HFR3 variant 2
34	RVTVTVDKSISTAYMELSRLRSDDTAVYYCAR	HFR3 variant 3
35	RVTVTVDKSISTAYMELSRLRSDDTAVYYCVK	HFR3 variant 4
36	WYQQKPGKSPKLLIY	LFR2 variant
37	GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC	LFR3 variant 1
38	GVPDRFSGSGSGTDFTLTISSLQPEDIATYYC	LFR3 variant 2
39	GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC	F73L mutation for variant 1-FR3
45	GVPDRFSGSGSGTDFTLTISSLQPEDIATYYC	F73L, A43S, S60D for LC variant 2-FR3

[00342] Table 4. The FR amino acid sequences for each humanized heavy and light chain variable regions for humanized antibody of 4B6.

VH or VL Name	FR1 (SEQ ID NO.)	FR2 (SEQ ID NO.)	FR3 (SEQ ID NO.)	FR4 (SEQ ID NO.)
Hu4B6_Hg.2	30	31	35	25
AM4B6_Hg.3	30	31	35	25
AM4B6_Hg.5	30	31	35	25
AM4B6_La.1	26	27	37	29
AM4B6_La.2	26	27	37	29
AM4B6_La.4	26	27	37	29
AM4B6_La.6	30	31	35	25

[00343] Table 5 below shows the 3 variants of humanized 4B6 heavy chain variable regions (i.e. Hu4B6_Hg.2, Hu4B6_Hg.3 and Hu4B6_Hg.5) and 4 variants of humanized 4B6 light chain variable regions (i.e. AM4B6_La.1, AM4B6_La.2, AM4B6_La.4, AM4B6_La.6).

Table 5. Amino acid sequences of the variable regions for humanized antibody of 4B6.

Antibody	VH	VL
AM4B6_Hg.2La.1	**Hu4B6_Hg.2, SEQ ID NO: 58** QVQLVQSGAEVKKPGASVKVSCKASG Y**V**FTDYYMNWVRQAPGQ**S**LEWMGDIN PNN**AQ**T**Q**YNHKFKGRVT**V**T**V**D**K**SIST AYMELSRLRSDDTAVYYC**VK**WGDGPF AYWGQGTLVTVSS	**AM4B6_La.1, SEQ ID NO: 62** DIQMTQSPSSLSASVGDRVTITCKA SQNV**KG**AVAWYQQKPGKAPKLLIYS ASNRYTGVPSRFSGSGSGTDFT**L**TI SSLQPEDIATYYCQQYSNYPTFGQG TKLEIK
AM4B6_Hg.2La.2	**Hu4B6_Hg.2, SEQ ID NO: 58** QVQLVQSGAEVKKPGASVKVSCKASG Y**V**FTDYYMNWVRQAPGQ**S**LEWMGDIN PNN**AQ**T**Q**YNHKFKGRVT**V**T**V**D**K**SIST AYMELSRLRSDDTAVYYC**VK**WGDGPF AYWGQGTLVTVSS	**AM4B6_La.2, SEQ ID NO: 63** DIQMTQSPSSLSASVGDRVTITCKA SQNV**P**AAVAWYQQKPGKAPKLLIYS ASNRYTGVPSRFSGSGSGTDFT**L**TI SSLQPEDIATYYCQQYSNYPTFGQG TKLEIK

AM4B6_Hg.2La.4	**Hu4B6_Hg.2, SEQ ID NO: 58** QVQLVQSGAEVKKPGASVKVSCKASG Y**V**FTDYYMNWVRQAPGQ**S**LEWMGDIN PNN**AQ**T**Q**YNHKFKGRVT**V**T**V**D**K**SIST AYMELSRLRSDDTAVYYC**VK**WGDGPF AYWGQGTLVTVSS	**AM4B6_La.4, SEQ ID NO: 64** DIQMTQSPSSLSASVGDRVTITCKA SQNVGAAVAWYQQKPGKAPKLLIYS **V**S**D**RYTGVPSRFSGSGSGTDFT**L**TI SSLQPEDIATYYCQQYSNYPTFGQG TKLEIK
AM4B6_Hg.2La.6	**Hu4B6_Hg.2, SEQ ID NO: 58** QVQLVQSGAEVKKPGASVKVSCKASG Y**V**FTDYYMNWVRQAPGQ**S**LEWMGDIN PNN**AQ**T**Q**YNHKFKGRVT**V**T**V**D**K**SIST AYMELSRLRSDDTAVYYC**VK**WGDGPF AYWGQGTLVTVSS	**AM4B6_La.6, SEQ ID NO: 65** DIQMTQSPSSLSASVGDRVTITCKA SQNVGAAVAWYQQKPGKAPKLLIYS **R**S**V**RYTGVPSRFSGSGSGTDFT**L**TI SSLQPEDIATYYCQQYSNYPTFGQG TKLEIK
AM4B6_Hg.3La.1	**AM4B6_Hg.3, SEQ ID NO: 59** QVQLVQSGAEVKKPGASVKVSCKASG Y**V**FTDYYMNWVRQAPGQ**S**LEWMGDIN PNN**AE**T**L**YNHKFKGRVT**V**T**V**D**K**SIST AYMELSRLRSDDTAVYYC**VK**WGDGPF AYWGQGTLVTVSS	**AM4B6_La.1, SEQ ID NO: 62** DIQMTQSPSSLSASVGDRVTITCKA SQNV**KG**AVAWYQQKPGKAPKLLIYS ASNRYTGVPSRFSGSGSGTDFT**L**TI SSLQPEDIATYYCQQYSNYPTFGQG TKLEIK
AM4B6_Hg.3La.2	**AM4B6_Hg.3, SEQ ID NO: 59** QVQLVQSGAEVKKPGASVKVSCKASG Y**V**FTDYYMNWVRQAPGQ**S**LEWMGDIN PNN**AE**T**L**YNHKFKGRVT**V**T**V**D**K**SIST AYMELSRLRSDDTAVYYC**VK**WGDGPF AYWGQGTLVTVSS	**AM4B6_La.2, SEQ ID NO: 63** DIQMTQSPSSLSASVGDRVTITCKA SQNV**P**AAVAWYQQKPGKAPKLLIYS ASNRYTGVPSRFSGSGSGTDFT**L**TI SSLQPEDIATYYCQQYSNYPTFGQG TKLEIK
AM4B6_Hg.3La.4	**AM4B6_Hg.3, SEQ ID NO: 59** QVQLVQSGAEVKKPGASVKVSCKASG Y**V**FTDYYMNWVRQAPGQ**S**LEWMGDIN PNN**AE**T**L**YNHKFKGRVT**V**T**V**D**K**SIST AYMELSRLRSDDTAVYYC**VK**WGDGPF AYWGQGTLVTVSS	**AM4B6_La.4, SEQ ID NO: 64** DIQMTQSPSSLSASVGDRVTITCKA SQNVGAAVAWYQQKPGKAPKLLIYS **V**S**D**RYTGVPSRFSGSGSGTDFT**L**TI SSLQPEDIATYYCQQYSNYPTFGQG TKLEIK
AM4B6_Hg.3La.6	**AM4B6_Hg.3, SEQ ID NO: 59** QVQLVQSGAEVKKPGASVKVSCKASG Y**V**FTDYYMNWVRQAPGQ**S**LEWMGDIN PNN**AE**T**L**YNHKFKGRVT**V**T**V**D**K**SIST AYMELSRLRSDDTAVYYC**VK**WGDGPF AYWGQGTLVTVSS	**AM4B6_La.6, SEQ ID NO: 65** QVQLVQSGAEVKKPGASVKVSCKAS GY**V**FTDYYMNWVRQAPGQ**S**LEWMGD INPNN**A**GTSYNHKFKGRVT**V**T**V**D**K**S ISTAYMELSRLRSDDTAVYYC**VK**WG DGPFAYWGQGTLVTVSS
AM4B6_Hg.5La.1	**AM4B6_Hg.5, SEQ ID NO: 60** QVQLVQSGAEVKKPGASVKVSCKASG Y**V**FTDYYMNWVRQAPGQ**S**LEWMGDIN PNN**AQ**T**V**YNHKFKGRVT**V**T**V**D**K**SIST AYMELSRLRSDDTAVYYC**VK**WGDGPF AYWGQGTLVTVSS	**AM4B6_La.1, SEQ ID NO: 62** DIQMTQSPSSLSASVGDRVTITCKA SQNV**KG**AVAWYQQKPGKAPKLLIYS ASNRYTGVPSRFSGSGSGTDFT**L**TI SSLQPEDIATYYCQQYSNYPTFGQG TKLEIK

AM4B6_Hg.5La.2	**AM4B6_Hg.5, SEQ ID NO: 60** QVQLVQSGAEVKKPGASVKVSCKASG Y**V**FTDYYMNWVRQAPGQ**S**LEWMGDIN PNN**AQ**T**V**YNHKFKGRVT**V**T**V**D**K**SIST AYMELSRLRSDDTAVYYC**VK**WGDGPF AYWGQGTLVTVSS	**AM4B6_La.2, SEQ ID NO: 63** DIQMTQSPSSLSASVGDRVTITCKA SQNV**P**AAVAWYQQKPGKAPKLLIYS ASNRYTGVPSRFSGSGSGTDFT**L**TI SSLQPEDIATYYCQQYSNYPTFGQG TKLEIK
AM4B6_Hg.5La.4	**AM4B6_Hg.5, SEQ ID NO: 60** QVQLVQSGAEVKKPGASVKVSCKASG Y**V**FTDYYMNWVRQAPGQ**S**LEWMGDIN PNN**AQ**T**V**YNHKFKGRVT**V**T**V**D**K**SIST AYMELSRLRSDDTAVYYC**VK**WGDGPF AYWGQGTLVTVSS	**AM4B6_La.4, SEQ ID NO: 64** DIQMTQSPSSLSASVGDRVTITCKA SQNVGAAVAWYQQKPGKAPKLLIYS **V**S**D**RYTGVPSRFSGSGSGTDFT**L**TI SSLQPEDIATYYCQQYSNYPTFGQG TKLEIK
AM4B6_Hg.5La.6	**AM4B6_Hg.5, SEQ ID NO: 60** QVQLVQSGAEVKKPGASVKVSCKASG Y**V**FTDYYMNWVRQAPGQ**S**LEWMGDIN PNN**AQ**T**V**YNHKFKGRVT**V**T**V**D**K**SIST AYMELSRLRSDDTAVYYC**VK**WGDGPF AYWGQGTLVTVSS	**AM4B6_La.6, SEQ ID NO: 65** QVQLVQSGAEVKKPGASVKVSCKAS GY**V**FTDYYMNWVRQAPGQ**S**LEWMGD INPNN**A**GTSYNHKFKGRVT**V**T**V**D**K**S ISTAYMELSRLRSDDTAVYYC**VK**WG DGPFAYWGQGTLVTVSS

[00344] In certain embodiments, the humanized anti-PD-L1 antibodies or antigen-binding fragments thereof provided herein are composed of substantially all human sequences except for the CDR sequences which are non-human. In some embodiments, the variable region FRs, and constant regions if present, are entirely or substantially from human immunoglobulin sequences. The human FR sequences and human constant region sequences may be derived from different human immunoglobulin genes, for example, FR sequences derived from one human antibody and constant region from another human antibody. In some embodiments, the humanized antibody or antigen-binding fragment thereof comprises human heavy chain HFR1-4, and/or light chain LFR1-4.

[00345] In some embodiments, the FR regions derived from human may comprise the same amino acid sequence as the human immunoglobulin from which it is derived. In some embodiments, one or more amino acid residues of the human FR are substituted with the corresponding residues from the parent non-human antibody. This may be desirable in certain embodiments to make the humanized antibody or its fragment closely approximate the non-human parent antibody structure, so as to optimize binding characteristics (for example, increase binding affinity). In certain embodiments, the humanized antibody or antigen-binding fragment thereof provided herein comprises no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residue substitutions in each of the human FR sequences, or no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residue substitutions in all the FR sequences of a heavy or a light chain variable domain. In some embodiments, such change in amino acid residue could be present in heavy chain FR regions only, in light chain FR regions only, or in both chains. In certain embodiments, one or more amino acids of the human FR sequences are randomly mutated to increase binding affinity. In certain embodiments, one or more amino acids of the human FR sequences are back mutated to the corresponding amino acid(s) of the parent non-human antibody so as to increase binding affinity.

[00346] In certain embodiments, the humanized anti-PD-L1 antibodies and antigen-binding fragments thereof of the present disclosure comprise a heavy chain HFR1 comprising the sequence of **QVQLVQSGAEVKKPGASVKVSCKASGYX$_9$FT** (SEQ ID NO: 40) or a homologous sequence of at least 80% sequence identity thereof, a heavy chain HFR2 comprising the sequence of **WVRQAPGQX$_{10}$LEWMG** (SEQ ID NO: 41) or a homologous sequence of at least 80% sequence identity thereof, a heavy chain HFR3 comprising the sequence of **RVTX$_{16}$TVDX$_{11}$SISTAYMELSRLRSDDTAVYYCX$_{12}$X$_{13}$** (SEQ ID NO: 42) or a homologous sequence of at least 80% sequence identity thereof, and a heavy chain HFR4 comprising the sequence of **WGQGTLVTVSS** (SEQ ID NO: 25) or a homologous sequence of at least 80% sequence identity thereof, wherein X_9 is T or V, X_{10} is G or S, X_{11} is T or K, X_{12} is A or V, and X_{13} is R or K.

[00347] In certain embodiments, the humanized anti-PD-L1 antibodies and antigen-binding fragments thereof of the present disclosure comprise a light chain LFR1 comprising the sequence of **DIQMTQSPSSLSASVGDRVTITC** (SEQ ID NO: 26) or a homologous sequence of at least 80% sequence identity thereof, a light chain LFR2 comprising the sequence of **WYQQKPGKX$_{14}$PKLLIY** (SEQ ID NO: 43) or a homologous sequence of at least 80% sequence identity thereof, a light chain LFR3 comprising the sequence of **GVPX$_{15}$RFSGSGSGTDFTX$_{17}$TISSLQPEDIATYYC** (SEQ ID NO: 44) or a homologous sequence of at least 80% sequence identity thereof, and a light chain LFR4 comprising the sequence of **FGQGTKLEIK** (SEQ ID NO: 29) or a homologous sequence of at least 80% sequence identity thereof, wherein X_{14} is A or S, X_{15} is S or D, X_{16} is M or V, and X_{17} is F or L.

[00348] In certain embodiments, the HFR1 comprises a sequence selected from the group consisting of SEQ ID NOs: 22 and 30, the HFR2 comprises a sequence selected from the group consisting of SEQ ID NOs: 23 and 31, the HFR3 comprises the sequence selected from the group consisting of SEQ ID NOs: 24 and 32-35, the HFR4 comprises a sequence of SEQ ID NOs: 25, the LFR1 comprises the sequence

from the group consisting of SEQ ID NO: 26, the LFR2 comprises a sequence selected from the group consisting of SEQ ID NOs: 27 and 36, the LFR3 comprises a sequence selected from the group consisting of SEQ ID NOs: 28, and 37-38, 39, 45, and the LFR4 comprises a sequence of SEQ ID NO: 29.

[00349] In certain embodiments, the humanized anti-PD-L1 antibodies and antigen-binding fragments thereof of the present disclosure comprise HFR1, HFR2, HFR3, and/or HFR4 sequences contained in a heavy chain variable region selected from a group consisting of: Hu4B6_Hg.2 (SEQ ID NO: 58), AM4B6_Hg.3 (SEQ ID NO: 59), AM4B6_Hg.5 (SEQ ID NO: 60).

[00350] In certain embodiments, the humanized anti-PD-L1 antibodies and antigen-binding fragments thereof of the present disclosure comprise LFR1, LFR2, LFR3, and/or LFR4 sequences contained in a light chain variable region selected from a group consisting of: AM4B6_La.1 (SEQ ID NO: 62), AM4B6_La.2 (SEQ ID NO: 63), AM4B6_La.4 (SEQ ID NO: 64), and AM4B6_La.6 (SEQ ID NO: 65).

[00351] In certain embodiments, the heavy chain variable region comprises a sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, and a homologous sequence thereof having at least 80% sequence identity thereof. In certain embodiments, the light chain variable region comprises a sequence selected from the group consisting of SEQ ID NO: 47, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, and a homologous sequence thereof having at least 80% sequence identity thereof. In certain embodiments, the antibody against PD-L1 or antigen-binding fragment thereof comprises a pair of heavy chain variable region and light chain variable region sequences selected from the group consisting of: SEQ ID NOs: 49/54, 50/54, 51/54, 52/54, 49/55, 50/55, 51/55, 52/55, 58/62, 58/63, 58/64, 58/65, 59/62, 59/63, 59/64, 59/65, 60/62, 60/63, 60/64, and 60/65.

[00352] These exemplary humanized anti-PD-L1 antibodies retained the specific binding capacity or affinity to PD-L1, and are better than, the parent mouse antibody 4B6 in that aspect.

[00353] In some embodiments, the anti-PD-L1 antibodies and antigen-binding fragments provided herein comprise all or a portion of the heavy chain variable domain and/or all or a portion of the light chain variable domain. In one embodiment, the anti-PD-L1 antibody or an antigen-binding fragment thereof provided herein is a single domain antibody which consists of all or a portion of the heavy chain variable domain provided herein. More information of such a single domain antibody is available in the art (see, e.g. U.S. Pat. No. 6,248,516).

[00354] In certain embodiments, the anti-PD-L1 antibodies or the antigen-binding fragments thereof provided herein further comprise an immunoglobulin (Ig) constant region, which optionally further comprises a heavy chain and/or a light chain constant region. In certain embodiments, the heavy chain constant region comprises CH1, hinge, and/or CH2-CH3 regions (or optionally CH2-CH3-CH4 regions). In certain embodiments, the anti-PD-L1 antibodies or the antigen-binding fragments thereof provided herein comprises heavy chain constant regions of human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2 or IgM. In certain embodiments, the light chain constant region comprises Cκ or Cλ. The constant region of the anti-PD-L1 antibodies or the antigen-binding fragments thereof provided herein may be identical to the wild-type constant region sequence or be different in one or more mutations.

[00355] In certain embodiments, the anti-PD-L1 antibodies or the antigen-binding fragments thereof provided herein have a specific binding affinity to human PD-L1 which is sufficient to provide for diagnostic and/or therapeutic use.

[00356] The anti-PD-L1 antibodies or antigen-binding fragments thereof provided herein can be a monoclonal antibody, a polyclonal antibody, a humanized

antibody, a chimeric antibody, a recombinant antibody, a bispecific antibody, a multi-specific antibody, a labeled antibody, a bivalent antibody, an anti-idiotypic antibody, or a fusion protein. A recombinant antibody is an antibody prepared in vitro using recombinant methods rather than in animals.

[00357] In certain embodiments, the PD-L1 binding moiety comprises an anti-PD-L1 antibody or antigen-binding fragment thereof, which competes for binding to PD-L1 with the antibody or antigen-binding fragment thereof comprising a pair of heavy chain variable region and light chain variable region sequences selected from the group consisting of: SEQ ID NOs: 49/54, 50/54, 51/54, 52/54, 49/55, 50/55, 51/55, 52/55, 58/62, 58/63, 58/64, 58/65, 59/62, 59/63, 59/64, 59/65, 60/62, 60/63, 60/64, and 60/65.

x. Antibody Variants

[00358] The anti-PD-L1 antibodies and antigen-binding fragments thereof provided herein also encompass various variants of the antibody sequences provided herein.

[00359] In certain embodiments, the antibody variants comprise one or more modifications or substitutions in one or more of the CDR sequences provided in Table 1 above, one or more of the non-CDR sequences of the heavy chain variable region or light chain variable region provided in Tables 3a , 3b and 5 above, and/or the constant region (e.g. Fc region). Such variants retain binding specificity to PD-L1 of their parent antibodies, but have one or more desirable properties conferred by the modification(s) or substitution(s). For example, the antibody variants may have improved antigen-binding affinity, improved glycosylation pattern, reduced risk of glycosylation, reduced deamination, reduced or depleted effector function(s), improved FcRn receptor binding, increased pharmacokinetic half-life, pH sensitivity, and/or compatibility to conjugation (e.g. one or more introduced cysteine residues).

[00360] The parent antibody sequence may be screened to identify suitable or preferred residues to be modified or substituted, using methods known in the art, for

example, “alanine scanning mutagenesis” (see, for example, Cunningham and Wells (1989) Science, 244:1081-1085). Briefly, target residues (e.g. charged residues such as Arg, Asp, His, Lys, and Glu) can be identified and replaced by a neutral or negatively charged amino acid (e.g. alanine or polyalanine), and the modified antibodies are produced and screened for the interested property. If substitution at a particular amino acid location demonstrates an interested functional change, then the position can be identified as a potential residue for modification or substitution. The potential residues may be further assessed by substituting with a different type of residue (e.g. cysteine residue, positively charged residue, etc.).

xi. Affinity Variants

[00361] Affinity variants of antibodies may contain modifications or substitutions in one or more CDR sequences provided in Table 1 above, one or more FR sequences provided in Tables 3b and 4 above, or the heavy or light chain variable region sequences provided in Tables 5 above. FR sequences can be readily identified by a person skilled in the art based on the CDR sequences in Table 1 above and variable region sequences in Table 5 above, as it is well-known in the art that a CDR region is flanked by two FR regions in the variable region. The affinity variants retain specific binding affinity to PD-L1 of the parent antibody, or even have improved PD-L1 specific binding affinity over the parent antibody. In certain embodiments, at least one (or all) of the substitution(s) in the CDR sequences, FR sequences, or variable region sequences comprises a conservative substitution.

[00362] A person skilled in the art will understand that in the CDR sequences provided in Table 1 and 3a above, and variable region sequences provided in Table 5 above, one or more amino acid residues may be substituted yet the resulting antibody or antigen-binding fragment still retain the binding affinity or binding capacity to PD-L1, or even have an improved binding affinity or capacity. Various methods known in the art can be used to achieve this purpose. For example, a library of antibody variants (such as Fab or scFv variants) can be generated and expressed with phage display technology, and then screened for the binding affinity to PD-L1. For

another example, computer software can be used to virtually simulate the binding of the antibodies to PD-L1, and identify the amino acid residues on the antibodies which form the binding interface. Such residues may be either avoided in the substitution so as to prevent reduction in binding affinity, or targeted for substitution to provide for a stronger binding.

[00363] In certain embodiments, the humanized anti-PD-L1 antibody or antigen-binding fragment thereof provided herein comprises one or more amino acid residue substitutions in one or more of the CDR sequences, and/or one or more of the FR sequences. In certain embodiments, an affinity variant comprises no more than 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 substitutions in the CDR sequences and/or FR sequences in total.

[00364] In certain embodiments, the anti-PD-L1 antibodies or antigen-binding fragments thereof comprise 1, 2, or 3 CDR sequences having at least 80% (e.g. at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity to that (or those) listed in Tables 1 and 3a above yet retaining the specific binding affinity to PD-L1 at a level similar to or even higher than its parent antibody.

[00365] In certain embodiments, the anti-PD-L1 antibodies or antigen-binding fragments thereof comprise one or more variable region sequences having at least 80% (e.g. at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity to that (or those) listed in Table 5 above yet retaining the specific binding affinity to PD-L1 at a level similar to or even higher than its parent antibody. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in a variable region sequence listed in Table 5 above. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the CDRs (e.g. in the FRs).

xii. Glycosylation Variants

[00366] The anti-PD-L1 antibodies or antigen-binding fragments thereof provided herein also encompass glycosylation variants, which can be obtained to

either increase or decrease the extent of glycosylation of the antibodies or antigen binding fragments thereof.

[00367] The anti-PD-L1 antibodies or antigen binding fragments thereof may comprise one or more modifications that introduce or remove a glycosylation site. A glycosylation site is an amino acid residue with a side chain to which a carbohydrate moiety (e.g. an oligosaccharide structure) can be attached. Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue, for example, an asparagine residue in a tripeptide sequence such as asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly to serine or threonine. Removal of a native glycosylation site can be conveniently accomplished, for example, by altering the amino acid sequence such that one of the above-described tripeptide sequences (for N-linked glycosylation sites) or serine or threonine residues (for O-linked glycosylation sites) present in the sequence in the is substituted. A new glycosylation site can be created in a similar way by introducing such a tripeptide sequence or serine or threonine residue.

[00368] In certain embodiments, the anti-PD-L1 antibodies and antigen-binding fragments provided herein comprise one or more mutationsto remove one or more deamidation site. In certain embodiments, the anti-PD-L1 antibodies and antigen-binding fragments provided herein comprise a mutation at G55 (for example, G55A) in the heavy chain. These mutations are tested and are believed not to negatively affect the binding affinity of the antibodies provided herein.

xiii. Cysteine-engineered Variants

[00369] The anti-PD-L1 antibodies or antigen-binding fragments thereof provided herein also encompass cysteine-engineered variants, which comprise one or more introduced free cysteine amino acid residues.

[00370] A free cysteine residue is one which is not part of a disulfide bridge. A cysteine-engineered variant is useful for conjugation with for example, a cytotoxic and/or imaging compound, a label, or a radioisoptype among others, at the site of the engineered cysteine, through for example a maleimide or haloacetyl. Methods for engineering antibodies or antigen-binding fragments thereof to introduce free cysteine residues are known in the art, see, for example, WO2006/034488.

xiv. Fc Variants

[00371] The anti-PD-L1 antibodies or antigen-binding fragments thereof provided herein also encompass Fc variants, which comprise one or more amino acid residue modifications or substitutions at the Fc region and/or hinge region, for example, to provide for altered effector functions such as ADCC and CDC. Methods of altering ADCC activity by antibody engineering have been described in the art, see for example, Shields RL. et al., J Biol Chem. 2001. 276(9): 6591-604; Idusogie EE. et al., J Immunol. 2000.164(8):4178-84; Steurer W. et al., J Immunol. 1995, 155(3): 1165- 74; Idusogie EE. et al., J Immunol. 2001, 166(4): 2571-5; Lazar GA. et al., PNAS, 2006, 103(11): 4005-4010; Ryan MC. et al., Mol. Cancer Ther., 2007, 6: 3009-3018; Richards JO,. et al., Mol Cancer Ther. 2008, 7(8): 2517-27; Shields R. L. et al., J. Biol. Chem, 2002, 277: 26733-26740; Shinkawa T. et al., J. Biol. Chem, 2003, 278: 3466-3473.

[00372] CDC activity of the antibodies or antigen-binding fragments provided herein can also be altered, for example, by improving or diminishing C1q binding and/or CDC (see, for example, WO99/51642; Duncan & Winter Nature 322:738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821); and WO94/29351 concerning other examples of Fc region variants. One or more amino acids selected from amino acid residues 329, 331 and 322 of the Fc region can be replaced with a different amino acid residue to alter C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC) (see, U.S. Pat. No. 6,194,551 by Idusogie et al.). One or more amino acid substitution(s) can also be introduced to alter the

ability of the antibody to fix complement (see PCT Publication WO 94/29351 by Bodmer et al.).

[00373] In certain embodiments, the Fc variants provided herein has reduced effector functions relative to a wildtype Fc (e.g. Fc of IgG1), and comprise one or more amino acid substitution(s) at a position selected from the group consisting of: 220, 226, 228, 229, 233, 234, 235, 236, 237, 238, 267, 268, 269, 270, 297, 309, 318, 320, 322, 325, 328, 329, 330, 331 and 332 of the Fc region (see, WO2016/196228; Richards *et al.* (2008) *Mol. Cancer Therap.* 7:2517; Moore *et al.* (2010) *mAbs* 2:181; and Strohl (2009) *Current Opinion in Biotechnology* 20:685-691), wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. Exemplary substitutions for reduced effector functions include, without limitation, 220S, 226S, 228P, 229S, 233P, 234V, 234G, 234A, 234F, 234A, 235A, 235G, 235E, 236E, 236R, 237A, 237K, 238S, 267R, 268A, 268Q, 269R, 297A, 297Q, 297G, 309L, 318A, 322A, 325L, 328R, 330S, 331S, or any combination thereof (see, WO2016/196228; and Strohl (2009) *Current Opinion in Biotechnology* 20:685-691).

[00374] In certain embodiments, the anti-PD-L1 antibodies or antigen-binding fragments thereof provided herein has reduced effector functions, and comprise one or more amino acid substitution(s) in IgG1 at a position selected from the group consisting of: 234, 235, 237, 238, 268, 297, 309, 330, and 331. In certain embodiments, the anti-PD-L1 antibodies or antigen-binding fragments thereof provided herein is of IgG1 isotype and comprise one or more amino acid substitution(s) selected from the group consisting of: N297A, N297Q, N297G, L235E, L234A, L235A, L234F, L235E, P331S, and any combination thereof.

[00375] In certain embodiments, the anti-PD-L1 antibodies or antigen-binding fragments thereof provided herein is of IgG1 isotype and comprise a L234A and L235A mutation. In certain embodiments, the anti-PD-L1 antibodies or antigen-binding fragments thereof provided herein is of IgG1 isotype and comprise L234F, L235E, and P331S. The L234F, L235E, and P331S set of substitutions (also

referred as FES triple mutation) located in the CH2 region of the Fc domain can abrogate FCγR and C1q binding resulting in an antibody unable to elicit ADCC or CDC (Oganesyan et al., Acta Crystallogr. D 64:700-704 (2008)). PCT/US2013/36872 has shown that combining these mutations in a variant Fc domain, e.g., a variant Fc domain in an antibody result in an Fc domain having reduced thermal stability compared to a wild type parent molecule, e.g., a wild type IgG1 Fc. In certain embodiments, the Fc variant comprises an amino acid sequence of SEQ ID NO: 81.

[00376] In certain embodiments, the anti-PD-L1 antibodies or antigen-binding fragments thereof provided herein is of IgG2 isotype, and comprises one or more amino acid substitution(s) selected from the group consisting of: H268Q, V309L, A330S, P331S, V234A, G237A, P238S, H268A, and any combination thereof (e.g. H268Q/V309L/A330S/P331S, V234A/G237A/P238S/H268A/V309L/A330S/ P331S). In certain embodiments, the anti-PD-L1 antibodies or antigen-binding fragments thereof provided herein is of IgG4 isotype, and comprises one or more amino acid substitution(s) selected from the group consisting of: S228P, N297A, N297Q, N297G, L235E, F234A, L235A, and any combination thereof. In certain embodiments, the anti-PD-L1 antibodies or antigen-binding fragments thereof provided herein is of IgG2/ IgG4 cross isotype. Examples of IgG2/ IgG4 cross isotype is described in Rother RP et al., Nat Biotechnol 25:1256–1264 (2007).

[00377] In certain embodiments, the anti-PD-L1 antibodies or antigen-binding fragments thereof comprise one or more amino acid substitution(s) that improves pH-dependent binding to neonatal Fc receptor (FcRn). Such a variant can have an extended pharmacokinetic half-life, as it binds to FcRn at acidic pH which allows it to escape from degradation in the lysosome and then be translocated and released out of the cell. Methods of engineering an antibody or antigen-binding fragment thereof to improve binding affinity with FcRn are well-known in the art, see, for example, Vaughn, D. *et al.*, *Structure*, 6(1): 63-73, 1998; Kontermann, R. *et al.*, *Antibody Engineering*, Volume 1, Chapter 27: Engineering of the Fc region for improved PK,

published by Springer, 2010; Yeung, Y. *et al.*, *Cancer Research*, 70: 3269-3277 (2010); and Hinton, P. *et al.*, *J. Immunology*, 176:346-356 (2006).

[00378] In certain embodiments, anti-PD-L1 antibodies or antigen-binding fragments thereof comprise one or more amino acid substitution(s) in the interface of the Fc region to facilitate and/or promote heterodimerization. These modifications comprise introduction of a protuberance into a first Fc polypeptide and a cavity into a second Fc polypeptide, wherein the protuberance can be positioned in the cavity so as to promote interaction of the first and second Fc polypeptides to form a heterodimer or a complex. Methods of generating antibodies with these modifications are known in the art, e.g. as described in U.S. Pat. No. 5,731,168.

xv. Antigen-binding Fragments

[00379] The PD-L1-binding moiety in the bi-functional molecules provided herein also encompass anti-PD-L1 antigen-binding fragments. Various types of antigen-binding fragments are known in the art and can be developed based on the anti-PD-L1 antibodies provided herein, including for example, the exemplary antibodies whose CDRs are shown in Tables 1 and 3a above, and variable sequences are shown in Tables 2, and 5, and their different variants (such as affinity variants, glycosylation variants, Fc variants, cysteine-engineered variants and so on).

[00380] In certain embodiments, an anti-PD-L1 antigen-binding fragment provided herein is a diabody, a Fab, a Fab', a F(ab')2, a Fd, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multispecific antibody, a camelized single domain antibody, a nanobody, a domain antibody, and a bivalent domain antibody.

[00381] Various techniques can be used for the production of such antigen-binding fragments. Illustrative methods include, enzymatic digestion of intact antibodies (see, e.g. Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); and Brennan *et al.*, *Science*, 229:81 (1985)),

recombinant expression by host cells such as *E. Coli* (e.g. for Fab, Fv and ScFv antibody fragments), screening from a phage display library as discussed above (e.g. for ScFv), and chemical coupling of two Fab'-SH fragments to form $F(ab')_2$ fragments (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). Other techniques for the production of antibody fragments will be apparent to a person skilled in the art.

[00382] In certain embodiments, the antigen-binding fragment is a scFv. Generation of scFv is described in, for example, WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. ScFv may be fused to an effector protein at either the amino or the carboxyl terminus to provide for a fusion protein (see, for example, Antibody Engineering, ed. Borrebaeck).

[00383] In certain embodiments, the anti-PD-L1 antibodies or antigen-binding fragments thereof provided herein are bivalent, tetravalent, hexavalent, or multivalent. Any molecule being more than bivalent is considered multivalent, encompassing for example, trivalent, tetravalent, hexavalent, and so on.

[00384] A bivalent molecule can be monospecific if the two binding sites are both specific for binding to the same antigen or the same epitope. This, in certain embodiments, provides for stronger binding to the antigen or the epitope than a monovalent counterpart. Similar, a multivalent molecule may also be monospecific. In certain embodiments, in a bivalent or multivalent antigen-binding moiety, the first valent of binding site and the second valent of binding site are structurally identical (i.e. having the same sequences), or structurally different (i.e. having different sequences albeit with the same specificity).

[00385] A bivalent can also be bispecific, if the two binding sites are specific for different antigens or epitopes. This also applies to a multivalent molecule. For example, a trivalent molecule can be bispecific when two binding sites are monospecific for a first antigen (or epitope) and the third binding site is specific for a second antigen (or epitope).

xvi. Bispecific Antibodies

[00386] In certain embodiments, the anti-PD-L1 antibodies or antigen-binding fragments thereof is bispecific. In certain embodiments, apart from the second moiety provided herein, the PD-L1 binding antibody or antigen-binding fragment thereof is further linked to an additional functional domain having a different binding specificity from said anti-PD-L1 antibody, or antigen binding fragment thereof.

[00387] In certain embodiments, the bispecific antibodies or antigen-binding fragments thereof provided herein are capable of specifically binding to a second antigen other than PD-L1 (and other than the target bound by the second moiety), or a second epitope on PD-L1 (or a second epitope on the target bound by the second moiety).

xvii. Bi-functional Molecules

[00388] In certain embodiments, the bi-functional molecule provided herein are capable of binding to both PD-L1 and the target bound by the second moiety. In certain embodiments, the bi-functional molecule provided herein are capable of binding to both PD-L1 and TGFβ, or binding to both PD-L1 and IL-1, or binding to both PD-L1 and IL-1R, or binding to both PD-L1 and MHCII, or binding to both PD-L1 and CD47, or binding to both PD-L1 and SIRPα.

[00389] In certain embodiments, the bi-functional molecule targeting PD-L1 and TGFβ of the present disclosure specifically binding to human TGFβ1 at an EC_{50} of no more than 2.0 nM (e.g. no more than 2.0 nM, no more than 1.2 nM, no more than 1.1 nM, no more than 1.0 nM, no more than 0.9 nM, no more than 0.8 nM) as measured by ELISA assay. In certain embodiments, the protein targeting PD-L1 and TGFβ of the present disclosure is capable of simultaneously binding to PD-L1 and TGFβ as measured by ELISA assay. In certain embodiments, the bi-functional molecule targeting PD-L1 and TGFβ of the present disclosure is capable of specifically binding to human PD-L1 at a K_D value of no more than 0.8 nM, no more than 0.7 nM, no more than 0.6 nM, no more than 0.5 nM, or no more than 0.4 nM as measured by Biacore assay. In certain embodiments, the bi-functional molecule

targeting PD-L1 and TGFβ of the present disclosure is capable of specifically binding to human TGFβ1 at a K_D value of no more than 2.0 nM (e.g. no more than 2.0 nM, no more than 1.2 nM, no more than 1.1 nM, no more than 1.0 nM, no more than 0.9 nM, no more than 0.8 nM) as measured by ELISA assay.

[00390] In certain embodiments, the bi-functional molecule targeting PD-L1 and TGFβ of the present disclosure is capable of exhibiting synergistic effect on tumor growth inhibition at a dose dependent manner.

[00391] In certain embodiments, the bi-functional molecule targeting PD-L1 and TGFβ of the present disclosure is capable of exhibiting enhanced infiltration of anti-tumor immune cells into a tumor microenvironment as compared to a molecule comprising the immune checkpoint molecule only.

[00392] In certain embodiments, the bi-functional molecule targeting PD-L1 and TGFβ of the present disclosure is capable of selectively reducing at least 90% (e.g. at least 80%, 70%, 60%, 50%, 40%, 30%, or 20%) of TGFβ1 in plasma and such reduction can be maintained for at least 10, 14, or 21 days.

[00393] In certain embodiments, the bi-functional molecule comprises heterodimeric heavy chains. The heavy chains are heterodimeric with respect to presence or position of the second moiety. In certain embodiments, the heterodimeric heavy chains comprise one heavy chain having the second moiety but the other heavy chain having not, wherein the second moiety comprises a CD47 binding domain (e.g. soluble SIRP α) or a SIRPα binding domain.

[00394] In the bi-functional molecule, the heterodimeric heavy chains comprise one heavy chain having the second moiety but the other heavy chain having not. The heterodimeric heavy chains can further comprise heterodimeric Fc regions that associate in a way that discourages homodimerization and/or favors heterodimerization. For example, the heterodimeric Fc regions can be selected so that they are not identical and that they preferentially form heterodimers between each

other rather than to form homodimers within themselves. In certain embodiments, the heterodimeric Fc regions are capable of associating into heterodimers via formation of knob-into-hole, hydrophobic interaction, electrostatic interaction, hydrophilic interaction, or increased flexibility. In certain embodiments, heterodimeric Fc regions comprise CH2 and/or CH3 domains which are respectively mutated to be capable of forming a knobs-into-holes. A knob can be obtained by replacement of a small amino acid residue with a larger one in the first CH2/CH3 polypeptide, and a hole can be obtained by replacement of a large residue with a smaller one. In certain embodiments, heterodimeric Fc regions comprise a first CH3 domain of the IgG1 isotype containing S354C and T366W substitution (SEQ ID NO: 96, knob) and a second CH3 domain of the IgG1 isotype containing Y349C, T366S, L368A and Y407V substitution (SEQ ID NO: 97, hole).

[00395] In certain embodiments, the bi-functional molecule comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 118 or SEQ ID NO: 120, and/or a light chain comprising an amino acid sequence of SEQ ID NO: 119 or SEQ ID NO: 121.

xviii. Conjugates

[00396] In some embodiments, the bi-functional molecule further comprise one or more conjugate moieties. The conjugate moiety can be linked to the bi-functional molecule. A conjugate moiety is a moiety that can be attached to the bi-functional molecule. It is contemplated that a variety of conjugate moieties may be linked to the bi-functional molecules provided herein (see, for example, "Conjugate Vaccines", Contributions to Microbiology and Immunology, J. M. Cruse and R. E. Lewis, Jr. (eds.), Carger Press, New York, (1989)). These conjugate moieties may be linked to the bi-functional molecule by covalent binding, affinity binding, intercalation, coordinate binding, complexation, association, blending, or addition, among other methods. In some embodiments, the bi-functional molecule can be linked to one or more conjugates via a linker.

[00397] In certain embodiments, the bi-functional molecule provided herein may be engineered to contain specific sites outside the epitope binding portion that may be utilized for binding to one or more conjugate moieties. For example, such a site may include one or more reactive amino acid residues, such as for example cysteine or histidine residues, to facilitate covalent linkage to a conjugate moiety.

[00398] In certain embodiments, the bi-functional molecules may be linked to a conjugate moiety indirectly, or through another conjugate moiety. For example, the bi-functional molecules provided herein may be conjugated to biotin, then indirectly conjugated to a second conjugate that is conjugated to avidin. In some embodiments, the conjugate moiety comprises a clearance-modifying agent (e.g. a polymer such as PEG which extends half-life), a chemotherapeutic agent, a toxin, a radioactive isotope, a lanthanide, a detectable label (e.g. a luminescent label, a fluorescent label, an enzyme-substrate label), a DNA-alkylator, a topoisomerase inhibitor, a tubulin-binder, a purification moiety or other anticancer drugs.

[00399] A "toxin" can be any agent that is detrimental to cells or that can damage or kill cells. Examples of toxin include, without limitation, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, MMAE, MMAF, DM1, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin and analogs thereof, antimetabolites (e.g. methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g. mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g. daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g. dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), anti-mitotic agents (e.g. vincristine and vinblastine), a topoisomerase inhibitor, and a tubulin-binders.

[00400] Examples of detectable label may include a fluorescent labels (e.g. fluorescein, rhodamine, dansyl, phycoerythrin, or Texas Red), enzyme-substrate labels (e.g. horseradish peroxidase, alkaline phosphatase, luceriferases, glucoamylase, lysozyme, saccharide oxidases or β-D-galactosidase), radioisotopes (e.g. 123I, 124I, 125I, 131I, 35S, 3H, 111In, 112In, 14C, 64Cu, 67Cu, 86Y, 88Y, 90Y, 177Lu, 211At, 186Re, 188Re, 153Sm, 212Bi, and 32P, other lanthanides), luminescent labels, chromophoric moieties, digoxigenin, biotin/avidin, DNA molecules or gold for detection.

[00401] In certain embodiments, the conjugate moiety can be a clearance-modifying agent which helps increase half-life of the bi-functional molecule. Illustrative examples include water-soluble polymers, such as PEG, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, copolymers of ethylene glycol/propylene glycol, and the like. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules.

[00402] In certain embodiments, the conjugate moiety can be a purification moiety such as a magnetic bead.

[00403] In certain embodiments, the bi-functional molecules provided herein is used as a base for a conjugate.

III. Polynucleotides and Recombinant Methods

[00404] The present disclosure provides isolated polynucleotides that encode the bi-functional molecules provided herein. The term “nucleic acid” or “polynucleotide” as used herein refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless otherwise indicated, a particular polynucleotide sequence also implicitly encompasses conservatively modified variants thereof (e.g. degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well

as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (see Batzer *et al.*, *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260:2605-2608 (1985); and Rossolini *et al.*, *Mol. Cell. Probes* 8:91-98 (1994)).

[00405] DNA encoding the monoclonal antibody is readily isolated and sequenced using conventional procedures (e.g. by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). The encoding DNA may also be obtained by synthetic methods.

[00406] The isolated polynucleotide that encodes the bi-functional molecule can be inserted into a vector for further cloning (amplification of the DNA) or for expression, using recombinant techniques known in the art. Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter (e.g. SV40, CMV, EF-1α), and a transcription termination sequence.

[00407] The present disclosure provides vectors comprising the isolated polynucleotides provided herein. In certain embodiments, the polynucleotide provided herein encodes the bi-functional molecule, at least one promoter (e.g. SV40, CMV, EF-1α) operably linked to the nucleic acid sequence, and at least one selection marker. Examples of vectors include, but are not limited to, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (e.g. herpes simplex virus), poxvirus, baculovirus, papillomavirus, papovavirus (e.g. SV40), lambda phage, and M13 phage, plasmid pcDNA3.3, pMD18-T, pOptivec, pCMV, pEGFP, pIRES, pQD-Hyg-GSeu, pALTER, pBAD, pcDNA, pCal, pL, pET, pGEMEX, pGEX, pCI, pEGFT, pSV2, pFUSE, pVITRO, pVIVO, pMAL, pMONO, pSELECT, pUNO, pDUO, Psg5L, pBABE, pWPXL, pBI, p15TV-L, pPro18, pTD, pRS10, pLexA,

pACT2.2, pCMV-SCRIPT.RTM., pCDM8, pCDNA1.1/amp, pcDNA3.1, pRc/RSV, PCR 2.1, pEF-1, pFB, pSG5, pXT1, pCDEF3, pSVSPORT, pEF-Bos etc.

[00408] Vectors comprising the polynucleotide sequence encoding the bi-functional molecule can be introduced to a host cell for cloning or gene expression. Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as Escherichia, e.g. E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, e.g. Salmonella typhimurium, Serratia, e.g. Serratia marcescans, and Shigella, as well as Bacilli such as B. subtilis and B. licheniformis, Pseudomonas such as P. aeruginosa, and Streptomyces.

[00409] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for the vectors encoding the bi-functional molecule. Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as Schizosaccharomyces pombe; Kluyveromyces hosts such as, e.g. K. lactis, K. fragilis (ATCC 12,424), K. bulgaricus (ATCC 16,045), K. wickeramii (ATCC 24,178), K. waltii (ATCC 56,500), K. drosophilarum (ATCC 36,906), K. thermotolerans, and K. marxianus; yarrowia (EP 402,226); Pichia pastoris (EP 183,070); Candida; Trichoderma reesia (EP 244,234); Neurospora crassa; Schwanniomyces such as Schwanniomyces occidentalis; and filamentous fungi such as, e.g. Neurospora, Penicillium, Tolypocladium, and Aspergillus hosts such as A. nidulans and A. niger.

[00410] Suitable host cells for the expression of glycosylated bi-functional molecule provided herein are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as Spodoptera

frugiperda (caterpillar), Aedes aegypti (mosquito), Aedes albopictus (mosquito), Drosophila melanogaster (fruiffly), and Bombyx mori have been identified. A variety of viral strains for transfection are publicly available, e.g. the L-1 variant of Autographa californica NPV and the Bm-5 strain of Bombyx mori NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of Spodoptera frugiperda cells. Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can also be utilized as hosts.

[00411] However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2). In some embodiments, the host cell is a mammalian cultured cell line, such as CHO, BHK, NS0, 293 and their derivatives.

[00412] Host cells are transformed with the above-described expression or cloning vectors for production of the bi-functional molecule and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. In another embodiment, the bi-functional molecule may be produced by homologous

recombination known in the art. In certain embodiments, the host cell is capable of producing the bi-functional molecule provided herein.

[00413] The present disclosure also provides a method of expressing the bi-functional molecule provided herein, comprising culturing the host cell provided herein under the condition at which the vector of the present disclosure is expressed. The host cells used to produce the bi-functional molecule provided herein may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium (MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., Meth. Enz. 58:44 (1979), Barnes et al., Anal. Biochem. 102:255 (1980), U.S. Pat. No. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCINTM drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to a person skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to a person skilled in the art.

[00414] When using recombinant techniques, the bi-functional molecule can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the bi-functional molecule is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Carter *et al.*, *Bio/Technology* 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic

space of E. coli. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the bi-functional molecule is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[00415] The bi-functional molecule prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, DEAE-cellulose ion exchange chromatography, ammonium sulfate precipitation, salting out, and affinity chromatography, with affinity chromatography being the preferred purification technique.

[00416] In certain embodiments, Protein A immobilized on a solid phase is used for immunoaffinity purification of the antibody and antigen-binding fragment thereof. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the bi-functional molecule. Protein A can be used to purify antibodies that are based on human gamma1, gamma2, or gamma4 heavy chains (Lindmark et al., J. Immunol. Meth. 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human gamma3 (Guss et al., EMBO J. 5:1567 1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABXTM resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSETM chromatography on an anion or cation exchange resin (such as a

polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[00417] Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g. from about 0-0.25M salt).

IV. Pharmaceutical Composition

[00418] The present disclosure further provides pharmaceutical compositions comprising the bi-functional molecule and one or more pharmaceutically acceptable carriers.

[00419] Pharmaceutical acceptable carriers for use in the pharmaceutical compositions disclosed herein may include, for example, pharmaceutically acceptable liquid, gel, or solid carriers, aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, anesthetics, suspending/dispending agents, sequestering or chelating agents, diluents, adjuvants, excipients, or non-toxic auxiliary substances, other components known in the art, or various combinations thereof.

[00420] Suitable components may include, for example, antioxidants, fillers, binders, disintegrants, buffers, preservatives, lubricants, flavorings, thickeners, coloring agents, emulsifiers or stabilizers such as sugars and cyclodextrins. Suitable antioxidants may include, for example, methionine, ascorbic acid, EDTA, sodium thiosulfate, platinum, catalase, citric acid, cysteine, thioglycerol, thioglycolic acid, thiosorbitol, butylated hydroxanisol, butylated hydroxytoluene, and/or propyl gallate. As disclosed herein, inclusion of one or more antioxidants such as methionine in a composition comprising the bi-functional molecule and conjugates provided herein decreases oxidation of the bi-functional molecule. This reduction in oxidation prevents or reduces loss of binding affinity, thereby improving antibody stability and

maximizing shelf-life. Therefore, in certain embodiments, pharmaceutical compositions are provided that comprise one or more bi-functional molecule as disclosed herein and one or more antioxidants such as methionine. Further provided are methods for preventing oxidation of, extending the shelf-life of, and/or improving the efficacy of a bi-functional molecule provided herein by mixing the bi-functional molecule with one or more antioxidants such as methionine.

[00421] To further illustrate, pharmaceutical acceptable carriers may include, for example, aqueous vehicles such as sodium chloride injection, Ringer's injection, isotonic dextrose injection, sterile water injection, or dextrose and lactated Ringer's injection, nonaqueous vehicles such as fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil, or peanut oil, antimicrobial agents at bacteriostatic or fungistatic concentrations, isotonic agents such as sodium chloride or dextrose, buffers such as phosphate or citrate buffers, antioxidants such as sodium bisulfate, local anesthetics such as procaine hydrochloride, suspending and dispersing agents such as sodium carboxymethylcelluose, hydroxypropyl methylcellulose, or polyvinylpyrrolidone, emulsifying agents such as Polysorbate 80 (TWEEN-80), sequestering or chelating agents such as EDTA (ethylenediaminetetraacetic acid) or EGTA (ethylene glycol tetraacetic acid), ethyl alcohol, polyethylene glycol, propylene glycol, sodium hydroxide, hydrochloric acid, citric acid, or lactic acid. Antimicrobial agents utilized as carriers may be added to pharmaceutical compositions in multiple-dose containers that include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Suitable excipients may include, for example, water, saline, dextrose, glycerol, or ethanol. Suitable non-toxic auxiliary substances may include, for example, wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, or agents such as sodium acetate, sorbitan monolaurate, triethanolamine oleate, or cyclodextrin.

[00422] The pharmaceutical compositions can be a liquid solution, suspension, emulsion, pill, capsule, tablet, sustained release formulation, or powder. Oral

formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrollidone, sodium saccharine, cellulose, magnesium carbonate, etc.

[00423] In certain embodiments, the pharmaceutical compositions are formulated into an injectable composition. The injectable pharmaceutical compositions may be prepared in any conventional form, such as for example liquid solution, suspension, emulsion, or solid forms suitable for generating liquid solution, suspension, or emulsion. Preparations for injection may include sterile and/or non-pyretic solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use, and sterile and/or non-pyretic emulsions. The solutions may be either aqueous or nonaqueous.

[00424] In certain embodiments, unit-dose parenteral preparations are packaged in an ampoule, a vial or a syringe with a needle. All preparations for parenteral administration should be sterile and not pyretic, as is known and practiced in the art.

[00425] In certain embodiments, a sterile, lyophilized powder is prepared by dissolving a bi-functional molecule as disclosed herein in a suitable solvent. The solvent may contain an excipient which improves the stability or other pharmacological components of the powder or reconstituted solution, prepared from the powder. Excipients that may be used include, but are not limited to, water, dextrose, sorbital, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent. The solvent may contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer known to a person skilled in the art at, in one embodiment, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to a person skilled in the art provides a desirable formulation. In one embodiment, the resulting solution will be apportioned into vials for lyophilization. Each vial can contain a single dosage or

multiple dosages of the bi-functional molecule or composition thereof. Overfilling vials with a small amount above that needed for a dose or set of doses (e.g. about 10%) is acceptable so as to facilitate accurate sample withdrawal and accurate dosing. The lyophilized powder can be stored under appropriate conditions, such as at about 4 °C to room temperature.

[00426] Reconstitution of a lyophilized powder with water for injection provides a formulation for use in parenteral administration. In one embodiment, for reconstitution the sterile and/or non-pyretic water or other liquid suitable carrier is added to lyophilized powder. The precise amount depends upon the selected therapy being given, and can be empirically determined.

V. Kits

[00427] In certain embodiments, the present disclosure provides a kit comprising the bi-functional molecule provided herein and/or the pharmaceutical composition provided herein. In certain embodiments, the present disclosure provides a kit comprising the bi-functional molecule provided herein, and a second therapeutic agent. In certain embodiments, the second therapeutic agent is selected from the group consisting of a chemotherapeutic agent, an anti-cancer drug, radiation therapy, an immunotherapy agent, an anti-angiogenesis agent, a targeted therapy, a cellular therapy, a gene therapy, a hormonal therapy, an antiviral agent, an antibiotic, an analgesics, an antioxidant, a metal chelator, and cytokines.

[00428] Such kits can further include, if desired, one or more of various conventional pharmaceutical kit components, such as, for example, containers with one or more pharmaceutically acceptable carriers, additional containers etc., as will be readily apparent to a person skilled in the art. Instructions, either as inserts or a labels, indicating quantities of the components to be administered, guidelines for administration, and/or guidelines for mixing the components, can also be included in the kit.

VI. Methods of Use

[00429] In another aspect, the present disclosure provides a method of treating, preventing or alleviating a PD-L1 related disease in a subject, comprising administering to the subject a therapeutically effective amount of the bi-functional molecule provided herein, or the pharmaceutical composition or kit provided herein.

[00430] In certain embodiments, the subject is human.

[00431] PD-1-related conditions and disorders can be immune related disease or disorder, cancers, autoimmune diseases, or infectious disease.

[00432] In certain embodiments, the PD-1-related conditions and disorders include cancers, for example, non-small cell lung cancer, small cell lung cancer, renal cell cancer, colorectal cancer, ovarian cancer, breast cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, head and neck cancer, thyroid cancer, sarcoma, prostate cancer, glioblastoma, cervical cancer, thymic carcinoma, leukemia, lymphomas, myelomas, mycoses fungoids, merkel cell cancer, and other hematologic malignancies, such as classical Hodgkin lymphoma (CHL), primary mediastinal large B-cell lymphoma, T-cell/histiocyte-rich B-cell lymphoma, EBV-positive and -negative PTLD, and EBV-associated diffuse large B-cell lymphoma (DLBCL), plasmablastic lymphoma, extranodal NK/T-cell lymphoma, nasopharyngeal carcinoma, and HHV8-associated primary effusion lymphoma, Hodgkin's lymphoma, neoplasm of the central nervous system (CNS), such as primary CNS lymphoma, spinal axis tumor, brain stem glioma. In certain embodiments, the tumors and cancers are metastatic, especially metastatic tumors expressing PD-L1.

[00433] In certain embodiments, the PD-1-related conditions and disorders include autoimmune diseases. Autoimmune diseases include, but are not limited to, Acquired Immunodeficiency Syndrome (AIDS, which is a viral disease with an autoimmune component), alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune diabetes, autoimmune

hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease (AIED), autoimmune lymphoproliferative syndrome (ALPS), autoimmune thrombocytopenic purpura (ATP), Behcet's disease, cardiomyopathy, celiac sprue-dermatitis hepetiformis; chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy (CIPD), cicatricial pemphigold, cold agglutinin disease, crest syndrome, Crohn's disease, Degos' disease, dermatomyositis-juvenile, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Graves' disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA nephropathy, insulin-dependent diabetes mellitus, juvenile chronic arthritis (Still's disease), juvenile rheumatoid arthritis, Meniere's disease, mixed connective tissue disease, multiple sclerosis, myasthenia gravis, pemacious anemia, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynaud's phenomena, Reiter's syndrome, rheumatic fever, rheumatoid arthritis, sarcoidosis, scleroderma (progressive systemic sclerosis (PSS), also known as systemic sclerosis (SS)), Sjogren's syndrome, stiff-man syndrome, systemic lupus erythematosus, Takayasu arteritis, temporal arteritis/giant cell arteritis, ulcerative colitis, uveitis, vitiligo and Wegener's granulomatosis.

[00434] In certain embodiments, the PD-1-related conditions and disorders include infectious disease. Infectious disease include, for example, chronic viral infection, for example, fungus infection, parasite/protozoan infection or chronic viral infection, for example, malaria, coccidioiodmycosis immitis, histoplasmosis, onychomycosis, aspergilosis, blastomycosis, candidiasis albicans, paracoccidioiomycosis, microsporidiosis, Acanthamoeba keratitis, Amoebiasis, Ascariasis, Babesiosis, Balantidiasis, Baylisascariasis, Chagas disease, Clonorchiasis, Cochliomyia, Cryptosporidiosis, Diphyllobothriasis, Dracunculiasis, Echinococcosis, Elephantiasis, Enterobiasis, Fascioliasis, Fasciolopsiasis, Filariasis, Giardiasis, Gnathostomiasis, Hymenolepiasis, Isosporiasis, Katayama fever, Leishmaniasis,

Lyme disease, Metagonimiasis, Myiasis, Onchocerciasis, Pediculosis, Scabies, Schistosomiasis, Sleeping sickness, Strongyloidiasis, Taeniasis, Toxocariasis, Toxoplasmosis, Trichinosis, Trichuriasis, Trypanosomiasis, helminth infection, infection of hepatitis B (HBV), hepatitis C (HCV), herpes virus, Epstein-Barr virus, HIV-1, HIV-2, cytomegalovirus, herpes simplex virus type I, herpes simplex virus type II, human papilloma virus, adenovirus, Kaposi West sarcoma associated herpes virus epidemics, thin ring virus (Torquetenovirus), human T lymphotrophic viruse I, human T lymphotrophic viruse II, varicella zoster, JC virus or BK virus.

[00435] In certain embodiments, the PD-L1 related disease is a PD-L1-expressing cancer, or a PD-L1-overexpressing cancer. A "PD-L1-expressing cancer" is one that involves cancer cells or tumor cells having PD-L1 protein present at their cell surface. A "PD-L1-overexpressing cancer" is one which has significantly higher levels of a PD-L1, at the cell surface of a cancer or tumor cell, compared to a noncancerous cell of the same tissue type.

[00436] PD-L1 expression or overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the PD-L1 present on the surface of a cell (e.g. via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of PD-L1-encoding nucleic acid in the cell, e.g. via fluorescent in situ hybridization (FISH; see WO98/45479 published October, 1998), southern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). One may also study PD-L1 overexpression by measuring shed antigen (e.g., PD-L1 ectodomain or soluble PD-L1) in a biological fluid such as serum. Aside from the above assays, various in vivo assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an anti-PD-L1 antibody which is optionally labeled with a detectable label, e.g. a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, e.g. by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody.

[00437] In some embodiments, the subject has been identified as being likely to respond to a PD-1 antagonist. The presence or level of PD-L1 on an interested biological sample can be indicative of whether the subject from whom the biological sample is derived could likely respond to a PD-1 antagonist. In some embodiments, the test sample is derived from a cancer cell or tissue, or tumor infiltrating immune cells. In certain embodiments, presence or up-regulated level of the PD-L1 in the test biological sample indicates likelihood of responsiveness. The term "up-regulated" as used herein, refers to an overall increase of no less than 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80% or greater, in the protein level of PD-L1 in the test sample, as compared to the PD-L1 protein level in a reference sample as detected using the same antibody. The reference sample can be a control sample obtained from a healthy or non-diseased individual, or a healthy or non-diseased sample obtained from the same individual from whom the test sample is obtained. For example, the reference sample can be a non-diseased sample adjacent to or in the neighborhood of the test sample (e.g. tumor).

[00438] In certain embodiments, the PD-L1 related disease is resistant to PD-L1/PD-1 monotherapy. "PD-L1/PD-1 monotherapy" as used herein refers to a monotherapy that acts by inhibiting or reducing PD-L1 and PD-1 interaction or signaling. Exemplary PD-L1/PD-1 monotherapy may include anti-PD-L1 antibody therapy, anti-PD-1 antibody therapy, or monotherapy involving small molecule inhibitors directed to PD-1 or PD-L1. By "resistant" it is meant that the disease has no or reduced responsiveness or sensitivity to a PD-L1/PD-1 monotherapy. Reduced responsiveness can be indicated by, for example, requirement of an increased dose to achieve a given efficacy. In certain embodiments, the disease can be non-responsive to PD-L1/PD-1 monotherapy. For example, the cancer cells or tumor size increases despite of the treatment with the PD-L1/PD-1 monotherapy, or the disease showed regression back to its former state, for example, return of previous symptoms following partial recovery. The resistance to PD-L1/PD-1 monotherapy can be *de novo* or acquired.

[00439] In another aspect, the present disclosure provides a method of treating, preventing or alleviating in a subject a disease or condition that would benefit from suppression of an immunosuppressive cytokine, from induction of sustained immune responses, or from stimulation of anti-tumor immunity, comprising administering an effective amount of the bi-functional molecule provided herein, or the pharmaceutical composition provided herein.

[00440] In some embodiments, the immunosuppressing cytokine is a TGFβ or IL-1. In some embodiments, the immunosuppressing cytokine is a TGFβ1 or IL-1β.

[00441] In some embodiments, the disease or condition is a TGFβ-related disease or condition. In some embodiments, the TGFβ-related disease is cancer, fibrotic disease, or kidney disease.

[00442] In certain embodiments, the TGFβ-related disease is cancer. In certain embodiments, the cancer is selected from the group consisting of: colorectal, breast, ovarian, pancreatic, gastric, prostate, renal, cervical, myeloma, lymphoma, leukemia, thyroid, endometrial, uterine, bladder, neuroendocrine, head and neck, liver, nasopharyngeal, testicular, small cell lung, cancer, non-small cell lung cancer, melanoma, basal cell, skin cancer, squamous cell skin cancer, dermatofibrosarcoma protuberans, Merkel cell carcinoma, glioblastoma, glioma, sarcoma, mesothelioma, and myelodisplastic syndromes.

[00443] In certain embodiments, the TGFβ-related disease is fibrotic disease. Fibrotic disease is a disease or condition that involves fibrosis. Fibrosis is a scarring process that is a common feature of chronic organ injury, for example in lungs, liver, kidney, skin, heart, gut or muscle. Fibrosis is characterized by elevated activity of transforming growth factor-beta (TGF-β) resulting in increased and altered deposition of extracellular matrix and other fibrosis-associated proteins.

[00444] Fibrotic disease can include fibrotic disease in lungs, liver, kidney, eyes, skin, heart, gut or muscle. Examples of fibrotic disease in lungs include pulmonary

fibrosis, cystic fibrosis, pulmonary hypertension, progressive massive fibrosis, bronchiolitis obliterans, airway remodeling associated with chronic asthma or idiopathic pulmonary. Examples of fibrotic disease in liver include cirrhosis or non-alcoholic steatohepatitis. Examples of fibrotic disease in kidney include such as renal fibrosis, ischemic renal injury, tubulointerstitial fibrosis, diabetic nephropathy, nephrosclerosis, or nephrotoxicity. Examples of fibrotic disease in eyes include such as corneal fibrosis, subretinal fibrosis. Examples of fibrotic disease in skin include such as nephrogenic systemic fibrosis, keloid or scleroderma. Examples of fibrotic disease in heart include endomyocardial fibrosis or old myocardial infarction.

[00445] In some embodiments, the disease or condition is an IL-1-related disease or condition. In some embodiments, the IL-1-related disease is autoinflammatory disease, metabolic syndrome, acute inflammation, chronic inflammation or malignancy.

[00446] In some embodiments, the disease or condition would benefit from induction of sustained immune responses by stimulating MHCII signaling with an immunostimulatory polypeptide, e.g., soluble LAG-3. In some embodiments, the disease or condition is cancer, viral infection, parasite infection, or a combination thereof.

[00447] In some embodiments, the disease or condition would benefit from stimulation of anti-tumor immunity by inhibiting an immunoinhibitory receptor signaling. In some embodiments, the immunoinhibitory receptor is SIRPα. In certain embodiments, the disease, disorder or condition is SIRPα related, such as cancer, solid tumor, a chronic infection, an inflammatory disease, multiple sclerosis, an autoimmune disease, a neurologic disease, a brain injury, a nerve injury, a polycythemia, a hemochromatosis, a trauma, a septic shock, fibrosis, atherosclerosis, obesity, type II diabetes, a transplant dysfunction, or arthritis. In some embodiments, the cancer is anal cancer, appendix cancer, astrocytoma, basal cell carcinoma, gallbladder cancer, gastric cancer, lung cancer, bronchial cancer, bone cancer, liver

and bile duct cancer, pancreatic cancer, breast cancer, liver cancer, ovarian cancer, testicle cancer, kidney cancer, renal pelvis and ureter cancer, salivary gland cancer, small intestine cancer, urethral cancer, bladder cancer, head and neck cancer, spine cancer, brain cancer, cervix cancer, uterine cancer, endometrial cancer, colon cancer, colorectal cancer, rectal cancer, anal cancer, esophageal cancer, gastrointestinal cancer, skin cancer, prostate cancer, pituitary cancer, vagina cancer, thyroid cancer, throat cancer, glioblastoma, melanoma, myelodysplastic syndrome, sarcoma, teratoma, chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), Hodgkin lymphoma, non-Hodgkin lymphoma, multiple myeloma, T or B cell lymphoma, GI organ interstitialoma, soft tissue tumor, hepatocellular carcinoma, and adenocarcinoma. In some embodiments, the cancer is a CD47-expressing cancer, or a CD47-overexpressing cancer.

[00448] The therapeutically effective amount of a bi-functional molecule provided herein will depend on various factors known in the art, such as for example body weight, age, past medical history, present medications, state of health of the subject and potential for cross-reaction, allergies, sensitivities and adverse side-effects, as well as the administration route and extent of disease development. Dosages may be proportionally reduced or increased by a person skilled in the art (e.g. physician or veterinarian) as indicated by these and other circumstances or requirements.

[00449] In certain embodiments, the bi-functional molecule provided herein may be administered at a therapeutically effective dosage of about 0.01 mg/kg to about 100 mg/kg. In certain embodiments, the administration dosage may change over the course of treatment. For example, in certain embodiments the initial administration dosage may be higher than subsequent administration dosages. In certain embodiments, the administration dosage may vary over the course of treatment depending on the reaction of the subject.

[00450] Dosage regimens may be adjusted to provide the optimum desired response (e.g. a therapeutic response). For example, a single dose may be administered, or several divided doses may be administered over time.

[00451] The bi-functional molecule provided herein may be administered by any route known in the art, such as for example parenteral (e.g. subcutaneous, intraperitoneal, intravenous, including intravenous infusion, intramuscular, or intradermal injection) or non-parenteral (e.g. oral, intranasal, intraocular, sublingual, rectal, or topical) routes.

[00452] In some embodiments, the bi-functional molecule provided herein may be administered alone or in combination with a therapeutically effective amount of a second therapeutic agent. For example, the bi-functional molecule disclosed herein may be administered in combination with a second therapeutic agent, for example, a chemotherapeutic agent, an anti-cancer drug, radiation therapy, an immunotherapy agent, an anti-angiogenesis agent, a targeted therapy, a cellular therapy, a gene therapy, a hormonal therapy, an antiviral agent, an antibiotic, an analgesics, an antioxidant, a metal chelator, or cytokines.

[00453] The term "immunotherapy" as used herein, refers to a type of therapy that stimulates immune system to fight against disease such as cancer or that boosts immune system in a general way. Examples of immunotherapy include, without limitation, checkpoint modulators, adoptive cell transfer, cytokines, oncolytic virus and therapeutic vaccines.

[00454] “Targeted therapy” is a type of therapy that acts on specific molecules associated with cancer, such as specific proteins that are present in cancer cells but not normal cells or that are more abundant in cancer cells, or the target molecules in the cancer microenvironment that contributes to cancer growth and survival. Targeted therapy targets a therapeutic agent to a tumor, thereby sparing of normal tissue from the effects of the therapeutic agent.

[00455] In certain of these embodiments, a bi-functional molecule provided herein that is administered in combination with one or more additional therapeutic agents may be administered simultaneously with the one or more additional therapeutic agents, and in certain of these embodiments the bi-functional molecule and the additional therapeutic agent(s) may be administered as part of the same pharmaceutical composition. However, a bi-functional molecule administered "in combination" with another therapeutic agent does not have to be administered simultaneously with or in the same composition as the agent. A bi-functional molecule administered prior to or after another agent is considered to be administered "in combination" with that agent as the phrase is used herein, even if the antibody or antigen-binding fragment and the second agent are administered via different routes. Where possible, additional therapeutic agents administered in combination with the antibodies or antigen-binding fragments thereof disclosed herein are administered according to the schedule listed in the product information sheet of the additional therapeutic agent, or according to the Physicians' Desk Reference 2003 (Physicians' Desk Reference, 57th Ed; Medical Economics Company; ISBN: 1563634457; 57th edition (November 2002)) or protocols well known in the art.

[00456] In another aspect, the present disclosure also provides use of the bi-functional molecule provided herein and/or the pharmaceutical composition provided herein in the manufacture of a medicament for treating a PD-L1 related disease, and/or a TGF-β-related disease and/or an IL-1 related disease and/or a CD47 related disease in a subject.

[00457] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. All specific compositions, materials, and methods described below, in whole or in part, fall within the scope of the present invention. These specific compositions, materials, and methods are not intended to limit the invention, but merely to illustrate specific embodiments falling within the scope of the invention. A person skilled in the art may develop equivalent compositions, materials, and methods without the exercise of

inventive capacity and without departing from the scope of the invention. It will be understood that many variations can be made in the procedures herein described while still remaining within the bounds of the present invention. It is the intention of the inventors that such variations are included within the scope of the invention.

EXAMPLES

Example 1: Generation, expression and purification of humanzied 4B6 antibodies

[00458] The anti-PD-L1 mAb 4B6, which originated in Patent WO2017161976A1 comprising a VH sequence of SEQ ID NO: 46 and a VL sequence of SEQ ID NO: 47 shown below, was a potent PD-1/PD-L1 blocker. This antibody was generated from mouse hybridoma antibody therefore it needed an appropriate humanization. The sequence of the variable domain of mouse antibody 4B6 was used to identify the germline sequence with the highest homology to their respective murine framework. Computer-modelling was used for designing the humanzied variants with complementarity-determining region (CDR) grafting and back mutations.

[00459] Mouse/chimeric heavy chain variable region (SEQ ID NO: 46):

*EVQLQQSGPELVKPGASVKISCKASGYVFT***DYYMN***WVKQSHGKSLEWIG***DINPNNGGTS***
YNHKFKG*KATVTVDKSSRTAYMELLSLTSEDSAVYYCVK***WGDGPFAY****WGQGTLVTVSA*

[00460] Mouse/chimeric light chain variable region (SEQ ID NO: 47):

*DIVMTQSQKFMSTSVGDRVSITC***KASQNVGAAVA***WYQQKPGQSPKLLIY***SASNRYT****GVP*
*DRFTGSGSGTDFTLTISNMQSEDLADYFC***QQYSNYPT****FGSGTKLGIK*

[00461] NOTE: The italic portion represents framework (FR), and the underlined portion represents CDR sequences. The order is FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

[00462] Human germline framework sequences VK/1-33 for light chain and VH/1-2 for heavy chain were used for CDR grafting, respectively.

[00463] Heavy chain variants 1, 2, 3 and 4 (i.e., VH variant 1, 2, 3, and 4) were obtained by direct grafting the three CDRs to the VH germline sequence (SEQ ID NO: 48), and in addition the back mutations of M69V, R71V for VH variant 1 (SEQ ID NO: 49), M69V, R71V, A93V, R94K for VH variant 2 (SEQ ID NO: 50), M69V, R71V, T73K, T28V for VH variant 3 (SEQ ID NO: 51) and M69V, R71V, A93V, R94K, T73K, T28V, G44S for VH variant 4 (SEQ ID NO: 52), respectively.

[00464] Germline sequence for 4B6 VH:

[00465] VH/1-2 (4B6-VH germline, SEQ ID NO: 48):

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMG
WINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAR

[00466] VH/1-2 variant 1 (4B6_Ha, SEQ ID NO: 49):

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMNWVRQAPGQGLEWMG
DINPNNGGTSYNHKFKGRVTVTVDTSISTAYMELSRLRSDDTAVYYCARWG
DGPFAYWGQGTLVTVSS

[00467] VH/1-2 variant 2 (4B6_Hb, SEQ ID NO: 50):

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMNWVRQAPGQGLEWMG
DINPNNGGTSYNHKFKGRVTVTVDTSISTAYMELSRLRSDDTAVYYCVKWG
DGPFAYWGQGTLVTVSS

[00468] VH/1-2 variant 3 (Hu4B6_Hc, SEQ ID NO: 51):

QVQLVQSGAEVKKPGASVKVSCKASGYVFTDYYMNWVRQAPGQGLEWMG
DINPNNGGTSYNHKFKGRVTVTVDKSISTAYMELSRLRSDDTAVYYCARWG
DGPFAYWGQGTLVTVSS

[00469] VH/1-2 variant 4 (Hu4B6_Hd, SEQ ID NO: 52):

QVQLVQSGAEVKKPGASVKVSCKASGYVFTDYYMNWVRQAPGQSLEWMG
DINPNNGGTSYNHKFKGRVTVTVDKSISTAYMELSRLRSDDTAVYYCVKWG
DGPFAYWGQGTLVTVSS

[00470] Light chain vairants 1 and 2 (VL variant 1 and 2) were obtained by direct grafting the three CDRs to the germline seqeunce (SEQ ID NO : 53), and in addition the back mutation of F73L mutation for VL variant 1 (SEQ ID NO: 54) and F73L, A43S, S60D for VL variant 2 (SEQ ID NO: 55), respectively.

[00471] Germline sequence for 4B6 VL:

[00472] VK/1-33 (4B6-VL-germline, SEQ ID NO: 53) :

DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDASN
LETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYDNLP

[00473] VK/1-33 variant 1 (Hu4B6_La, SEQ ID NO: 54) :

DIQMTQSPSSLSASVGDRVTITCKASQNVGAAVAWYQQKPGKAPKLLIYSAS
NRYTGVPSRFSGSGSGTDFTLTISSLQPEDIATYYCQQYSNYPTFGQGTKLEIK

[00474] VK/1-33 variant 2 (Hu4B6_Lb, SEQ ID NO: 55) :

DIQMTQSPSSLSASVGDRVTITCKASQNVGAAVAWYQQKPGKSPKLLIYSAS
NRYTGVPDRFSGSGSGTDFTLTISSLQPEDIATYYCQQYSNYPTFGQGTKLEIK

[00475] cDNAs of the variable regions of the above heavy chains and light chains were synthesized and then fused with the sequences of the constatnt region of human IgG1 and human kappa. The resulting antibody gene sequences were cloned into an expression vector. Large-scale DNA was prepared by using Plasmid Maxiprep System from Qiagen for humanized 4B6 variants expression, as shown in Table 6, and cell transfection was carried out using the ExpiFectamine™ CHO Reagent from Invitrogen according to the manufacturer's protocol. Supernatant was harvested when cell viability was more than 60% and filtered through 0.22um filtration capsule to remove cell debris. The filtered supernatant was subsequently loaded onto a

pre-equilibrated Protein-A affinity column. Protein A resin was washed with equilibration buffer (PBS), and 25 mM citrate (pH3.5) was then used to elute antibody. The purified antibody solution was adjusted to pH 6.0-7.0 by using 1M Tris-base (pH 9.0). The endotoxin was controlled below 1EU/mg. Finally, the purified antibody was characterized by SDS-PAGE.

[00476] Table 6. Expression of humanized 4B6 variants

Humanized sequence combinations for murine antobody 4B6			
	Hu4B6_L0	Hu4B6_La (SEQ ID NO : 54)	Hu4B6_Lb (SEQ ID NO : 55)
Hu4B6_H0			
Hu4B6_Ha (SEQ ID NO : 49)		Hu4B6_HaLa	Hu4B6_HaLb
Hu4B6_Hb (SEQ ID NO : 50)		Hu4B6_HbLa	Hu4B6_HbLb
Hu4B6_Hc (SEQ ID NO : 51)		Hu4B6_HcLa	Hu4B6_HcLb
Hu4B6_Hd (SEQ ID NO : 52)		Hu4B6_HdLa	Hu4B6_HdLb

[00477] NOTE : This table shows various sequence combinations of different mutations. For example, Hu4B6_HaLa indicates that two kinds of mutation (heavy chain Hu4B6_Ha and light chain Hu4B6_La) are present on the humanized murine antibody Hu4B6_HaLa, and so on. Hu4B6_L0 and Hu4B6_H0 are obtained by CDR-grafting, which are lack of the key back mutations, so they are not used for expression.

Example 2: Binding to human PD-L1 by an ELISA assay

[00478] Binding of the humanized antibodies were evaluated by an ELISA method. Briefly, human PD-L1-His was immobilized on the plate. Humanized 4B6 antibodies set forth in Table 6 were serial diluted in PBS and added for 1h incubation. Next, Goat pAb to human IgG-HRP and TMB were added for detection of binding at OD450nm.

[00479] As shown in Figure 1, all humanized variants were tested in order to screen the best one. All the variants retained their binding activity, and Hu4B6_HdLa showed a better binding activity than the others.

[00480] We analyzed the CDR sequence of this antibody and found that there is a NG motif in CDR2 of the heavy chain. There may be a risk of deamination in expression and purification. To remove the deamidation hot spot, we introduced the mutation of G55A (bolded and enlarged below) into the Hu4B6_Hd. Then Hu4B6_Hg (SEQ ID NO: 56) was obtained, and the affinty to human PD-L1 was not affected, as shown in Figure 2.

[00481] Hu4B6_Hg (SEQ ID NO: 56) :

QVQLVQSGAEVKKPGASVKVSCKASGYVFTDYYMNWVRQAPGQSLEWMG
DINPNN**A**GTSYNHKFKGRVTVTVDKSISTAYMELSRLRSDDTAVYYCVKWG
DGPFAYWGQGTLVTVSS

Example 3: Monoclonal phage ELISA and sequence analysis

[00482] Although Hu4B6-HgLa has retained activity from chimeric 4B6, as an antagonist drug, a higher affinity was preferable. Based on the Hu4B6-HgLa sequence, site-directed mutagenesis in the CDRs and several cycles of panning for off-rate-dependent selection in vitro was further used for affinity maturation. First of all, the VL and VH domains of 4B6-HgLa, were amplified and attached by a peptide linker$(G_4S)3$ to form the scFv by overlapping PCR, then subcloned into the phagemid

vector pComb3x(Wuhan MiaoLingBio, P0862), as a wild-type sequence for affinity maturation via SfiI cleavage sites.

[00483] To investigate the individual contributions of CDR1 and CDR2 of both heavy and light chain to 4B6 affinity maturation, one SPM (small perturbation mutagenesis) phage library for each CDR above would be constructed, as antibody CDR3 of both chains usually plays an important role in antigen binding. The CDR1 and CDR2 sequences of both chains were aligned with germline sequences and the germline of variable region of heavy chain and light chain was IGHV1-2 and IGKV1-33, respectively. The bioinformatics analysis results of the germline CDR sequences are used to guide design of the library.

[00484] After determining amino acid mutation sites and substitution sequences, the degenerate primers were designed for increasing diversity of mutation library. The diversified CDR fragment was amplified to construct 4B6 scFv gene mutant library. The scFv genes were ligated with pComb3XSS phage display vector to generate the scFv libraries. The codon-based primers of each CDR (including HCDR1, HCDR2, LCDR1 and LCDR2, listed in Table 2) was established as an independent library, and 4B6 affinity maturation library was divided into 4 libraries. The capacities were 1.76×10^8 CFM for HCDR1, 1.81×10^8 CFM for HCDR2, 2.34×10^8 CFM for LCDR1 and 2.00×10^8 CFM for LCDR2. 5 or 6 clones of each library were picked randomly for sequencing of colony. The results showed that the insertion rate of the constructed library was 100%.

[00485] 10 μg/ml hPD-L1 (Acro Biosystems, PD1-H5229) antigens was coated to the ELISA plate and were reacted with 200 μL of phages (1×10^{10} pfu/ml of phage display library) at 37°C for 1 h. After washing, TG1(Lucigen, 60502-2) with OD600 around 0.5 was added into the well directly for infection and incubated with phage 15 min. Sufficient volume of M13KO7 helper phage (NEB, N0315S) to mid-log phase culture for library phagemid rescue, and the phages were generated and purified for the next round of screening. The screening process was repeated for 3 rounds, and

concentration of antigen was reduced to 2.5 μg/ml for the 2nd round and 1 μg/ml for the 3rd round.

[00486] ELISA binding assay was carried out for detecting titer of these polyclonal phage variants. After 3 rounds of panning, 3 libraries, including 4B6-H-CDR2, 4B6-L–CDR1 and 4B6-L-CDR2, are obviously enriched.

[00487] For these 3 libraries, 96 clones of each library were picked and subjected to phage ELISA to detect their binding activity. Briefly, 1 μg/ml hPD-L1 (Acro Biosystems, PD1-H5229) antigens was coated to the ELISA plate and left overnight at 4°C. Then 300 μL of 3% (w/v) skim milk was added for blocking at room temperature for 1 h. After 1 h, 100 μl of supernatant containing monoclonal antibody fragment phage was added with PBS as a negative control, and incubated at 37°C for 1h. 0.5% PBS+Tween-20 were used for washing for 3 times, and 100 μl HRP coupled anti-M13 mAb (1:20000, Sino Biological, 11973-MM05T-H) was added. After incubation at room temperature for 1h, mixed TMB (InnoReagents, TMB-S-003) substrate reagent was added and the plate was incubated at room temperature for 5 min. 0.1M H_2SO_4 was added for stopping reaction, then OD450 nm was recorded. The positive clones were picked for DNA sequencing by Genewiz (Suzhou, China). The sequences were shown in Table 7.

[00488] Table 7. The sequences of positive clones of 4B6 scFv phage library

AM4B6_Hg		QVQLVQSGAEVKKPGASVKVSCKASGY**V**FTDYYMNWVRQAPGQ**S**L EWMGDINPNN**A**GTSYNHKFKGRVT**V**T**V**D**K**SISTAYMELSRLRSDD TAVYYC**VK**WGDGPFAYWGQGTLVTVSS(SEQ ID NO: 56)
Hu4B6_Hg.2	G57Q, S59Q	QVQLVQSGAEVKKPGASVKVSCKASGY**V**FTDYYMNWVRQAPGQ**S**L EWMGDINPNN**AQ**T**Q**YNHKFKGRVT**V**T**V**D**K**SISTAYMELSRLRSDD TAVYYC**VK**WGDGPFAYWGQGTLVTVSS(SEQ ID NO: 58)
AM4B6_Hg.3	G57E, S59L	QVQLVQSGAEVKKPGASVKVSCKASGY**V**FTDYYMNWVRQAPGQ**S**L EWMGDINPNN**AE**T**L**YNHKFKGRVT**V**T**V**D**K**SISTAYMELSRLRSDD TAVYYC**VK**WGDGPFAYWGQGTLVTVSS(SEQ ID NO: 59)
AM4B6_Hg.5	G57Q, S59V	QVQLVQSGAEVKKPGASVKVSCKASGY**V**FTDYYMNWVRQAPGQ**S**L EWMGDINPNN**AQ**T**V**YNHKFKGRVT**V**T**V**D**K**SISTAYMELSRLRSDD TAVYYC**VK**WGDGPFAYWGQGTLVTVSS(SEQ ID NO: 60)

Hu4B6_La		DIQMTQSPSSLSASVGDRVTITCKASQNVGAAVAWYQQKPGKAPKLLIYSASNRYTGVPSRFSGSGSGTDFT**L**TISSLQPEDIATYYCQQYSNYPTFGQGTKLEIK(SEQ ID NO: 61)
AM4B6_La.1	G30K, A31G	DIQMTQSPSSLSASVGDRVTITCKASQNV**KG**AVAWYQQKPGKAPKLLIYSASNRYTGVPSRFSGSGSGTDFT**L**TISSLQPEDIATYYCQQYSNYPTFGQGTKLEIK(SEQ ID NO: 62)
AM4B6_La.2	G30P	DIQMTQSPSSLSASVGDRVTITCKASQNV**P**AAVAWYQQKPGKAPKLLIYSASNRYTGVPSRFSGSGSGTDFT**L**TISSLQPEDIATYYCQQYSNYPTFGQGTKLEIK(SEQ ID NO: 63)
AM4B6_La.4	A51V, N53D	DIQMTQSPSSLSASVGDRVTITCKASQNVGAAVAWYQQKPGKAPKLLIYS**V**S**D**RYTGVPSRFSGSGSGTDFT**L**TISSLQPEDIATYYCQQYSNYPTFGQGTKLEIK(SEQ ID NO: 64)

Note: the sequences underlined refer to CDR sequences, and the amino acids bolded refer to mutated amino acids.

Example 4: Bio-lay interferometry (BLI) for detection of binding affinity

[00489] Bio-Lay interferometry (BLI) was used for testing the binding affinity of 4B6 scFv variants to human PD-L1-Fc (Sino Biological, 70110-D02H) antigen. The materials and procedure were shown in Table 8 and Table 9, respectively. The results were shown in Tables 10-12. According to the binding affinity results, 4 light chain variants (L-CDR1-2, L-CDR1-3, L-CDR2-2, L-CDR2-3) and 3 heavy chain variants (H-CDR2-2, H-CDR2-3, H-CDR2-5) were selected for future construction.

[00490] Table 8 Samples and materials used in BLI assay

Well Type	Sample ID
Baseline	KD buffer
Load	PD-L1-FC (3.25 μg/ml)
Baseline2	KD buffer
Sample	4B6 scFvs (5.2 μg/ml)
Reference Well	KD buffer
Regeneration	Regeneration buffer
Neutralization	KD buffer

[00491] Table 9 BLI assay procedure

Step Type	Assay Time
Baseline	60s
Loading	180s
Baseline2	120s

Association	180s
Dissociation	240s
Regeneration	5s

[00492] Table 10. Binding affinity ranking of 4B6-L-CDR1 mutation variants

Sample No.	**Sequence**	**K_{on}($\times10^{5}M^{-1}s^{-1}$)**	**K_{off}($\times10^{-3}M^{-1}s^{-1}$)**	**K_{d}($\times10^{-9}M$)**	**Fold increase**
L-CDR1-WT	KASQNVGAAVA (SEQ ID NO: 4)	0.358	1.01	28.2	----
L-CDR1-1	KASQNVGAIVA (SEQ ID NO: 7)	0.412	0.285	6.91	4.08
L-CDR1-2	KASQNVPAAVA (SEQ ID NO: 8)	2.71	1.54	5.68	4.96
L-CDR1-3	KASQNVKGAVA (SEQ ID NO: 9)	0.911	0.253	2.78	10.14

[00493] Table 11. Binding affinity ranking of 4B6-L-CDR2 mutation variants

Sample No.	**Sequence**	**Kon($\times10^{5}M^{-1}s^{-1}$)**	**Koff($\times10^{-3}M^{-1}s^{-1}$)**	**Kd(×10-9M)**	**Fold increase**
L-CDR2-WT	SASNRYT (SEQ ID NO: 5)	0.381	1.05	27.6	----
L-CDR2-1	SNSHRYT (SEQ ID NO: 10)	0.468	0.377	8.06	3.42
L-CDR2-2	SRSVRYT (SEQ ID NO: 11)	1.62	0.540	3.34	8.26
L-CDR2-3	SVSDRYT (SEQ ID NO: 12)	1.24	0.543	4.37	6.32

[00494] Table 12. Binding affinity ranking of 4B6-H-CDR2 mutation variants

Sample No.	**Sequence**	**K_{on}($\times10^{5}M^{-1}s^{-1}$)**	**K_{off}($\times10^{-3}M^{-1}s^{-1}$)**	**K_{d}($\times10^{-9}M$)**	**Fold increase**
H-CDR2-WT (G55A)	DINPNNAGTSYNHKFKG (SEQ ID NO: 18)	0.463	1.24	26.7	----

H-CDR2-1	DINPNNADTMYNHKFKG (SEQ ID NO: 13)	1.41	0.495	3.52	7.6
H-CDR2-2	DINPNNAQTQYNHKFKG (SEQ ID NO: 14)	1.01	0.244	2.42	11.03
H-CDR2-3	DINPNNAETLYNHKFKG (SEQ ID NO: 15)	1.16	0.283	2.43	10.99
H-CDR2-4	DINPNNGLTSYNHKFKG (SEQ ID NO: 16)	1.17	0.517	4.43	6.03
H-CDR2-5	DINPNNAQTVYNHKFKG (SEQ ID NO: 17)	1.02	0.245	2.40	11.13

Example 5: Construction and expression of AM-4B6-hIgG1-TGFβRII (1-136) fusion protein

[00495] The selected heavy chain and light chain variants were cross combined and expressed with hIgG1-TGFβRII (1-136) fusion protein. The TGFβRII (1-136) has an amino acid sequence set forth in SEQ ID NO: 79:

TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS
ICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKK
KPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

The amino acid sequence of hIgG1 is as follows (SEQ ID NO: 80):

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK

The TGFβRII (1-136) was linked to the carboxyl terminus of the hIgG1 via a peptide linker $(G4S)_4G$ (SEQ ID NO: 68).

The amino acid sequence of hKappa is as follows (SEQ ID NO: 82):

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC

[00496] The intact antibody variants were prefixed by AM (affinity matured). The sequence construct of heavy chain and light chain was shown in Table 13 and the design of intact antibody was shown in Table 14. For example, as shown in Table 13 and Table 14, the bi-functional molecule "AM-4B6-hIgG1-TGFβRII variant 1" has a heavy chain and a light chain, where the heavy chain from N terminus to C terminus includes: Hu4B6_Hg.2 -_hIgG1 -(G4S)4G- TGFβRII (1-136); and the light chain from N terminus to C terminus includes: Hu4B6_La.1 – hKappa. The same nomenclature applies to the other variants in Table 14.

[00497] The co-transfection of heavy chain and light chain was carried out using the ExpiFectamine™ CHO Reagent (Thermo, A29129) from Invitrogen according to the manufacturer's protocol. The supernatant was harvested on day 10 and purified by affinity chromatography.

[00498] Table 13 The list of AM-4B6-hIgG1-TGF RII heavy chain and light chain variants

Name	Mutation site	Region	Sequence
Hu4B6_Hg.2_hIgG1 -(G4S)4G- TGFβRII	G57Q, S59Q	H-CDR2-2	DINPNNAQTQYNHKFKG
Hu4B6_Hg.3_hIgG1 -(G4S)4G- TGFβRII	G57E, S59L	H-CDR2-3	DINPNNAETLYNHKFKG
Hu4B6_Hg.5_hIgG1 -(G4S)4G- TGFβRII	G57Q, S59V	H-CDR2-5	DINPNNAQTVYNHKFKG
Hu4B6_La.1_hKappa	G30K, A31G	L-CDR1-3	KASQNVKGAVA
Hu4B6_La.2_hKappa	G30P	L-CDR1-2	KASQNVPAAVA
Hu4B6_La.4_hKappa	A51V, N53D	L-CDR2-2	SRSVRYT
Hu4B6_La.6_hKappa	A51R, N53V	L-CDR2-3	SVSDRYT

[00499] Table 14. The list of AM-4B6-hIgG1-TGFβRII antibody variants

Sample No.	Heavy chain	Light chain

AM-4B6-hIgG1-TGFβRII variant 1	Hu4B6_Hg.2 (SEQ ID NO: 58)	Hu4B6_La.1 (SEQ ID NO: 62)
AM-4B6-hIgG1-TGFβRII variant 2		Hu4B6_La.2 (SEQ ID NO: 63)
AM-4B6-hIgG1-TGFβRII variant 3		Hu4B6_La.4 (SEQ ID NO: 64)
AM-4B6-hIgG1-TGFβRII variant 4		Hu4B6_La.6 (SEQ ID NO: 66)
AM-4B6-hIgG1-TGFβRII variant 5	Hu4B6_Hg.3 (SEQ ID NO: 59)	Hu4B6_La.1 (SEQ ID NO: 62)
AM-4B6-hIgG1-TGFβRII variant 6		Hu4B6_La.2 (SEQ ID NO: 63)
AM-4B6-hIgG1-TGFβRII variant 7		Hu4B6_La.4 (SEQ ID NO: 64)
AM-4B6-hIgG1-TGFβRII variant 8		Hu4B6_La.6 (SEQ ID NO: 65)
AM-4B6-hIgG1-TGFβRII variant 9	Hu4B6_Hg.5 (SEQ ID NO: 60)	Hu4B6_La.1 (SEQ ID NO: 62)
AM-4B6-hIgG1-TGFβRII variant 10		Hu4B6_La.2 (SEQ ID NO: 63)
AM-4B6-hIgG1-TGFβRII variant 11		Hu4B6_La.4 (SEQ ID NO: 64)
AM-4B6-hIgG1-TGFβRII variant 12		Hu4B6_La.6 (SEQ ID NO: 65)

Example 6: Binding affinity of AM-4B6-hIgG1-TGFβRII variants to hPD-L1

[00500] Binding to hPD-L1 by an ELISA assay

[00501] 1 μg/ml hPD-L1 (Acro Biosystems, PD1-H5229) antigens was coated to the ELISA plate and left overnight at 4°C. Then 300 μl of 3% (w/v) skim milk was added for blocking at room temperature for 1 h. After 1 h, 100 μl of

AM-4B6-hIgG1-TGFβRII variants or original 4B6-hIgG1-TGFβRII at concentrations ranging from 100 nM to 0.006 nM (four-fold serial dilutions) were added with PBS as a negative control, and incubated at room temperature for 1 h. 0.5% PBS+Tween-20 were used for washing for 3 times, and 100 μl HRP-conjugated anti-human Fc antibody (1:20000,Abcam, ab98624) was added After incubation at room temperature for 1 h, mixed TMB substrate reagent (InnoReagents, TMB-S-003) was added and incubated at room temperature for 5 min, and then stopped by adding 0.1M H_2SO_4. OD450 nm was recorded by Microplate Reader.

[00502] As shown in Figure 3A - Figure 3C, when compared to the original 4B6-hIgG1-TGFβRII, all of the variants had enhanced binding signals and affinities. Given the limited differences between these variants, Surface Plasmon Resonance technology was used for further evaluation of their binding affinity.

[00503] **Binding to hPD-L1 by Biacore**

[00504] 4 μg/ml AM-4B6-hIgG1-TGFβRII variants or original 4B6-hIgG1-TGFβRII were immobilized on the surface of S series Protein A chip. The human PD-L1 was diluted to an appropriated concentration gradient (0 nM, 1.875 nM, 3.75 nM, 7.5 nM, 15 nM, 30 nM, 60 nM) and injected into the sample channel of Biacore 2000. The results are shown in Table 15. The binding affinity of AM-4B6-hIgG1-TGFβRII variant 7 to human PD-L1 was improved about 15 folds than that of original 4B6-hIgG1-TGFβRII.

[00505] **Table 15. Binding affinity of AM-4B6-hIgG1-TGFβRII to hPD-L1 by Biacore**

Analyte	Sample NO.	Sample name	ka(1/Ms)	kd(1/s)	KD(M)
PD-L1 his	1	AM-4B6-hIgG1-TGFβRII variant 5	2.060E+5	1.487E-4	7.218E-10
	2	AM-4B6-hIgG1-TGFβRII variant 7	3.815E+5	1.479E-4	3.876E-10
	3	AM-4B6-hIgG1-TGFβRII variant 2	3.231E+5	1.676E-4	5.189E-10
	4	AM-4B6-hIgG1-TGFβRII variant 10	2.444E+5	1.476E-4	6.039E-10
	5	AM-4B6-hIgG1-TGFβRII variant 11	2.688E+5	1.366E-4	5.083E-10

	6	4B6-hIgG1-TGFβRII	2.107E+5	0.001156	5.489E-9

[00506] Binding to PD-L1 expressing on cell surface by a FACS assay

[00507] 293T-PD-L1-CD3L cell was generated by MabSpace Bioscience for characterization of PD-L1 antibodies. The cell was transfected with both human PD-L1 and anti-CD3 scFv. AM-4B6-hIgG1-TGFβRII variants or original 4B6-hIgG1-TGFβRII were serially diluted (5-fold dilutions) to obtain 8 concentrations in dilution buffer (PBS with 2%BSA). 293T-PD-L1-CD3L cells were harvested and centrifuged. They were resuspended in PBS with a density of 2×10^6 cells/ml and then added to the plate with 100 ul per well. After centrifugation and removal of supernatant，the diluted antibodies were added to the plate and incubated in 4°C for 30min. After washing twice with dilution buffer, PE conjugated donkey anti-human IgG(H+L)(Jacksonimmuno, 709-116-149) was added to the plate and incubated in 4°C for 30 min. After washing, cells were resuspended in 200 μl PBS and analyzed by flow cytometry.

[00508] As shown in Figure 4, these 5 variants bound to PD-L1 expressed on surface of 293T-PD-L1-CD3L cells with a similar EC50. Variant 7 had a slight lower EC50 than others, which was consistent with the binding affinity results measured by Biacore.

Example 7: PD-1/PD-L1 blockade activity of AM-4B6-hIgG1-TGFbRII variants

[00509] PD-1/PD-L1 and B7-1/PD-L1 blockade by an ELISA assay

To test ligand/receptor blocking activity, 0.5 μg/ml hPD-L1-Fc antigen was coated to the ELISA plate and left overnight at 4°C. 300 μL blocking buffer was added for blocking at room temperature for 1 h. After 1 h, 50 μl of AM-4B6-hIgG1-TGFβRII variant 7 or original 4B6-hIgG1-TGFβRII at concentrations ranging from 100 nM to 0.024 nM (four-fold serial dilutions) with 50 μl PD-L1-his, concentration of which is 1 μg/ml, were added and incubated at room temperature for 1 h. 0.5% PBS+Tween-20 were used for washing for 3 times, and 100 μl HRP-conjugated streptavidin (1:5000,

Abcam, cat#ab7403) was added. After incubation at room temperature for 1.5 h, mixed TMB substrate reagent was added and incubated at room temperature for 5 min, then stopped by adding 0.1 M H_2SO_4. OD450nm was recorded by Microplate Reader. As shown in Figure 5A - Figure 5B, both samples could block PD-L1/PD-1 or PD-L1/B7-1 while AM-4B6-hIgG1-TGFβRII variant 7 with a lower IC50, indicating variant 7 has the improved activity.

[00510] PD-1/PD-L1 blockade by a cell-based assay

[00511] In this assay, 293T-PD-L1-CD3L cell expresses PD-L1 and anti-CD3 scFv and Jurkat-NFAT-Luc-PD1 cell expresses PD-1 and carrying NFAT signal which can be activated with CD3 stimulation. NFAT activation leads to downstream luciferase gene transcription and expression, which can be detected by its substrate. The two cells were generated by MabSpace Bioscience.

[00512] Briefly, 293T-PD-L1-CD3L cells were harvested and resuspended at a density of 2×10^6 cells/ml. 20 μl cells per well were added into half well plate. AM-4B6-hIgG1-TGFβRII variants or original 4B6-hIgG1-TGFβRII were serially diluted (3-fold dilutions) to obtain 8 concentrations in RPMI medium with 2%FBS. 20 μl antibodies per well were added into half well plate, and the plate was incubated at 37°C, 5% CO_2 for 30min. Jurkat-NFAT-Luc-PD1 cells were harvested and resuspended at a density of 4×10^6 cells/ml in RPMI medium with 2%FBS. Finally, 20 μl cells per well with 5 ng/ml TGF-beta (R&D, 240-B-010) were added into half well plate and incubated in 37°C, 5% CO_2 for 5 h. 60 μl OneGlo detection reagent (Promega, E6120) was added to each well and incubated at room temperature for 5 minutes. The luminescent signal was read by Microplate Reader. The data was analyzed by GraphPad Prism.

[00513] As shown in Figure 6, consistent with the previous ELISA results, variant 7 had the most potent blockade activity in this cell-based assay as compared with the other variants. Therefore, the 4B6 Fab part of AM-4B6-hIgG1-TGFβRII

variant 7 was abbreviated as AM4B6, and AM4B6-hIgG1-TGFβRII fusion protein was further evaluated in the following experiments.

Example 8: Generation and characterization of AM4B6-hIgG1-TGFβRII' *in vitro*

[00514] AM4B6-hIgG1-TGFβRII cloning and expression

[00515] It was reported that the truncated TGFβRII ECD_20-136 was soluble and retained the ability to bind TGFβ1 (Kim-Ming Lo, et al, US9676863 B2, 2017; Christian C., et al), Protein Expression and Purification, 2000, 20: 98–104). Next, we replaced the full length of extracellular domain of TGFβRII_1-136 with the truncated one and evaluated the developability and stability. The SDS-PAGE results from the stable cell line showed that the protein expression is good for both truncated and full-length TGFβRII ECD, but the protein stability is much better for truncated TGFβRII ECD_20-136 than full length TGFβRII ECD (Figure 7). The sequences of the truncated TGFβRII ECD_20-136 are as below:

[00516] Sequence of stable TGF-β trap, TGF-βRII extracellular domain (20-136)

GAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCH
DPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
(SEQ ID NO : 66)

[00517] The bi-functional molecules comprising the truncated TGF-βRII (i.e. TGF-βRII (20-136), SEQ ID NO: 66) was used in this example and also in Examples 9-11. Such bi-functional molecules' names were indicated with TGFβRII', to distinguish from the TGF-βRII(1-136). For example, AM4B6-hIgG1-TGFβRII' indicates a molecule having TGF-βRII(20-136).

[00518] Binding to PD-L1 from various species by an ELISA assay

[00519] 1 μg/ml human PD-L1 (Acro Biosystems, PD1-H5229) or cyno PD-L1 antigens were coated to the ELISA plate and left overnight at 4°C. Then 300 μl of 3%(w/v) skim milk was added for blocking at room temperature for 1 h. After 1 h, 100 μl of AM4B6-hIgG1-TGFβRII' or control hIgG1-TGFβRII' at concentrations ranging from 100 nM to 0.02 nM (four-fold serial dilutions) were added, and incubated at room temperature for 1 h. 0.5% PBS+Tween-20 were used for washing for 3 times, and 100 μl HRP-conjugated anti-human Fc antibody (1:20000,Abcam, ab98624) was added, After incubation at room temperature for 1 h, mixed TMB substrate reagent (InnoReagents, TMB-S-003) was added and incubated at room temperature for 5 min, and stopped by adding 0.1M H_2SO_4. OD450 nm was recorded by Microplate Reader.

[00520] As shown in Figure 8A and Figure 8B, AM4B6-hIgG1-TGFβRII' cross-reacted with cyno PD-L1 with a similar EC50 to that of human PD-L1.

[00521] **Binding to TGFβ1, TGFβ2 and TGFβ3 from various species by an ELISA**

[00522] According to the sequences of TGFβ1, TGFβ2 and TGFβ3 from 4 common species: human, cynomolgus, mouse and rat, which were published on the Uniport website (https://www.uniprot.org/), TGFβ members are quite conservative. The sequences of human TGFβ1 and cynomolgus TGFβ1 are identical; mouse TGFβ1 and rat TGFβ1 are identical; human TGFβ2 and cynomolgus TGFβ2 are identical; mouse TGFβ2 and rat TGFβ2 are identical; human TGFβ3, cynomolgus TGFβ3 and mouse TGFβ3 are identical.

[00523] For TGFβ1 and TGFβ3, the procedure is as below: 0.5 μg/ml human TGFβ1 (Sino Biological, 10804-HNAC) or Mouse TGFβ1 (Novoprotein, CK33) or Human TGFβ3 (Genscript, Z03430) or Rat TGFβ3 (Novoprotein, CJ44) antigens were coated to the ELISA plate and left overnight at 4°C. Then 300 μl of 3%(w/v) skim milk was added for blocking at room temperature for 1 h. After 1 h, 100 μl of

AM4B6-hIgG1-TGFβRII' or control hIgG1-TGFβRII' at concentrations ranging from 100 nM to 0.006 nM (four-fold serial dilutions) were added, and incubated at room temperature for 1 h. 0.5% PBS+Tween-20 were used for washing for 3 times, and 100 μl HRP-conjugated anti-human Fc antibody (1:20000, Abcam, ab98624) was added, After incubation at room temperature for 1 h, mixed TMB substrate reagent (InnoReagents, TMB-S-003) was added and incubated at room temperature for 5 min, and stopped by adding 0.1M H_2SO_4. OD450 nm was recorded by Microplate Reader.

[00524] For TGFβ2, the test procedure is different: 2 μg/ml AM4B6-hIgG1-TGFβRII' or control hIgG1-TGFβRII' were coated to the ELISA plate and left overnight at 4°C. Then 300 μl of 3%(w/v) skim milk was added for blocking at room temperature for 1 h. After 1 h, 100 μl of human TGFβ2 or mouse TGFβ2 at concentrations ranging from 39.4 nM to 0.3 nM (two-fold serial dilutions) were added, and incubated at room temperature for 1 h. 0.5% PBS+Tween-20 were used for washing for 3 times, and 100 μl TGFβ2 Biotinylated antibody (1:10000, R&D, BAF302) was added. After incubation at room temperature for 1 h and washing, 100 μl HRP-streptavidin (1:5000, Abcam, ab7403) was added and the plate was incubated at room temperature for 1 h. After washing, mixed TMB substrate reagent (InnoReagents, TMB-S-003) was added and incubated at room temperature for 5 min and stopped by adding 0.1M H_2SO_4. OD450 nm was recorded by Microplate Reader.

[00525] Results were summarized in Table 16. For binding affinity to TGFβ1, the EC50 values were quite similar among different species. Furthermore, the binding affinity to TGFβ1 and TGFβ3 was significantly higher than TGFβ2, indicating the blocking activity to TGFβ1 and TGFβ3 may be more potent than TGFβ2.

[00526] Table 16. Binding of AM4B6-hIgG1-TGFβRII' to TGFβ1, TGFβ2 and TGFβ3

Binding to TGFβ1, TGFβ2 and TGFβ3 from various species by ELISA (EC_{50}, nM)

TGFβ1		TGFβ2		TGFβ3	
Human	Mouse	Human	Mouse	Human	Rat
0.748	0.800	8.459	8.884	5.257	3.733

[00527] Binding to other members within the B7 family or TGFβ superfamily by an ELISA assay

[00528] For B7 family, 0.5 μg/ml hPD-L1 (Acro Biosystems, PD1-H5229) or hPD-L2 or B7-2 or B7-1 or B7-H2 or B7-H3 or B7-H4 or VISTA were coated to the ELISA plate and left overnight at 4°C. For TGFβ superfamily, 0.5 μg/ml human Activin A, BMP-2, LAP or TGFβ1 were coated at 4°C overnight. Then 300 μl of 3%(w/v) skim milk was added for blocking at room temperature for 1 h. After 1 h, 100 μl of AM4B6-hIgG1-TGFβRII' at concentrations ranging from 100 nM to 0.006 nM (serial diluted) were added, and incubated at room temperature for 1 h. 0.5% PBS+Tween-20 were used for washing for 3 times, and 100 μl HRP-conjugated anti-human Fc antibody (1:20000,Abcam, ab98624) was added, After incubation at room temperature for 1 h, mixed TMB substrate reagent (InnoReagents, TMB-S-003) was added and incubated at room temperature for 5 min, and stopped by adding 0.1M H_2SO_4. OD450 nm was recorded by Microplate Reader.

[00529] As shown in Figure 9A - Figure 9B, AM4B6-hIgG1-TGFβRII' specifically bound to PD-L1 rather than the other antigens that also belong to the B7 family. As shown in Figure 9C, AM4B6-hIgG1-TGFβRII' specifically bound to TGF-β1 rather than the other antigens that also belong to the TGFβ superfamily.

[00530] Binding to PD-L1 expressing cells of AM4B6-hIgG1-TGFβRII'

[00531] MC38/hPD-L1 was generated by deleting mPD-L1 via CRISPR-Cas9 system, followed by transduction of hPD-L1 using lenti-virus. This cell line was a courtesy of Professor Qin Xiaofeng's laboratory at the Center of Systems Medicine, Chinese Academy of Medical Sciences Suzhou Institute of Systems Medicine (Huang, Anfei, et al. Scientific Reports 7 (2017): 42687.). MC-38/hPD-L1 cells were cultured

in RPMI1640+10%FBS. EMT-6/hPD-L1, is a mouse breast cancer cell line that stably expresses transfected human PD-L1 gene. EMT-6/hPD-L1 cells were cultured in Waymouth's (1 x) MB752/1 +15% FBS. NCI-H460 cells were purchased from COBIOER Ltd. It's a human lung epithelial tumor cell line with PD-L1 expression. NCI-H460 cells were cultured in RPMI1640+10%FBS. NCI-H292 cells were purchased from COBIOER Ltd. It's a human lung epithelial tumor cell line with PD-L1 expression. NCI-H292 cells were cultured in RPMI1640+10%FBS+ 1nM sodium pyruvate solution. The protocol of FACS analysis was the same with Example 6 section 3.

[00532] Human or cynomolgus PBMC (TPCS, Cat#PB025C) was recovered from liquid nitrogen and resuspended in RPMI1640 with 10%FBS. 5μg/ml PHA (Sigma, Cat# L8902) was added to stimulate PBMC activation and cells were cultured for 3 days. Activated PBMC were harvested and centrifuged and resuspended in PBS with density of 2×10^6 cells/ml and added to the plate with 100 ul per well. AM4B6-hIgG1-TGFβRII' or AM4B6 or control hIgG1-TGFβRII' were serially diluted (5-fold dilutions) to obtain 10 concentrations in dilution buffer (PBS with 2%BSA). After centrifugation and removal of the supernatant in the plate, the diluted antibodies were added to the plate with the activated PBMC and incubated in 4°C for 1 hour. After washing twice with dilution buffer, Alexa488-labeled mouse anti-human CD3 (Biolegend, Cat#300320) and APC-labeled anti-human IgG secondary antibody (BD, Cat#550931) were added and incubate at 4°C for 30 mins. After washing, cells were resuspended in 150 μl PBS and analyzed by flow cytometry.

[00533] As shown in Figure 10A - Figure 10F, AM4B6-hIgG1-TGFβRII' could bind to PD-L1 expressed on these cancer cell lines and the activated human or cynomolgus T cells with the similar affinity to AM4B6 mAb alone.

[00534] Binding to activated human T cells

[00535] Human PBMC (TPCS, Cat#PB025C) was recovered from liquid nitrogen and resuspended in RPMI1640 with 10%FBS. 5μg/ml PHA (Sigma, Cat# L8902) was added to stimulate PBMC activation and cells were cultured for 3 days. Activated PBMC were harvested and centrifuged and resuspended in PBS with density of 2×10^6 cells/ml and added to the plate with 100 ul per well. AM4B6-hIgG1-TGFβRII' or AM4B6 or control hIgG1-TGFβRII' were serially diluted (5-fold dilutions) to obtain 10 concentrations in dilution buffer (PBS with 2%BSA). After centrifugation and removal of the supernatant in the plate, the diluted antibodies were added to the plate with the activated PBMC and incubated in 4°C for 1 hour. After washing twice with dilution buffer, Alexa488-labeled mouse anti-human CD3 (Biolegend, Cat#300320) and APC-labeled anti-human IgG secondary antibody (BD, Cat#550931) were added and incubate at 4°C for 30 mins. After washing, cells were resuspended in 150 μl PBS and analyzed by flow cytometry.

[00536] As shown in Figure 11, AM4B6-hIgG1-TGFβRII' could bind to PD-L1 expressed on the activated human T cells.

[00537] **Blockade of hPD-L1/hPD-1 and cynoPD-L1/cynoPD-1 by an ELISA assay**

[00538] 0.5 μg/ml hPD-L1-Fc or 0.5 μg/ml cynoPD-L1-Fc was coated to the ELISA plate and left overnight at 4°C. 300 μL of 3%(w/v) skim milk was added for blocking at room temperature for 1 h. After 1 h, 100 μl of AM4B6-hIgG1-TGFβRII' or AM4B6 at concentrations ranging from 100 nM to 0.02 nM (four-fold serial dilutions) with 0.5 μg/ml hPD-1-Fc-biotin or cyno PD-1-Fc-biotin were added and incubated at room temperature for 1 h. 0.5% PBS+Tween-20 were used for washing for 3 times, and 100 μl HRP-conjugated streptavidin (1:5000) was added. After incubation at room temperature for 1 h, mixed TMB substrate reagent was added and incubated at room temperature for 5 min, then stopped by adding 0.1 M H_2SO_4. OD450nm was recorded by Microplate Reader.

[00539] As shown in Figure 12A - Figure 12B, AM4B6-hIgG1-TGFβRII' could also completely block cyno PD-L1/cyno PD-1 with a similar IC50 to that of blocking human PD-L1/human PD-1.

[00540] **Simultaneously binding to hPD-L1 and hTGFβ1**

[00541] 0.5 μg/ml hTGFβ-1 was coated to the ELISA plate and left overnight at 4°C. Then 300 μl of blocking buffer was added for blocking at room temperature for 1 h. After 1 h, 100 μl of AM4B6-hIgG1-TGFβRII' or AM4B6 or control hIgG1-TGFβRII' at concentrations ranging from 100 nM to 0.02 nM (four-fold serial dilutions) were added, and incubated at room temperature for 1 h. 0.5% PBS+Tween-20 were used for washing for 3 times, and then 0.5 μg/ml hPD-L1-biotin was added into each well. 1h later, 100 μl HRP-conjugated streptavidin (1:5000) was added. After incubation at room temperature for 1 h, mixed TMB substrate reagent (InnoReagents, TMB-S-003) was added and incubated at room temperature for 5 min, and stopped by adding 0.1M H_2SO_4. OD450 nm was recorded by Microplate Reader.

[00542] As shown in Figure 13, AM4B6-hIgG1-TGFβRII', which was composed of anti-PD-L1 antibody AM4B6 and TGFβRII', could bind the two targets at the same time, indicating its bispecific or bifunctional character.

[00543] **Blockade of hPD-L1/hPD-1 by a reporter cell assay**

[00544] Protocol was the same as Example 15 section 2. As shown in Figure 14, AM4B6-hIgG1-TGFβRII could block the inhibition effect of PD-L1/PD-1 and subsequently reverse the signaling activation, same as AM4B6 mAb alone.

[00545] **Blockade of TGFβ1 signaling by a reporter cell assay**

[00546] TGFβ reporter HEK-293 cell line was purchased from Genomeditech (Cat: GM-C05346) and cultured in DMEM media containing 10% FBS, 4 μg/ml blasticidin, 400 μg/ml neomycin, 125μg/ml hygromycin, 0.75 μg/ml puromycin, and 1% Pen/Strep in 37 °C incubator with 5% carbon dioxide.

[00547] Cells were collected in the log-growth phase and resuspended in DMEM media and planted in 96-well plate in density of 2×10^4 cells/100 μl per well. After cells were cultured overnight, the medium was replaced with 75 μl of culture media containing 10 ng/ml of human TGFβ1. 75 μl of AM4B6-hIgG1-TGFβRII' or AM4B6 were added at the final concentration of ranging from 100 nM to 0.02 nM (three-fold serial dilutions). The plate was incubated at 37 °C incubator for 16 hours. The ONE-GloTM luciferase assay system was added at 150 μl/well and after incubation at room temperature for 10 minutes, the plate was read with the microplate reader.

[00548] As shown in Figure 15, AM4B6-hIgG1-TGFβRII' displayed a potent blocking activity on TGFβ1 signaling with an IC50 of 0.35nM, while AM4B6 mAb alone had no blocking activity, indicating the blocking activity is TGFβ1 specific.

[00549] Effect of AM4B6-hIgG1-TGFβRII' on IFN-γ release of PBMC stimulated by tuberculin (TB)

[00550] Human PBMC was recovered from liquid nitrogen and resuspend the cells at density of 2×10^6/mL. Add TB to a final concentration of 1.33μg/mL; cultured at 37°C for 5 days. On the sixth day, the induced PBMC were collected and centrifuged, washed once with PBS, resuspended in fresh medium, adjusted to a density of 1×10^6/ml, and seeded into a 96-well cell plate, 180μL/well. Add diluted antibodies to the 96-well cell culture plate, 20μL/well. Control group and blank group were added with 20μL PBS. Cell culture plates were incubated at 37°C for 3 days in a 5% CO_2 incubator. At the end of incubation, the cell supernatant was diluted 10-fold, and the secretion level of IFNγ was detected with an IFN-γ ELISA detection kit (R&D, DY285B).

[00551] As shown in Figure 16, AM4B6-hIgG1-TGFβRII' induced a significantly higher level of IFN-γ release than AM4B6 mAb alone, indicating its activation activity is more potent due to its bispecific binding and blocking activity.

[00552] ADCC/CDC activity of AM4B6-hIgG1-TGFβRII'

[00553] For ADCC assay, the effector cell: Jurkat-NFAT Luc-FcγRIIIa-158V cell line was constructed by Mabspace Biosciences (Suzhou) Co., Limited. The target cell: HEK-293T-hPD-L1 cells (purchased from Crown Biosciences Inc., Cat: 2005).

[00554] HEK-293T-hPD-L1 cells were added to the cell culture plates at 10,000 cells/12.5 μl per well. AM4B6-hIgG1-TGFβRII' dilutions at final concentrations ranging from 200nM to 0.003nM were then added at 12.5 μl/well. The plates were then placed in the incubator at 37 °C to allow the antibody and cells incubation for 30 minutes. Then Jurkat-NFAT Luc-FcγRIIIa-158V cells were added to the wells at 60,000 cells/25 μl per well. The plates were then placed in the incubator at 37 °C for 6 hours. The ONE-GloTM luciferase assay system was added at 50 μl/well and after incubation at room temperature for 10 minutes, the plate was read with the microplate reader.

[00555] For CDC assay, the target cell is also HEK-293T-hPD-L1 cells. HEK-293T-hPD-L1 cells were added to the cell culture plates at 10,000 cells/25 per well. AM4B6-hIgG1-TGFβRII' dilutions at final concentrations ranging from 200nM to 0.3nM were then added at 12.5 μl/well. The plates were then placed in the incubator at 37 °C to allow the antibody and cells incubation for 30 minutes. The HEK-293T-hPD-L1 cells were treated with 40% complements at 50 μl/well (final concentration is 20%), then incubated at 37 °C for 80 min. The ONE-GloTM luciferase assay system was added at 100 μl/well and after incubation at room temperature for 10 minutes, the plate was read with the microplate reader.

[00556] The results suggested that AM4B6-hIgG1-TGFβRII' had neither ADCC nor CDC activity on HEK-293T-hPD-L1 cells (data not shown).

Example 9: Efficacy of AM4B6-hIgG1-TGFβRII' *in vivo*

[00557] MC38-hPD-L1 tumor model on C57BL/6 mice

[00558] Endogenous mouse PD-L1 in mouse tumor cell line MC38 (ATCC) was knocked out using a highly efficient CRISPR/Cas9 system we recently developed. Briefly, sgRNA targeting the first coding exon of mouse PD-L1 gene was designed, and the cells were transfected by hit-and-run CRISPR/Cas9+sgRNA constructs and selected for knock out cells. The cells with complete knock out of endogenous mouse PD-L1 were identified by FACS analysis for cell surface expression of PD-L1 in steady state or stimulated by interferon gamma, and subsequently verified by TA cloning and sequencing of the targeted genomic region. To generate human PD-L1 replacement cell line, the coding sequence of human PD-L1 cDNA was cloned into a FG12 derived lentiviral vector. The mouse PD-L1 knock out cells were then infected with the human PD-L1 expressing lentivirus, and a high level and stable expression of human PD-L1 in the established cell line was confirmed by FACS analysis. This engineered cells of MC38 was named as MC38-hPD-L1.

[00559] MC38-hPD-L1 cells were maintained *in vitro* as a monolayer culture in RPMI1640 medium supplemented with 10% heat inactivated fetal bovine serum at 37°C in an atmosphere with 5% CO_2 in air. The tumor cells growing in an exponential growth phase were harvested and counted for tumor inoculation. Each female SPF grade C57BL/6 mouse was inoculated with mixed 2×10^6 MC38-hPD-L1 cells with 50% matri-gel. When the tumor size around 90mm^3, tumor bearing mice were selected and randomized to 5 groups (n=8). Animals were treated with 2.5mg/kg isotype control, 3mg/kg isotype control-TGFβRII', 2.5mg/kg AM4B6, 0.3mg/kg AM4B6-hIgG1-TGFβRII', 1mg/kg AM4B6-hIgG1-TGFβRII' and 3mg/kg AM4B6-hIgG1-TGFβRII'. All the antibodies were administrated twice a week for 4 weeks by i.p. injection. Tumor size was measured twice or triple times a week in two dimensions using a caliper (INSIZE) and the volume was expressed in mm^3 using the formula: V=0.5 a×b^2 where a and b ate the long and shirt diameters of the tumor, respectively. Results were analyzed using Prism GraphPad and expressed as mean±S.E.M. Comparisons between two groups were made by T-test, and the difference is considered significant if p is *<0.05 and **<0.01.

[00560] As shown in Figure 17A- Figure 17B, 3mg/kg isotype control-TGFβRII' didn't inhibit tumor growth at all, indicating TGFβRII' alone had little efficacy. 2.5mg/kg AM4B6 had only partial inhibition effect, similar to that of 0.3mg/kg AM4B6-hIgG1-TGFβRII', which seemed not sufficient to control tumor growth. With the increase of dosage, the tumor volumes were getting smaller and smaller. 3mg/kg AM4B6-hIgG1-TGFβRII' almost completely stopped the tumor growth, with 84% TGI (Table 17).

[00561] Table 17. Tumor Growth Inhibition (TGI) of antibodies in MC38-hPD-L1 tumor model on Day 32 (mean±S.E.M., n=8)

Treatment	Tumor size (±SEM, mm^3)	TGI (%)	p value vs. Isotype Control
Isotype Control 2.5 mg/kg	3877.77±712.67		
Isotype control-TGFβRII' 3 mg/kg	3489.60±880.53	10.01	0.6713
AM4B6 2.5 mg/kg	2105.51±443.56	45.70	0.0183
AM4B6-hIgG1-TGFβRII' 0.3 mg/kg	2411.61±742.60	37.81	0.0931
AM4B6-hIgG1-TGFβRII' 1 mg/kg	1646.12±517.06	57.55	0.0063
AM4B6-hIgG1-TGFβRII' 3 mg/kg	601.31±197.10	84.49	0.00006

[00562] H460 tumor and human PBMC co-inoculated model on NOD-SCID mice

[00563] H460 cell was purchased from COBIOER Ltd. H460 cells were maintained *in vitro* as a monolayer culture in RPMI1640 medium supplemented with 10% heat inactivated fetal bovine serum at 37°C in an atmosphere with 5% CO_2 in air. The tumor cells growing in an exponential growth phase were harvested and counted for tumor inoculation. Each female SPF grade NOD-SCID mouse was inoculated with mixed 3×10^6 H460 cells (Model group) or 3×10^6 H460 cells mixed with 1.5×10^6 human PBMC. All the cell suspension was mixed well with Matrigel as 1:1 ratio before inoculation. Approximately 4 hours after inoculations, animals were grouped to 7 groups (n=10) and dosed differently. Animals were treated with either 16.7mg/kg

control hIgG1, or 20mg/kg control hIgG1-TGFβRII', or 16.7mg/kg AM4B6, or 5mg/kg AM4B6-hIgG1-TGFβRII', or 10mg/kg AM4B6-hIgG1-TGFβRII', or 20mg/kg AM4B6-hIgG1-TGFβRII'. Model group was treated with nothing. All the antibodies were administrated twice a week for 5 weeks by i.p. injection. Tumor size was measured twice or triple times a week in two dimensions using a caliper (INSIZE) and the volume was expressed in mm^3 using the formula: V=0.5 a×b^2 where a and b are the long and short diameters of the tumor, respectively. Results were analyzed using GraphPad Prism and expressed as mean±S.E.M. Comparisons between two groups were made by T-test, and the difference is considered significant if p is *<0.05 and **<0.01.

[00564] As shown in Figure 18A - Figure 18B, 20mg/kg isotype control-TGFβRII' didn't inhibit tumor growth at all. 16.7mg/kg AM4B6 had only partial inhibition effect, while 5mg/kg AM4B6-hIgG1-TGFβRII' already had significantly better tumor inhibition, indicating TGFβRII fusion increased the anti-tumor efficacy of AM4B6 mAb alone. In addition, an obvious dose-response of AM4B6-hIgG1-TGFβRII' was observed in this model, again suggesting the efficacy is depending on AM4B6-hIgG1-TGFβRII'.

[00565] EMT6-hPD-L1 tumor model on C57BL/6 mice

[00566] Endogenous mouse PD-L1 in mouse tumor cell line EMT6 (ATCC) was knocked out and human PD-L1 was knocked in the cells, the engineered cells of EMT6 were named as EMT6-hPD-L1.

[00567] Mice were subcutaneously inoculated with EMT6/hPD-L1 tumor cells and randomly divided into 7 groups thereafter according to the tumor volume with 10 mice per group. After grouping, animals from group 1 to 7 were administered with 24.9 mg/kg Control hIgG1, 30 mg/kg control hIgG1-TGFβRII', 24.9 mg/kg AM4B6, 3 mg/kg AM4B6-hIgG1-TGFβRII', 10 mg/kg AM4B6-hIgG1-TGFβRII' or 30 mg/kg AM4B6-hIgG1-TGFβRII' respectively, by intraperitoneal injection twice a week for

4 weeks. The tumor volume and body weight of tumor bearing mice were observed twice weekly. As shown in Figure 19 A - Figure 19B, AM4B6-hIgG1-TGFβRII' dose-dependently inhibited tumor growth with TGI of 21.43%, 46.83% and 79.39% at 3, 10 and 30 mg/kg respectively on Day 29 post dose. At equal molar quantity, the anti-tumor activity of AM4B6-hIgG1-TGFβRII' at 30 mg/kg was more pronounced than AM4B6 at 24.9 mg/kg, in that group, TGI was 29.67% on Day 29.

Example 10: Impact of AM4B6-hIgG1-TGFβRII' treatment on tumor infiltrating lymphocytes (TIL) in MC38-hPD-L1 tumor model

[00568] MC38-hPD-L1 tumor cells were cultured and inoculated following the same process of Example 9. When the tumor size was 250-300 mm^3, tumor bearing mice were selected and randomized to 4 groups (n=6). Animals were treated with PBS, or 3mg/kg isotype control-TGFβRII', or 2.5mg/kg AM4B6, or 3mg/kg AM4B6-hIgG1-TGFβRII'. All the antibodies were administrated twice a week for 1 or 2 weeks by i.v. injection. Tumors were harvested 24 hours after the 2nd dosing and 24 hours after the 4th dosing, respectively, followed by dissociation with gentle MACS Dissociator (Miltenyi Biotec, 130-093-235) and digested with mouse Tumor Dissociation Kit (Miltenyi Biotec, 130-096-730) for 40 min at 37°C. Isolated single tumor cell suspension of each group was analyzed for TIL sub-population percentage using FACS after being stained by PE anti-mouse CD45 (BD bioscience, Cat#553081), APC anti-mouse CD8a (Biolegend, Cat#100712), APC anti-mouse NK1.1 (Biolegend, Cat#108710), FITC anti-mouse Granzyme B (Biolegend, Cat#515403) and FITC anti-mouse IFN gamma (Invitrogen, Cat#11-7311-82), shown in Table 18 and Table 19.

[00569] Table 18. TIL analysis on MC38-hPD-L1 tumor model 24 hours after 2nd dosing.

Sub-population of TILs (%) / Group(n=3)	CD8+/CD45+	CD8+GZMB+ /CD45+	NK1.1+/CD45+	NK1.1+GZM B+ /CD45+	CD8+IFNg+ /CD45+	NK1.1+IFNg+ /CD45+

PBS	11.77%±0.95%	1.18%±0.00%	1.78%±0.57%	0.40%±0.07%	0.31%±0.03%	0.32%±0.03%
isotype control-TGFβRII' 3mpk	13.53%±3.64%	0.91%±0.36%	2.02%±0.48%	0.44%±0.14%	0.22%±0.02%	0.21%±0.02%
AM4B6 2.5mpk	13.80%±4.88%	1.26%±0.49%	2.70%±0.35%	0.63%±0.06%	0.30%±0.06%	0.27%±0.06%
AM4B6-hIgG1-TGFβRII' 3mpk	11.32%±2.97%	1.11%±0.31%	2.59%±0.39%	0.72%±0.21%	0.26%±0.06%	0.27%±0.05%

[00570] Table 19. TIL analysis on MC38-hPD-L1 tumor model 24 hours after 4th dosing.

Sub-population of TILs (%) / Group(n=3)	**CD8+/CD45+**	**CD8+GZMB+ /CD45+**	**NK1.1+/CD45+**	**NK1.1+GZMB+ /CD45+**	**CD8+IFNg+ /CD45+**	**NK1.1+IFNg+ /CD45+**
PBS	9.36%±3.79%	0.72% ± 0.07%	1.54%±0.21%	0.31% ± 0.05%	0.30% ± 0.07%	0.31%±0.05%
isotype control-TGFβRII' 3mpk	10.53%±1.86%	0.66% ± 0.10%	1.90%±0.14%	0.27% ± 0.05%	0.21% ± 0.05%	0.20%±0.06%
AM4B6 2.5mpk	17.36%±3.16%	1.03% ± 0.24%	2.73%±0.46%	0.33% ± 0.02%	0.26% ± 0.04%	0.21%±0.03%
AM4B6-hIgG1-TGFβRII' 3mpk	17.77%±0.88%	1.37% ± 0.15%	4.19%±1.12%	0.62% ± 0.16%	0.24% ± 0.04%	0.30%±0.04%

[00571] After the 2nd dosing, there was no significant changes in percentage of sub-population of TILs among different treatment groups (Table 18). But after the 4th dosing, CD8+/CD45+% of AM4B6 group and AM4B6-hIgG1-TGFβRII' group significantly increased, comparing to that of the isotype control-TGFβRII' group

(Table 19). CD8+GZMB+% and NK1.1+% also increased a lot, as compared to that of the isotype control-TGFβRII' group. These findings indicates the CD8+T cells and NK1.1 T cells might be stimulated by AM4B6 or AM4B6-hIgG1-TGFβRII' to activate and proliferate, and also enriched in tumor microenvironment to facilitate tumor cell killing. When compared to AM4B6, AM4B6-hIgG1-TGFβRII' had an even higher CD8+GZMB+% and NK1.1+%, which was correlated with its more potent anti-tumor activity as measured by TGI above.

Example 11: Pharmacokinetics and pharmacodynamics study of AM4B6-hIgG1-TGFβRII' *in vivo*

[00572] C57BL/6 female mice were randomized to 6 groups (n=3). Animals were treated with 3mg/kg isotype control-TGFβRII', or 2.5mg/kg AM4B6, or 0.3mg/kg AM4B6-hIgG1-TGFβRII', or 1mg/kg AM4B6-hIgG1-TGFβRII', or 3mg/kg AM4B6-hIgG1-TGFβRII', or 3mg/kg M7824-analog. M7824-analog was generated by MabSpace Biosciences according to the sequence disclosed in US9676863. All the antibodies were administrated by i.v. single injection. After injection, 200μl blood of each mice was collected at different time points: Predose, 30 min, 2 h, 8 h, 24 h, 48 h, D4, D7, D10, D14, D21 post injection. 80μl plasma of each mice was collected and tested antibody concentration.

[00573] To measure antibody concentration in plasma, two methods were used. The first one is to detect whole bi-functional molecule, including both AM4B6 and TGFβRII' arms. Generally, 1 μg/ml of human PD-L1-his was coated on the 96-well ELISA plate at room temperature for 2 hours. After blocking, serially diluted standard and plasma samples were added and incubated for 1.5 hours. After washing, 0.1 μg/ml biotinylated anti-human TGFβRII' was added, and then after washing, streptavidin-HRP was added. Finally, TMB was added to develop color, which was stopped by diluted sulfuric acid. The plates were read of OD450nm and OD620nm by a microplate reader. Data were analyzed by OD450nm-OD620nm.

[00574] The second one is to only detect AM4B6 antibody arm. Similar to the procedure above, 1 μg/ml of human PD-L1-his was coated, and serially diluted standard and plasma samples were added, and incubated for 1.5 hours. After washing, diluted goat HRP conjugated anti-human IgG Fc antibody was added. Finally, TMB was added to develop color, which was stopped by diluted sulfuric acid. The plates were read of OD450nm and OD620nm by a microplate reader. Data were analyzed by OD450nm-OD620nm.

[00575] To evaluate the correlation between antibody concentration and change of TGFβ in plasma, the concentration changes of TGFβ1 and TGFβ2 in plasma were tested. Briefly, 4 μg/ml of mouse TGF-β1 capture antibody or 2 μg/ml of mouse TGF-β2 capture antibody was coated on the 96-well ELISA plate at room temperature for 2 hours. 10 μl of 1 N HCl were added to 50 μl of each plasma sample and incubated for 10 minutes at room temperature. The acidified samples were neutralized by adding 10 μl of 1.2N NaOH/0.5M HEPES to ensure the final pH within 7.2-7.6. After blocking, serially diluted standard and plasma samples were added and incubated for 1.5 hours. After washing, TGF-β1 or TGF-β2 detection antibody was added, and then after washing, streptavidin-HRP was added. Finally, TMB was added to develop color, which was stopped by diluted sulfuric acid. The plates were read of OD450nm and OD620nm by a microplate reader. Data were analyzed by OD450nm-OD620nm.

[00576] Figure 20A showed the antibody concentration change in plasma. There was no significant difference in PK profiles using the two methods, indicating the whole bifunctional molecule AM4B6-hIgG1-TGFβRII' was quite stable without abnormal cleavage and clearance *in vivo*, like that of AM4B6 mAb. And at the same time, AM4B6-hIgG1-TGFβRII' depleted TGF-β1 within 30min after i.v. injection even at the lowest dose of 0.3mg/kg, as shown in Figure 20B. M7824-analog and isotype control-TGFβRII' also depleted TGF-β1, but M7824-analog could not maintain that effect from Day 2 while AM4B6-hIgG1-TGFβRII' could maintain a low level of TGF-β1 to Day 21. This results also corresponded to their PK exposure

(Figure 20C), indicating TGF-β1 may serve as a good pharmacodynamic marker for AM4B6-hIgG1-TGFβRII' target engagement in plasma. No obvious depletion of TGF-β2 in mice plasma was detected (data now shown).

Example 12: Construction and Expression of AM4B6-hIgG1-IL-1RA fusion protein

[00577] The selected heavy chain and light chain variants were cross combinated and expressed with hIgG1-IL-1RA (34-177) (UniProtKB, P18510) fusion protein. The sequence of heavy chain and light chain was shown in Table 20.

[00578] Table 20. The list of AM4B6-hIgG1-IL-1RA heavy chain and light chain variants

Name	Mutation site	Region	Sequence
AM4B6_Hg.3_hIgG1 -(G4S)4G- IL-1RA	G57E, S59L	H-CDR2-3	DINPNNAETLYNHKFKG
AM4B6_La.4_hKappa	A51V, N53D	L-CDR2-2	SRSVRYT

[00579] Similar to AM4B6-hIgG1-TGFβRII' bi-functional molecule, the truncated human IL-1RA _34-177 was fused with AM4B6 to obtain better activity and stability. AM4B6-hIgG1-IL-1RA was short for AM4B6-hIgG1-IL-1RA (34-177). The sequences of the truncated human IL-1RA _34-177 are as below: (SEQ ID NO: 67)

KMQAFRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKIDVVPIEPHALFLGI
HGGKMCLSCVKSGDETRLQLEAVNITDLSENRKQDKRFAFIRSDSGPTTSFES
AACPGWFLCTAMEADQPVSLTNMPDEGVMVTKFYFQEDE

[00580] The co-transfection of heavy chain and light chain was carried out using the ExpiFectamine™ CHO Reagent (Thermo, A29129) from Invitrogen according to the manufacturer's protocol. The supernatant was harvested on day 10 and purified by affinity chromatography.

Example 13: Affinity of AM4B6- hIgG1-IL-1RA bi-functional molecule to hPD-L1

[00581] Binding to human PD-L1 based on ELISA assay

[00582] 1 μg/ml hPD-L1 (Acro Biosystems, PD1-H5229) antigens was coated to the ELISA plate and coated overnight at 4°C. Then 300 μl of 2% (w/v) BSA was added for blocking at room temperature for 1 h. After 1h incubation, 100 μl of AM4B6-hIgG1-IL-1RA bi-functional molecule or AM4B6-hIgG1 monoclonal antibody at concentrations ranging from 10 nM to 0.00017 nM (three-fold serial dilutions) were added with PBST as negative control, and incubated at room temperature for 1 h. PBS with 0.5% Tween-20 were used for washing for 3 times, and 100 μl HRP-conjugated anti-human Fc antibody (1:20000, Abcam, ab98624) was added, After incubation at room temperature for 1 h, mixed TMB substrate reagent (InnoReagents, TMB-S-003) was added and incubated at room temperature for 5 min, and stopped by adding 0.1M H_2SO_4. OD450 nm was recorded by Microplate Reader. The data was analyzed by Graphpad prism.

[00583] As shown in Figure 21, comparing to the AM4B6 monoclonal antibody, AM4B6-hIgG1-IL-1RA bi-functional molecule have similar binding signals and affinities.

[00584] Binding to PD-L1 expressing on cell surface of AM4B6-hIgG1-IL-1RA by a FACS assay

[00585] 293T-PD-L1-CD3L cell was generated by MabSpace Biosciences for characterization of PD-L1 antibodies. The cell was transfected with both human PD-L1 and anti-CD3 scFv. AM4B6-hIgG1-IL-1RA bi-functional molecule or AM4B6 monoclonal antibody were serially diluted with 3-fold dilutions to obtain 11 concentrations in dilution buffer (PBS with 2%BSA). 293T-PD-L1-CD3L cells were harvested and centrifuged. Then they were resuspended in PBS with density of 2×10^6 cells/ml and added to the plate with 100 μl per well. After centrifugation and

removing the supernatant, the diluted antibodies were added to the plate and incubated in 4°C for 30min. After washing twice with dilution buffer, PE conjugated donkey anti-human IgG (H+L) (Jacksonimmuno, 709-116-149) was added to the plate and incubated in 4°C for 30 min. After washing, cells were resuspended in 200 μl PBS and analyzed by flow cytometry. The data was analyzed by Graphpad prism.

[00586] As shown in Figure 22, AM4B6-hIgG1-IL-1RA bi-functional molecule and AM4B6-hIgG1 could bind to PD-L1 expressed on surface of cells with similar EC50 which was consistent with affinity results measured by ELISA.

Example 14: PD1/PD-L1 blockade activity of AM4B6-hIgG1-IL-1RA

[00587] In this assay, 293T-PD-L1-CD3L cell was expressing PD-L1 and anti-CD3 scFv, and Jurkat-NFAT-Luc-PD1 cell was expressing PD-1 and carrying NFAT signal which can be activated by CD3 stimulation. NFAT activation will lead to luciferase gene transcription and expression, which can be detected by its substrate. Both two cells were generated by MabSpace Biosciences.

[00588] Briefly, 293T-PD-L1-CD3L cells was harvested and resuspended at density of 2×10^6 cells/ml. 20 μl cells per well was added into half well plate. AM4B6-hIgG1-IL-1RA bi-functional molecule and AM4B6-hIgG1 were serially diluted (3-fold dilutions) to obtain 8 concentrations in RPMI medium with 2%FBS. 20 μl antibodies per well was added into half well plate, and the plate was incubated at 37°C, 5% CO_2 for 30min. Jurkat-NFAT-Luc-PD1 cells were harvested and resuspended at density of 4×10^6 cells/ml in RPMI medium with 2%FBS. Finally, 20 μl cells per well was added into half well plate and incubated in 37°C, 5% CO_2 for 5 h. 60 μl OneGlo detection reagent (Promega, E6120) was added to each well and incubated at room temperature for 5 minutes. The luminescent signal was read by Microplate Reader. The data was analyzed by GraphPad Prism.

[00589] As shown in Figure 23, AM4B6-hIgG1-IL-1RA bi-functional molecule and AM4B6-hIgG1 had similar blockade activity to PD-L1 in this cell-based assay.

Example 15: Blocking activity of AM4B6-hIgG1-IL-1RA to human IL-1β

[00590] Blocking activity of AM4B6-hIgG1-IL-1RA BsAb to hIL-1β based on ELISA

[00591] To test ligand/receptor blocking activity, 5 μg/ml Human IL-1β protein (Sino Biological, Cat#10139) was coated to the ELISA plate and incubated overnight at 4°C. 300 μl blocking buffer was added for blocking at room temperature for 1 h. After 1 h, 50 μl of AM4B6-hIgG1-IL-1RA BsAb or IL-1RA protein(Sino Biological, Cat#10123-HNAE) at serial concentrations ranging from 200 nM to 0.03 nM (three-fold serial dilutions) with 50μl 10nM Human IL-1RI-his (Sino Biological, Cat#10126-H08H) were added to the well and incubate 1hr at room temperature. PBS with 0.5% Tween-20 were used for washing for 3 times, and 100 μl HRP-conjugated his-tag Antibody (1:2000 dilution, Genscript, Cat#A00612) was added, incubate for 1hr in room temperature. Then, mixed TMB substrate reagent (InnoReagents, Cat#: TMB-S-003) was added and incubated at room temperature for 5 min, then stopped by adding 0.1 M H_2SO_4. OD450nm was recorded by Microplate Reader. The data was analyzed by Graphpad prism.

[00592] As shown in Figure 24, AM4B6-hIgG1-IL-1RA can block IL-1β dose dependently, and the blocking activity of AM4B6-hIgG1-IL-1RA to IL-1RI was better than that of IL-1RA protein.

[00593] **Blocking activity of AM4B6-hIgG1-IL-1RA Bi-Functional Molecule to hIL-1β on reporter cell**

[00594] In this assay, HEK-Blue™ CD40L cells were purchase from Invivogen (Cat# hkb-cd40), These cells were generated by stable transfection of HEK293 cells with the human CD40 gene and an NF-kB inducible SEAP construct. Binding of CD40L to its receptor CD40 triggers cascade leading to the activation of NF-kB and subsequent production of SEAP which can monitored by QUANTI-Blue. HEK293 cells express endogenously the receptor for the cytokines IL-1β which share a

common signaling pathway with CD40L. So, IL-1b -mediated SEAP production can be blocked using neutralizing antibody.

[00595] Briefly, collect HEK293-CD40L cells at log phase cells and seed cells at density of 5×10^4/well (100μl/well) into 96-well plate to adhere overnight. AM4B6-hIgG1 -IL-1RA bispecific antibody and IL-1RA protein were serially diluted (5-fold dilutions) to obtain 10 concentrations in complete culture medium. Add 50μl/well diluted antibody (or IL-1RA protein) and 50μl/well human IL-1β to the cells, incubate at 37°C for 24h. Next day add 160μl of QUANTI-Blue ™ Solution (Invivogen, Cat#rep-qbs) to a new plate, and 40μl cell culture supernatant were added the plates. Incubate the plates at 37°C for 2h. Determine SEAP level using a spectrophotometer at 620nm. The The data was analyzed by Graphpad prism.

[00596] As shown in Figure 25, AM4B6-hIgG1-IL-1RA can block IL-1β in a dose dependent manner, and the blocking activity of AM4B6-hIgG1-IL-1RA to IL-1β was stronger than IL-1RA protein, which was consistent with blocking results measured by ELISA.

Example 16: Construction, expression, purification of AM4B6-SIRPα bifunctional antibodies

[00597] The SIRPα_CV1 is an engineered high-affinity SIRPα variant, which potently antagonized CD47 on cancer cells but did not induce macrophage phagocytosis on their own (*Kipp Weiskopf et al. Science 341, 88 (2013)*). We invented bifunctional antibodies targeting both PD-L1 and CD47, including symmetrical antibodies (AM4B6-hIgG1-SIRP α and 3280A-hIgG1-SIRP α) and asymmetric antibodies (AM4B6-hIgG1-SIRP α (KIH) and 3280A-hIgG1-SIRP α (KIH)), wherein KIH is short for knob into hole. The constructions of these molecules are described in Table 21, and the SIRPα_CV1 sequenced is also listed below.

[00598] SIRPα_CV1 sequence (SEQ ID NO: 84):

[00599] EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPG RVLIYNQRQGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFRKGSP DDVEFKSGAGTELSVRAKPS

[00600] Table 21 construction of anti-PD-L1- SIRPα bifunctional antibodies

ID	Light chain sequence	Heavy chain sequence 1	Heavy chain sequence 2	Note
AM4B6-hIgG1-SIRPα	AM4B6-La.4-hKappa SEQ ID NO: 90	AM4B6_Hg.3_hIgG1_L234F_L235E_P331S - (G4S)4G - SIRPa_CV1 SEQ ID NO: 91	NA	SIRPα_CV1 sequence is from Kipp Weiskopf et al. Science 341, 88 (2013)
3280A-hIgG1-SIRPα	3280A-L-hKappa SEQ ID NO: 92	3280A-H-hIgG1(N297A)-(G4S)4G-SIRPα_CV1 SEQ ID NO: 93	NA	3280A is short for Atezolizumab, anti-PD-L1 antibody of Roche
AM4B6-hIgG1-SIRPα(KIH)	AM4B6-La.4-hKappa SEQ ID NO: 90	AM4B6_Hg.3_hIgG1_L234F_L235E_P331S (knob) - SIRPa_CV1 SEQ ID NO: 94	AM4B6_Hg.3_hIgG1_L234F_L235E_P331S (hole) SEQ ID NO: 95	Knob: S354C, T366W Hole: Y349C、T366S、L368A、Y407V
3280A-hIgG1-SIRPα(KIH)	3280A-L-hKappa 3280A-L-hKappa SEQ ID NO: 92	3280A_H_hIgG1_L234F_L235E_P331S (knob) - SIRPa_CV1 SEQ ID NO: 96	3280A_H_hIgG1_L234F_L235E_P331S (hole) SEQ ID NO: 97	3280A is short for Atezolizumab, anti-PD-L1 antibody of Roche

[00601] All of the 4 bifunctional antibodies were expressed with Expi-CHO cell according to the manufacture's protocol. For the two symmetrical bifunctional

antibodies, high purity antibodies were obtained with one-step Mabselect SuRe purification, but for the asymmetric bifunctional antibodies, high purity antibody cannot be obtained by conventional one-step Mabselect SuRe purification. To obtain the high purity asymmetric antibodies, we used a HiTrap PrismA resin to polish purify the antibodies, and the purity of the asymmetric antibodies was better than 95%. The polish purification procedure is described as follows.

[00602] Buffer used:

[00603] Equilibration buffer: 50mM Tris-HAc, 150mM NaCl, pH7.4.

[00604] Wash buffer: 50mM NaAc/HAc, 500mM NaCl, 5% PEG, pH5.5.

[00605] Elution buffer: 50mM HAc, 500mM NaCl, 5% PEG, pH 3.0.

[00606] Equilibrate the HiTrap PrismA column with equilibration buffer for at least 5 column volumes (CV), until the UV baseline, eluent pH, and conductivity are unchanged.

[00607] Load the sample onto a pre-equilibrated HiTrap PrismA column.

[00608] Wash with 5 to 10 CV wash buffer, until the UV trace returns to baseline.

[00609] Elute with 0-100% elution buffer in 10-20 CV and collect 5-10 fractions with several tubes separately. The pH was adjusted to about 6.0-7.0 with 1M Tris-base (pH 9.0)

[00610] The fractions are then characterized by SEC-HPLC. Figure 26 shows that the purity of 3280A-hIgG1-SIRPα (KIH) and AM4B6-hIgG1-SIRPα (KIH) is 95.33% and 96.5%, respectively.

[00611] The affinity to human PD-L1 or human CD47 were tested with ELISA. Figures 27 and 28 show that the affinities to antigen of bifunctional antibodies are

comparable with that of parent monoclonal antibody (4B6 mAb control) or fusion proteins (SIRPα-Fc (FES)).

[00612] Example 17: Construction and Expression of IgG-scFv-ACZ885-AM4B6 and IgG-scFv-XOMA052-AM4B6 bispecific antibodies (bsAbs)

[00613] The sequence of single chain fragments (scFvs) AM4B6 are shown in the table below. The anti-IL-1 β antibodies Gevokizumab (XOMA052) and Canakinumab (ACZ885) were from Novartis.-The scFvs of AM4B6 were connected to anti-IL-1β antibody heavy chain C-terminal to obtain better activity and stability. The scFvs have the GS linker (GGGGSGGGGSGGGGSGGGGS) that connected VH to VL, and contain an interdomain disulfide bond between the residues H44C and L100C (Kabat numbering). The sequence of the anti- IL-1β antibodies (XOMA052 and ACZ885) are shown in Table 22. The constructed bispecific antibodies were named IgG-scFv-ACZ885-AM4B6 and IgG-scFv-XOMA052-AM4B6, respectively.

[00614] The co-transfection of heavy chain and light chain of the bsAbs were carried out using the ExpiFectamine™ CHO Reagent (Thermo, A29129) from Invitrogen according to the manufacturer's protocol. The supernatant was harvested on day 10 and purified by affinity chromatography.

[00615] Example 18: Binding activities of IgG-scFv-ACZ885-AM4B6 and IgG-scFv-XOMA052-AM4B6 bsAbs to hIL-1β

[00616] Binding to human IL-1β protein based on ELISA assay

[00617] 100μL 1 μg/ml hIL-1β protein (SinoBiological, Cat# 10139 -HNAE) was coated to the ELISA plate and coated overnight at 4°C. Then 200 μl of 2% (w/v) BSA was added for blocking at room temperature for 2 h. After the incubation, 100 μl of IgG-scFv-ACZ885-AM4B6, IgG-scFv-XOMA052-AM4B6 bsAbs, ACZ885, and XOMA052 at the concentrations ranging from 20 nM to 0.000339 nM (three-fold

serial dilutions) were added with PBST as negative control, and incubated at room temperature for 1 h. PBS with 0.5% Tween-20 were used for washing for 3 times, and 100 μl HRP-conjugated anti-human Fc antibody (1:20000, Abcam, ab98624) was added. After incubation at room temperature for 1 h, mixed TMB substrate reagent (InnoReagents, TMB-S-003) was added and incubated at room temperature for 5 min, and stopped by adding 0.1M H_2SO_4. OD450 nm was recorded by Microplate Reader. The data was analyzed by Graphpad prism.

[00618] As shown in Figure 29, comparing to the ACZ885 and XOMA052 monoclonal antibody, IgG-scFv-ACZ885-AM4B6 and IgG-scFv-XOMA052-AM4B6 bsAbs have similar binding activity to hIL-1β protein, respectively.

[00619] Example 19: Binding activities of IgG-scFv-ACZ885-AM4B6 and IgG-scFv-XOMA052-AM4B6 bsAbs to hPD-L1

[00620] Binding to human PD-L1 based on ELISA assay

[00621] 100μL 1 μg/ml hPD-L1 (Acro Biosystems, PD1-H5229) antigen was coated to the ELISA plate and coated overnight at 4°C. Then 300 μl of 2% (w/v) BSA was added for blocking at room temperature for 1 h. After 1h incubation, 100 μl of IgG-scFv-ACZ885-AM4B6, IgG-scFv-XOMA052-AM4B6 bsAbs or AM4B6-hIgG1 monoclonal antibody (AM4B6 mAb) at the concentrations ranging from 20 nM to 0.000339 nM (three-fold serial dilutions) were added with PBST as negative control, and incubated at room temperature for 1 h. PBS with 0.5% Tween-20 were used for washing for 3 times, and 100 μl HRP-conjugated anti-human Fc antibody (1:20000, Abcam, ab98624) was added. After incubation at room temperature for 1 h, mixed TMB substrate reagent (InnoReagents, TMB-S-003) was added and incubated at room temperature for 5 min, and stopped by adding 0.1M H_2SO_4. OD450 nm was recorded by Microplate Reader. The data was analyzed by Graphpad prism.

[00622] As shown in Figure 30, comparing to the AM4B6 monoclonal antibody, IgG-scFv-ACZ885-AM4B6, IgG-scFv-XOMA052-AM4B6 bsAbs have similar binding activity to hPD-L1 protein.

[00623] Binding of IgG-scFv-ACZ885-AM4B6 and IgG-scFv-XOMA052-AM4B6 to PD-L1 expressing 293T cells by FACS method

[00624] 293T-PD-L1-CD3L cell was generated by MabSpace Biosciences for characterization of PD-L1 antibodies. The cell was transfected with both human PD-L1 and anti-CD3 scFv. IgG-scFv-ACZ885-AM4B6, IgG-scFv-XOMA052-AM4B6 or AM4B6 mAb were serially diluted with 4-fold dilutions to obtain 9 concentrations in dilution buffer (PBS with 2%BSA). 293T-PD-L1-CD3L cells were harvested and centrifuged. Then they were resuspended in PBS with density of 2×10^6 cells/ml and added to the plate with 100 μl per well. After centrifugation and removing the supernatant, the diluted antibodies were added to the plate and incubated in 4°C for 30min. After washing twice with dilution buffer, PE conjugated donkey anti-human IgG (H+L) (Jacksonimmuno, 709-116-149) was added to the plate and incubated in 4°C for 30 min. After washing, cells were resuspended in 200 μl PBS and analyzed by flow cytometry. The data was analyzed by Graphpad prism.

[00625] As shown in Figure 31, IgG-scFv-ACZ885-AM4B6 and IgG-scFv-XOMA052-AM4B6 could bind to PD-L1 expressed on surface of cells with similar EC_{50} which was consistent with affinity results measured by ELISA.

Example 20: PD1/PD-L1 blockade activity of IgG-scFv-ACZ885-AM4B6 and IgG-scFv-XOMA052-AM4B6

[00626] In this assay, 293T-PD-L1-CD3L cell was expressing PD-L1 and anti-CD3 scFv, and Jurkat-NFAT-Luc-PD1 cell was expressing PD-1 and carrying NFAT signal which can be activated by CD3 stimulation. NFAT activation will lead

to luciferase gene transcription and expression, which can be detected by its substrate. The two cell lines were generated by MabSpace Biosciences.

[00627] Briefly, 293T-PD-L1-CD3L cells was harvested and resuspended at density of 2×10^6 cells/ml. 20 μl cells per well was added into half well plate. IgG-scFv-ACZ885-AM4B6, IgG-scFv-XOMA052-AM4B6 and AM4B6-hIgG1 were serially diluted (3-fold dilutions) to obtain 9 concentrations in RPMI medium with 2%FBS. 20 μl antibodies per well was added into half well plate, and the plate was incubated at 37°C, 5% CO_2 for 30min. Jurkat-NFAT-Luc-PD1 cells were harvested and resuspended at density of 4×10^6 cells/ml in RPMI medium with 2%FBS. Finally, 20 μl cells per well was added into half well plate and incubated in 37°C, 5% CO_2 for 5 h. 60 μl OneGlo detection reagent (Promega, E6120) was added to each well and incubated at room temperature for 5 minutes. The luminescent signal was read by Microplate Reader. The data was analyzed by GraphPad Prism.

[00628] As shown in Figure 32, IgG-scFv-ACZ885-AM4B6, IgG-scFv-XOMA052-AM4B6 and AM4B6-hIgG1 had similar blockade activity to PD-L1 in this cell-based assay.

Example 21: Blocking activity of IgG-scFv-XOMA052-AM4B6 to human IL-1β on human dermal fibroblast (HDF) cells

[00629] Blocking activity of IgG-scFv-XOMA052-AM4B6 to human IL-1β on HDF cells

[00630] To test ligand/receptor blocking activity, $4x10^4$/mL HDF cells with 100 μL/well were stimulated with 50 pg/mL of recombinant human IL-1β (Sino Biological, Cat#10139) while cells without IL-1β stimulation as the negative control. Then, 100uL/well IgG-scFv-XOMA052-AM4B6 and XOMA052 at serial concentrations ranging from 100 nM to 0.00038 nM (four-fold serial dilutions) were added to the cultures and incubated overnight (16 -17 hr) at room temperature. After

stimulation, IL-6 release in the cell cultured supernatant was detected using IL-6 ELISA Kit (R&D, DY206, P209026) guided by the kit instruction.

[00631] As shown in Figure 33, IgG-scFv-XOMA052-AM4B6 and XOMA052 can block IL-1β dose dependently, and the blocking activity of IgG-scFv-XOMA052-AM4B6 to IL-1β was similar to that of XOMA052 on HDF cells.

[00632] Blocking activity of IgG-scFv-ACZ885-AM4B6 to hIL-1β on reporter cell

[00633] In this assay, HEK-Blue™ CD40L cells were purchase from Invivogen (Cat# hkb-cd40), These cells were generated by stable transfection of HEK293 cells with the human CD40 gene and an NF-kB inducible SEAP construct. Binding of CD40L to its receptor CD40 triggers cascade leading to the activation of NF-kB and subsequent production of SEAP which can be monitored by QUANTI-Blue. HEK293 cells express endogenously the receptor for the cytokines IL-1β which share a common signaling pathway with CD40L. So, IL-1β-mediated SEAP production can be blocked using neutralizing antibody.

[00634] Briefly, collect HEK293-CD40L cells at log phase cells and seed cells at density of 5×10^4/well (100μl/well) into 96-well plate to adhere overnight. IgG-scFv-ACZ885-AM4B6and ACZ885 were serially diluted from 100 nM (4-fold dilutions) to obtain 9 concentrations in complete culture medium. Add 50μl/well diluted antibodies and 50μl/well human IL-1β (1ng/mL) to the cells, incubate at 37°C for 24h. Next day, add 160μl of QUANTI-Blue™ Solution (Invivogen, Cat#rep-qbs) to a new plate, and 40μl cell culture supernatant were added the plates. Incubate the plates at 37°C for 2h. Determine SEAP level using a spectrophotometer at 620nm. The data was analyzed by Graphpad prism.

[00635] As shown in Figure 34, IgG-scFv-ACZ885-AM4B6 and ACZ885(Canakinumab) can block IL-1β in a dose dependent manner, and the

blocking activity of IgG-scFv-ACZ885-AM4B6 was similar to ACZ885(Canakinumab) on HEK293-CD40L reporter cells.

[00636] Table 22. Amino acid sequences mentioned in the present disclosure

SED ID NO.	Sequences	Region
1	DYYMN	4B6_HCDR1
2	DINPNNGGTSYNHKFKG	4B6_HCDR2
3	WGDGPFAY	4B6_HCDR3
4	KASQNVGAAVA	4B6_LCDR1
5	SASNRYT	4B6_LCDR2
6	QQYSNYPT	4B6_LCDR3
7	KASQNVGAIVA	4B6-L-CDR1-1
8	KASQNVPAAVA	4B6-L-CDR1-2
9	KASQNVKGAVA	4B6-L-CDR1-3
10	SNSHRYT	4B6-L-CDR2-1
11	SRSVRYT	4B6-L-CDR2-2
12	SVSDRYT	4B6-L-CDR2-3
13	DINPNNADTMYNHKFKG	4B6-H-CDR2-1
14	DINPNNAQTQYNHKFKG	4B6-H-CDR2-2
15	DINPNNAETLYNHKFKG	4B6-H-CDR2-3
16	DINPNNGLTSYNHKFKG	4B6-H-CDR2-4
17	DINPNNAQTVYNHKFKG	4B6-H-CDR2-5
18	DINPNNAGTSYNHKFKG	H-CDR2-WT (G55A)
19	DINPNN$X_1X_2TX_3$YNHKFKG	HCDR2
20	KASQNV$X_4X_5X_6$VA	LCDR1
21	SX_7SX_8RYT	LCDR2
22	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	HFR1
23	WVRQAPGQGLEWMG	HFR2
24	RVTMTRDTSISTAYMELSRLRSDDTAVYYCAR	HFR3
25	WGQGTLVTVSS	HFR4
26	DIQMTQSPSSLSASVGDRVTITC	LFR1
27	WYQQKPGKAPKLLIY	LFR2
28	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	LFR3
29	FGQGTKLEIK	LFR4
30	QVQLVQSGAEVKKPGASVKVSCKASGYVFT	HFR1 variant
31	WVRQAPGQSLEWMG	HFR2 variant
32	RVTVTVDTSISTAYMELSRLRSDDTAVYYCAR	HFR3 variant 1
33	RVTVTVDTSISTAYMELSRLRSDDTAVYYC	HFR3 variant 2

	VK	
34	RVTVTVDKSISTAYMELSRLRSDDTAVYY CAR	HFR3 variant 3
35	RVTVTVDKSISTAYMELSRLRSDDTAVYY CVK	HFR3 variant 4
36	WYQQKPGKSPKLLIY	LFR2 variant
37	GVPSRFSGSGSGTDFTLTISSLQPEDIATYY C	LFR3 variant 1
38	GVPDRFSGSGSGTDFTLTISSLQPEDIATYY C	LFR3 variant 2
39	GVPSRFSGSGSGTDFTLTISSLQPEDIATYY C	F73L mutation for variant 1-FR3
40	QVQLVQSGAEVKKPGASVKVSCKASGYX$_9$ FT	HFR1
41	WVRQAPGQX$_{10}$LEWMG	HFR2
42	RVTX$_{16}$TVDX$_{11}$SISTAYMELSRLRSDDTAVY YCX$_{12}$X$_{13}$	HFR3
43	WYQQKPGKX$_{14}$PKLLIY	LFR2
44	GVPX$_{15}$RFSGSGSGTDFTX$_{17}$TISSLQPEDIAT YYC	LFR3
45	GVPDRFSGSGSGTDFTLTISSLQPEDIATYY C	F73L, A43S, S60D for LC variant 2-FR3
46	EVQLQQSGPELVKPGASVKISCKASGYVFT DYYMNWVKQSHGKSLEWIGDINPNNGGT SYNHKFKGKATVTVDKSSRTAYMELLSLT SEDSAVYYCVKWGDGPFAYWGQGTLVTV SA	4B6_VH
47	DIVMTQSQKFMSTSVGDRVSITCKASQNV GAAVAWYQQKPGQSPKLLIYSASNRYTGV PDRFTGSGSGTDFTLTISNMQSEDLADYFC QQYSNYPTFGSGTKLGIK	4B6_VL
48	QVQLVQSGAEVKKPGASVKVSCKASGYTF TGYYMHWVRQAPGQGLEWMGWINPNSG GTNYAQKFQGRVTMTRDTSISTAYMELSR LRSDDTAVYYCAR	Humanized 4B6, VH germline
49	QVQLVQSGAEVKKPGASVKVSCKASGYTF TDYYMNWVRQAPGQGLEWMGDINPNNG GTSYNHKFKGRVTVTVDTSISTAYMELSRL RSDDTAVYYCARWGDGPFAYWGQGTLVT VSS	Humanized 4B6, VH variant 1
50	QVQLVQSGAEVKKPGASVKVSCKASGYTF TDYYMNWVRQAPGQGLEWMGDINPNNG GTSYNHKFKGRVTVTVDTSISTAYMELSRL RSDDTAVYYCVKWGDGPFAYWGQGTLVT	Humanized 4B6, VH variant 2

	VSS	
51	QVQLVQSGAEVKKPGASVKVSCKASGYVF TDYYMNWVRQAPGQGLEWMGDINPNNG GTSYNHKFKGRVTVTVDKSISTAYMELSRL RSDDTAVYYCARWGDGPFAYWGQGTLVT VSS	Humanized 4B6, VH variant 3
52	QVQLVQSGAEVKKPGASVKVSCKASGYVF TDYYMNWVRQAPGQSLEWMGDINPNNGG TSYNHKFKGRVTVTVDKSISTAYMELSRLR SDDTAVYYCVKWGDGPFAYWGQGTLVTV SS	Humanized 4B6, VH variant 4
53	DIQMTQSPSSLSASVGDRVTITCQASQDISN YLNWYQQKPGKAPKLLIYDASNLETGVPS RFSGSGSGTDFTFTISSLQPEDIATYYCQQY DNLP	Humanized 4B6, VL germline
54	DIQMTQSPSSLSASVGDRVTITCKASQNVG AAVAWYQQKPGKAPKLLIYSASNRYTGVP SRFSGSGSGTDFTLTISSLQPEDIATYYCQQ YSNYPTFGQGTKLEIK	Humanized 4B6, VL variant 1
55	DIQMTQSPSSLSASVGDRVTITCKASQNVG AAVAWYQQKPGKSPKLLIYSASNRYTGVP DRFSGSGSGTDFTLTISSLQPEDIATYYCQQ YSNYPTFGQGTKLEIK	Humanized 4B6, VL variant 2
56	QVQLVQSGAEVKKPGASVKVSCKASGYVF TDYYMNWVRQAPGQSLEWMGDINPNNAG TSYNHKFKGRVTVTVDKSISTAYMELSRLR SDDTAVYYCVKWGDGPFAYWGQGTLVTV SS	Hu4B6_Hg
57	QVQLVQSGAEVKKPGASVKVSCKASGYVF TDYYMNWVRQAPGQSLEWMGDINPNNAG TSYNHKFKGRVTVTVDKSISTAYMELSRLR SDDTAVYYCVKWGDGPFAYWGQGTLVTV SS	AM4B6_Hg
58	QVQLVQSGAEVKKPGASVKVSCKASGYVF TDYYMNWVRQAPGQSLEWMGDINPNNAQ TQYNHKFKGRVTVTVDKSISTAYMELSRL RSDDTAVYYCVKWGDGPFAYWGQGTLVT VSS	Hu4B6_Hg.2
59	QVQLVQSGAEVKKPGASVKVSCKASGYVF TDYYMNWVRQAPGQSLEWMGDINPNNAE TLYNHKFKGRVTVTVDKSISTAYMELSRLR SDDTAVYYCVKWGDGPFAYWGQGTLVTV	AM4B6_Hg.3

	SS	
60	QVQLVQSGAEVKKPGASVKVSCKASGYVF TDYYMNWVRQAPGQSLEWMGDINPNNAQ TVYNHKFKGRVTVTVDKSISTAYMELSRL RSDDTAVYYCVKWGDGPFAYWGQGTLVT VSS	AM4B6_Hg.5
61	DIQMTQSPSSLSASVGDRVTITCKASQNVG AAVAWYQQKPGKAPKLLIYSASNRYTGVP SRFSGSGSGTDFTLTISSLQPEDIATYYCQQ YSNYPTFGQGTKLEIK	Hu4B6_La
62	DIQMTQSPSSLSASVGDRVTITCKASQNVK GAVAWYQQKPGKAPKLLIYSASNRYTGVP SRFSGSGSGTDFTLTISSLQPEDIATYYCQQ YSNYPTFGQGTKLEIK	AM4B6_La.1
63	DIQMTQSPSSLSASVGDRVTITCKASQNVP AAVAWYQQKPGKAPKLLIYSASNRYTGVP SRFSGSGSGTDFTLTISSLQPEDIATYYCQQ YSNYPTFGQGTKLEIK	AM4B6_La.2
64	DIQMTQSPSSLSASVGDRVTITCKASQNVG AAVAWYQQKPGKAPKLLIYSVSDRYTGVP SRFSGSGSGTDFTLTISSLQPEDIATYYCQQ YSNYPTFGQGTKLEIK	AM4B6_La.4
65	DIQMTQSPSSLSASVGDRVTITCKASQNVG AAVAWYQQKPGKAPKLLIYSRSVRYTGVP SRFSGSGSGTDFTLTISSLQPEDIATYYCQQ YSNYPTFGQGTKLEIK	AM4B6_La.6
66	GAVKFPQLCKFCDVRFSTCDNQKSCMSNC SITSICEKPQEVCVAVWRKNDENITLETVC HDPKLPYHDFILEDAASPKCIMKEKKKPGE TFFMCSCSSDECNDNIIFSEEYNTSNPD	truncated TGF-beta receptor II sequence 20-136
67	KMQAFRIWDVNQKTFYLRNNQLVAGYLQ GPNVNLEEKIDVVPIEPHALFLGIHGGKMC LSCVKSGDETRLQLEAVNITDLSENRKQDK RFAFIRSDSGPTTSFESAACPGWFLCTAME ADQPVSLTNMPDEGVMVTKFYFQEDE	truncated IL-1 R-34-177
68	(G4S)4G	linker

69	LQCFCHLCTKDNFTCVTDGLCFVSVTETTD KVIHNSMCIAEIDLIPRDRPFVCAPSSKTGS VTTTYCCNQDHCNKIELPTTVKSSPGLGPV ELAAVIAGPVCFVCISLMLMVYICHNRTVI HHRVPNEEDPSLDRPFISEGTTLKDLIYDMT TSGSGSGLPLLVQRTIARTIVLQESIGKGRF GEVWRGKWRGEEVAVKIFSSREERSWFRE AEIYQTVMLRHENILGFIAADNKDNGTWT QLWLVSDYHEHGSLFDYLNRYTVTVEGMI KLALSTASGLAHLHMEIVGTQGKPAIAHRD LKSKNILVKKNGTCCIADLGLAVRHDSATD TIDIAPNHRVGTKRYMAPEVLDDSINMKHF ESFKRADIYAMGLVFWEIARRCSIGGIHED YQLPYYDLVPSDPSVEEMRKVVCEQKLRP NIPNRWQSCEALRVMAKIMRECWYANGA ARLTALRIKKTLSQLSQQEGIKM	TGFβRI
70	TIPPHVQKSVNNDMIVTDNNGAVKFPQLC KFCDVRFSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDF ILEDAASPKCIMKEKKKPGETFFMCSCSSD ECNDNIIFSEEYNTSNPDLLLVIFQVTGISLL PPLGVAISVIIIFYCYRVNRQQKLSSTWETG KTRKLMEFSEHCAIILEDDRSDISSTCANNI NHNTELLPIELDTLVGKGRFAEVYKAKLK QNTSEQFETVAVKIFPYEEYASWKTEKDIF SDINLKHENILQFLTAEERKTELGKQYWLIT AFHAKGNLQEYLTRHVISWEDLRKLGSSL ARGIAHLHSDHTPCGRPKMPIVHRDLKSSN ILVKNDLTCCLCDFGLSLRLDPTLSVDDLA NSGQVGTARYMAPEVLESRMNLENVESFK QTDVYSMALVLWEMTSRCNAVGEVKDYE PPFGSKVREHPCVESMKDNVLRDRGRPEIP SFWLNHQGIQMVCETLTECWDHDPEARLT AQCVAERFSELEHLDRLSGRSCSEEKIPEDG SLNTTK	TGFβRII isoform A

71	TIPPHVQKSDVEMEAQKDEIICPSCNRTAHP LRHINNDMIVTDNNGAVKFPQLCKFCDVR FSTCDNQKSCMSNCSITSICEKPQEVCVAV WRKNDENITLETVCHDPKLPYHDFILEDAA SPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDLLLVIFQVTGISLLPPLGVAI SVIIIFYCYRVNRQQKLSSTWETGKTRKLM EFSEHCAIILEDDRSDISSTCANNINHNTELL PIELDTLVGKGRFAEVYKAKLKQNTSEQFE TVAVKIFPYEEYASWKTEKDIFSDINLKHE NILQFLTAEERKTELGKQYWLITAFHAKGN LQEYLTRHVISWEDLRKLGSSLARGIAHLH SDHTPCGRPKMPIVHRDLKSSNILVKNDLT CCLCDFGLSLRLDPTLSVDDLANSGQVGTA RYMAPEVLESRMNLENVESFKQTDVYSMA LVLWEMTSRCNAVGEVKDYEPPFGSKVRE HPCVESMKDNVLRDRGRPEIPSFWLNHQGI QMVCETLTECWDHDPEARLTAQCVAERFS ELEHLDRLSGRSCSEEKIPEDGSLNTTK	TGFβRII isoform B
72	GPEPGALCELSPVSASHPVQALMESFTVLS GCASRGTTGLPQEVHVLNLRTAGQGPGQL QREVTLHLNPISSVHIHHKSVVFLLNSPHPL VWHLKTERLATGVSRLFLVSEGSVVQFSSA NFSLTAETEERNFPHGNEHLLNWARKEYG AVTSFTELKIARNIYIKVGEDQVFPPKCNIG KNFLSLNYLAEYLQPKAAEGCVMSSQPQN EEVHIIELITPNSNPYSAFQVDITIDIRPSQED LEVVKNLILILKCKKSVNWVIKSFDVKGSL KIIAPNSIGFGKESERSMTMTKSIRDDIPSTQ GNLVKWALDNGYSPITSYTMAPVANRFHL RLENNAEEMGDEEVHTIPPELRILLDPGALP ALQNPPIRGGEGQNGGLPFPFPDISRRVWN EEGEDGLPRPKDPVIPSIQLFPGLREPEEVQ GSVDIALSVKCDNEKMIVAVEKDSFQASG YSGMDVTLLDPTCKAKMNGTHFVLESPLN GCGTRPRWSALDGVVYYNSIVIQVPALGD SSGWPDGYEDLESGDNGFPGDMDEGDASL FTRPEIVVFNCSLQQVRNPSSFQEQPHGNIT FNMELYNTDLFLVPSQGVFSVPENGHVYV EVSVTKAEQELGFAIQTCFISPYSNPDRMSH YTIIENICPKDESVKFYSPKRVHFPIPQADM DKKRFSFVFKPVFNTSLLFLQCELTLCTKM EKHPQKLPKCVPPDEACTSLDASIIWAMM QNKKTFTKPLAVIHHEAESKEKGPSMKEPN	TGFβRIII

	PISPPIFHGLDTLTVMGIAFAAFVIGALLTG ALWYIYSHTGETAGRQQVPTSPPASENSSA AHSIGSTQSTPCSSSSTA	
73	LEADKCKEREEKIILVSSANEIDVRPCPLNP NEHKGTITWYKDDSKTPVSTEQASRIHQHK EKLWFVPAKVEDSGHYYCVVRNSSYCLRI KISAKFVENEPNLCYNAQAIFKQKLPVAGD GGLVCPYMEFFKNENNELPKLQWYKDCKP LLLDNIHFSGVKDRLIVMNVAEKHRGNYT CHASYTYLGKQYPITRVIEFITLEENKPTRP VIVSPANETMEVDLGSQIQLICNVTGQLSDI AYWKWNGSVIDEDDPVLGEDYYSVENPA NKRRSTLITVLNISEIESRFYKHPFTCFAKNT HGIDAAYIQLIYPVTNFQKHMIGICVTLTVII VCSVFIYKIFKIDIVLWYRDSCYDFLPIKAS DGKTYDAYILYPKTVGEGSTSDCDIFVFKV LPEVLEKQCGYKLFIYGRDDYVGEDIVEVI NENVKKSRRLIIILVRETSGFSWLGGSSEEQI AMYNALVQDGIKVVLLELEKIQDYEKMPE SIKFIKQKHGAIRWSGDFTQGPQSAKTRFW KNVRYHMPVQRRSPSSKHQLLSPATKEKL QREAHVPLG	IL-1RI
74	SERCDDWGLDTMRQIQVFEDEPARIKCPLF EHFLKFNYSTAHSAGLTLIWYWTRQDRDL EEPINFRLPENRISKEKDVLWFRPTLLNDTG NYTCMLRNTTYCSKVAFPLEVVQKDSCFN SPMKLPVHKLYIEYGIQRITCPNVDGYFPSS VKPTITWYMGCYKIQNFNNVIPEGMNLSFL IALISNNGNYTCVVTYPENGRTFHLTRTLT VKVVGSPKNAVPPVIHSPNDHVVYEKEPG EELLIPCTVYFSFLMDSRNEVWWTIDGKKP DDITIDVTINESISHSRTEDETRTQILSIKKVT SEDLKRSYVCHARSAKGEVAKAAKVKQK VPAPRYTVELACGFGATVLLVVILIVVYHV YWLEMVLFYRAHFGTDETILDGKEYDIYV SYARNAEEEEFVLLTLRGVLENEFGYKLCI FDRDSLPGGIVTDETLSFIQKSRRLLVVLSP NYVLQGTQALLELKAGLENMASRGNINVI LVQYKAVKETKVKELKRAKTVLTVIKWK GEKSKYPQGRFWKQLQVAMPVKKSPRRSS SDEQGLSYSSLKNV	IL-1RAP

75	FTLQPAAHTGAARSCRFRGRHYKREFRLE GEPVALRCPQVPYWLWASVSPRINLTWHK NDSARTVPGEEETRMWAQDGALWLLPAL QEDSGTYVCTTRNASYCDKMSIELRVFENT DAFLPFISYPQILTLSTSGVLVCPDLSEFTRD KTDVKIQWYKDSLLLDKDNEKFLSVRGTT HLLVHDVALEDAGYYRCVLTFAHEGQQY NITRSIELRIKKKKEETIPVIISPLKTISASLGS RLTIPCKVFLGTGTPLTTMLWWTANDTHIE SAYPGGRVTEGPRQEYSENNENYIEVPLIFD PVTREDLHMDFKCVVHNTLSFQTLRTTVK EASSTFSWGIVLAPLSLAFLVLGGIWMHRR CKHRTGKADGLTVLWPHHQDFQSYPK	IL-1RII
76	RPSGRKSSKMQAFRIWDVNQKTFYLRNNQ LVAGYLQGPNVNLEEKIDVVPIEPHALFLGI HGGKMCLSCVKSGDETRLQLEAVNITDLS ENRKQDKRFAFIRSDSGPTTSFESAACPGW FLCTAMEADQPVSLTNMPDEGVMVTKFYF QEDE	IL-1RA
77	LQCFCHLCTKDNFTCVTDGLCFVSVTETTD KVIHNSMCIAEIDLIPRDRPFVCAPSSKTGS VTTTYCCNQDHCNKIELPTTVKSSPGLGPV EL	ECD of human TGFβRI 34-126
78	IPPHVQKSVNNDMIVTDNNGAVKFPQLCK FCDVRFSTCDNQKSCMSNCSITSICEKPQEV CVAVWRKNDENITLETVCHDPKLPYHDFIL EDAASPKCIMKEKKKPGETFFMCSCSSDEC NDNIIFSEEYNTSNPD	ECD of human TGFβRII 24-159

79	GPEPGALCELSPVSASHPVQALMESFTVLS GCASRGTTGLPQEVHVLNLRTAGQGPGQL QREVTLHLNPISSVHIHHKSVVFLLNSPHPL VWHLKTERLATGVSRLFLVSEGSVVQFSSA NFSLTAETEERNFPHGNEHLLNWARKEYG AVTSFTELKIARNIYIKVGEDQVFPPKCNIG KNFLSLNYLAEYLQPKAAEGCVMSSQPQN EEVHIIELITPNSNPYSAFQVDITIDIRPSQED LEVVKNLILILKCKKSVNWVIKSFDVKGSL KIIAPNSIGFGKESERSMTMTKSIRDDIPSTQ GNLVKWALDNGYSPITSYTMAPVANRFHL RLENNAEEMGDEEVHTIPPELRILLDPGALP ALQNPPIRGGEGQNGGLPFPFPDISRRVWN EEGEDGLPRPKDPVIPSIQLFPGLREPEEVQ GSVDIALSVKCDNEKMIVAVEKDSFQASG YSGMDVTLLDPTCKAKMNGTHFVLESPLN GCGTRPRWSALDGVVYYNSIVIQVPALGD SSGWPDGYEDLESGDNGFPGDMDEGDASL FTRPEIVVFNCSLQQVRNPSSFQEQPHGNIT FNMELYNTDLFLVPSQGVFSVPENGHVYV EVSVTKAEQELGFAIQTCFISPYSNPDRMSH YTIIENICPKDESVKFYSPKRVHFPIPQADM DKKRFSFVFKPVFNTSLLFLQCELTLCTKM EKHPQKLPKCVPPDEACTSLDASIIWAMM QNKKTFTKPLAVIHHEAESKEKGPSMKEPN PISPPIFHGLDTLTV	ECD of human TGFβRIII 21-787
80	ASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	hIgG1 aa sequence:

81	ASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDKTHTCPPCPAPEFEGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPASIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLS PGA	hIgG1_FES (L234F/L235E/P331 S)
82	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC	hKappa aa sequence
83	ASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEFEGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPASIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG A	hIgG1_FES (L234F/L235E/P331 S)
84	EEELQIIQPDKSVLVAAGETATLRCTITSLFP VGPIQWFRGAGPGRVLIYNQRQGPFPRVTT VSDTTKRNNMDFSIRIGNITPADAGTYYCIK FRKGSPDDVEFKSGAGTELSVRAKPS	SIRPa_CV1
85	DIQMTQSPSSLSASVGDRVTITCRASQDVST AVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYL YHPATFGQGTKVEIK	3280A Light chain variable region
86	EVQLVESGGGLVQPGGSLRLSCAASGFTFS DSWIHWVRQAPGKGLEWVAWISPYGGSTY YADSVKGRFTISADTSKNTAYLQMNSLRAE DTAVYYCARRHWPGGFDYWGQGTLVTVS S	3280A Heavy chain variable region
87	ASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN	hIgG1(N297A)

	TKVDKKVEPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG A	
88	ASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEFEGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPASIEKTISKAKGQPREPQVYTLPPCRDE LTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG A	hIgG1_L234F_L235 E_P331S (knob)S354C/ T366W
89	ASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEFEGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPASIEKTISKAKGQPREPQVCTLPPSRDEL TKNQVSLSCAVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK	hIgG1_L234F_L235 E_P331S(hole) T366S/ L368A/Y407V
90	DIQMTQSPSSLSASVGDRVTITCKASQNVG AAVAWYQQKPGKAPKLLIYSVSDRYTGVPS RFSGSGSGTDFTLTISSLQPEDIATYYCQQYS NYPTFGQGTKLEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGE C	AM4B6-La.4-hKappa
91	QVQLVQSGAEVKKPGASVKVSCKASGYVF TDYYMNWVRQAPGQSLEWMGDINPNNAE TLYNHKFKGRVTVTVDKSISTAYMELSRLR SDDTAVYYCVKWGDGPFAYWGQGTLVTVS	AM4B6_Hg.3_hIgG1 _L234F_L235E_P33 1S-(G4S)4G-SIRPa_ CV1

	SASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEFEGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPASIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG AGGGGSGGGGSGGGGSGGGGSGEEELQII QPDKSVLVAAGETATLRCTITSLFPVGPIQW FRGAGPGRVLIYNQRQGPFPRVTTVSDTTK RNNMDFSIRIGNITPADAGTYYCIKFRKGSP DDVEFKSGAGTELSVRAKPS	
92	DIQMTQSPSSLSASVGDRVTITCRASQDVST AVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYL YHPATFGQGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRG EC	3280A-L-hKappa
93	EVQLVESGGGLVQPGGSLRLSCAASGFTFS DSWIHWVRQAPGKGLEWVAWISPYGGSTY YADSVKGRFTISADTSKNTAYLQMNSLRAE DTAVYYCARRHWPGGFDYWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG AGGGGSGGGGSGGGGSGGGGSGEEELQII QPDKSVLVAAGETATLRCTITSLFPVGPIQW FRGAGPGRVLIYNQRQGPFPRVTTVSDTTK RNNMDFSIRIGNITPADAGTYYCIKFRKGSP DDVEFKSGAGTELSVRAKPS	3280A-H-hIgG1(N297A)-(G4S)4G-SIRPα_CV1

94	QVQLVQSGAEVKKPGASVKVSCKASGYVF TDYYMNWVRQAPGQSLEWMGDINPNNAE TLYNHKFKGRVTVTVDKSISTAYMELSRLR SDDTAVYYCVKWGDGPFAYWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEFEGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPASIEKTISKAKGQPREPQVYTLPPCRDE LTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG AGGGGSGGGGSGGGGSGGGGSGEEELQII QPDKSVLVAAGETATLRCTITSLFPVGPIQW FRGAGPGRVLIYNQRQGPFPRVTTVSDTTK RNNMDFSIRIGNITPADAGTYYCIKFRKGSP DDVEFKSGAGTELSVRAKPS	AM4B6_Hg.3_hIgG1_L234F_L235E_P331S(knob) - SIRPa_CV1
95	QVQLVQSGAEVKKPGASVKVSCKASGYVF TDYYMNWVRQAPGQSLEWMGDINPNNAE TLYNHKFKGRVTVTVDKSISTAYMELSRLR SDDTAVYYCVKWGDGPFAYWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEFEGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPASIEKTISKAKGQPREPQVCTLPPSRDEL TKNQVSLSCAVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK	AM4B6_Hg.3_hIgG1_L234F_L235E_P331S(hole)
96	EVQLVESGGGLVQPGGSLRLSCAASGFTFS DSWIHWVRQAPGKGLEWVAWISPYGGSTY YADSVKGRFTISADTSKNTAYLQMNSLRAE DTAVYYCARRHWPGGFDYWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEFEGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE	3280A_H_hIgG1_L234F_L235E_P331S(knob) - SIRPa_CV1

	DPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPASIEKTISKAKGQPREPQVYTLPPCRDE LTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG AGGGGSGGGGSGGGGSGGGGSGEEELQII QPDKSVLVAAGETATLRCTITSLFPVGPIQW FRGAGPGRVLIYNQRQGPFPRVTTVSDTTK RNNMDFSIRIGNITPADAGTYYCIKFRKGSP DDVEFKSGAGTELSVRAKPS	
97	EVQLVESGGGLVQPGGSLRLSCAASGFTFS DSWIHWVRQAPGKGLEWVAWISPYGGSTY YADSVKGRFTISADTSKNTAYLQMNSLRAE DTAVYYCARRHWPGGFDYWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEFEGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPASIEKTISKAKGQPREPQVCTLPPSRDEL TKNQVSLSCAVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK	3280A_H_hIgG1_L234F_L235E_P331S(hole)
98	EEELQVIQPDKSVLVAAGETATLRCTVTSLI PVGPIQWFRGAGPGRELIYNQKEGHFPRVT TVSDSTKRNNMDFSIRIGNITPADAGTYYC VKFRKGSPDDVEFKSGAGTELSVRAKPS	SIRPa_WT allele 1
99	LQPGAEVPVVWAQEGAPAQLPCSPTIPLQD LSLLRRAGVTWQHQPDSGPPAAAPGHPLA PGPHPAAPSSWGPRPRRYTVLSVGPGGLRS GRLPLQPRVQLDERGRQRGDFSLWLRPARR ADAGEYRAAVHLRDRALSCRLRLRLGQAS MT	LAG3 D1
100	LQPGAEVPVVWAQEGAPAQLPCSPTIPLQD LSLLRRAGVTWQHQPDSGPPAAAPGHPLA PGPHPAAPSSWGPRPRRYTVLSVGPGGLRS GRLPLQPRVQLDERGRQRGDFSLWLRPARR ADAGEYRAAVHLRDRALSCRLRLRLGQAS MTASPPGSLRASDWVILNCSFSRPDRPASV HWFRNRGQGRVPVRESPHHHLAESFLFLPQ VSPMDSGPWGCILTYRDGFNVSIMYNLTVL	LAG3 D1+D2

	GLEPPTPLTVYAGAGSRVGLPC	
101	GPPAAAPGHPLAPGPHPAAPSSWGPRPRR	LAG3 extra-loop
102	QVQLQESGPGLVKPSQTLSLTCSFSGFSLST SGMGVGWIRQPSGKGLEWLAHIWWDGDE SYNPSLKSRLTISKDTSKNQVSLKITSVTAA DTAVYFCARNRYDPPWFVDWGQGTLVTVS S	XOMA052 VH
103	DIQMTQSTSSLSASVGDRVTITCRASQDISN YLSWYQQKPGKAVKLLIYYTSKLHSGVPSR FSGSGSGTDYTLTISSLQQEDFATYFCLQGK MLPWTFGQGTKLEIK	XOMA052 VL
104	TSGMGVG	XOMA052 HCDR1
105	HIWWDGDESYNPSLKS	XOMA052 HCDR2
106	NRYDPPWFVD	XOMA052 HCDR3
107	RASQDISNYLS	XOMA052 LCDR1
108	YTSKLHS	XOMA052 LCDR2
109	LQGKMLPWT	XOMA052 LCDR3
110	QVQLVESGGGVVQPGRSLRLSCAASGFTFS VYGMNWVRQAPGKGLEWVAIIWYDGDN QYYADSVKGRFTISRDNSKNTLYLQMNGL RAEDTAVYYCARDLRTGPFDYWGQGTLV TVSS	ACZ885 VH
111	EIVLTQSPDFQSVTPKEKVTITCRASQSIGSS LHWYQQKPDQSPKLLIKYASQSFSGVPSRF SGSGSGTDFTLTINSLEAEDAAAYYCHQSS SLPFTFGPGTKVDIK	ACZ885 VL
112	VYGMN	ACZ885 HCDR1

113	IIWYDGDNQYYADSVKG	ACZ885 HCDR2
114	DLRTGPFDY	ACZ885 HCDR3
115	RASQSIGSSLH	ACZ885 LCDR1
116	YASQSFS	ACZ885 LCDR2
117	HQSSSLPFT	ACZ885 LCDR3
118	QVQLQESGPGLVKPSQTLSLTCSFSGFSLST SGMGVGWIRQPSGKGLEWLAHIWWDGDE SYNPSLKSRLTISKDTSKNQVSLKITSVTAA DTAVYFCARNRYDPPWFVDWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEFEGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPASIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG AGGGGSGGGGSGGGGSGGGGSQVQLVQS GAEVKKPGASVKVSCKASGYVFTDYYMN WVRQAPGQSLEWMGDINPNNAETLYNHK FKGRVTVTVDKSISTAYMELSRLRSDDTAV YYCVKWGDGPFAYWGQGTLVTVSSGGGG SGGGGSGGGGSGGGGSDIQMTQSPSSLSAS VGDRVTITCKASQNVGAAVAWYQQKPGKA PKLLIYSVSDRYTGVPSRFSGSGSGTDFTLTI SSLQPEDIATYYCQQYSNYPTFGQGTKLTV L	IgG-scFv-XOMA052 -AM4B6 HC
119	DIQMTQSTSSLSASVGDRVTITCRASQDISN YLSWYQQKPGKAVKLLIYYTSKLHSGVPSR FSGSGSGTDYTLTISSLQQEDFATYFCLQGK MLPWTFGQGTKLEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDN	IgG-scFv-XOMA052 -AM4B6 LC

	ALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRG EC	
120	QVQLVESGGGVVQPGRSLRLSCAASGFTFS VYGMNWVRQAPGKGLEWVAIIWYDGDNQ YYADSVKGRFTISRDNSKNTLYLQMNGLR AEDTAVYYCARDLRTGPFDYWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDKTHTCPPCPAPEFEGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPASIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP GAGGGGSGGGGSGGGGSGGGGSQVQLVQ SGAEVKKPGASVKVSCKASGYVFTDYYM NWVRQAPGQCLEWMGDINPNNAETLYNH KFKGRVTVTVDKSISTAYMELSRLRSDDTA VYYCVKWGDGPFAYWGQGTLVTVSSGGG GSGGGGSGGGGSGGGGSDIQMTQSPSSLSA SVGDRVTITCKASQNVGAAVAWYQQKPGK APKLLIYSVSDRYTGVPSRFSGSGSGTDFTL TISSLQPEDIATYYCQQYSNYPTFGCGTKLT VL	IgG-scFv-ACZ885-A M4B6 HC
121	EIVLTQSPDFQSVTPKEKVTITCRASQSIGSS LHWYQQKPDQSPKLLIKYASQSFSGVPSRF SGSGSGTDFTLTINSLEAEDAAAYYCHQSSS LPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNAL QSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGE C	IgG-scFv-ACZ885-A M4B6 LC
122	GGGGSGGGGSGGGGSGGGGS	G4S

WHAT IS CLAIMED IS:

1. A bi-functional molecule comprising a first moiety that binds to an immune checkpoint molecule, and a second moiety that blocks activity of Interleukin-1 (IL-1).

2. The bi-functional molecule of claim 1, wherein the first moiety comprises an agonist of immunostimulatory check point molecule, optionally selected from the group consisting of: CD27, CD70, CD28, CD80 (B7-1), CD86 (B7-2), CD40, CD40L (CD154), CD122, CD137, CD137L, OX40 (CD134), OX40L (CD252), GITR, ICOS (CD278), and ICOSLG (CD275), CD2, , ICAM-1, LFA-1 (CD11a/CD18), CD30, BAFFR, HVEM, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, and CD83.

3. The bi-functional molecule of claim 1, wherein the first moiety comprises an antagonist of immunoinhibitory check point molecule, optionally selected from the group consisting of: A2AR, B7-H3 (CD276), B7-H4 (VTCN1), BTLA (CD272), CTLA-4 (CD152), IDO1, IDO2, TDO, KIR, LAG3, NOX2, PD-1, PD-L1, PD-L2, TIM-3, VISTA, SIGLEC7 (CD328), TIGIT, PVR(CD155), SIGLEC9 (CD329), CD160, LAIR1, 2B4 (CD244), CD47, B7-H5.

4. The bi-functional molecule of claim 3, wherein the immune checkpoint molecule is PD-L1.

5. The bi-functional molecule of any of the preceding claims, wherein the first moiety comprises an antibody against PD-L1 or an antigen-binding fragment thereof, and the second moiety comprises an IL-1-binding moiety or an IL-1 Receptor (IL-1R)-binding moiety.

6. The bi-functional molecule of claim 5, wherein the IL-1-binding moiety comprises an IL-1R or a fragment or variant thereof, or an antibody against IL-1 or an antigen-binding fragment thereof.

7. The bi-functional molecule of claim 6, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises a heavy chain variable region and/or a light variable region from an anti-IL-1α antibody selected from the group consisting of: XB2001, lutikizumab, LY2189102 and bermekimab, or from an anti-IL-1β

antibody selected from the group consisting of: SSGJ-613, CDP484, canakinumab and gevokizumab.

8. The bi-functional molecule of claim 6, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 104 or SEQ ID NO: 112, a HCDR2 comprising a sequence of SEQ ID NO: 105 or SEQ ID NO: 113, and a HCDR3 comprising a sequence of SEQ ID NO: 106 or SEQ ID NO: 114, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 107 or SEQ ID NO: 115, a LCDR2 comprising a sequence of SEQ ID NO: 108 or SEQ ID NO: 116, and a LCDR3 comprising a sequence of SEQ ID NO: 109 or SEQ ID NO: 117.

9. The bi-functional molecule of claim 6, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 104, a HCDR2 comprising a sequence of SEQ ID NO: 105, and a HCDR3 comprising a sequence of SEQ ID NO: 106, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 107, a LCDR2 comprising a sequence of SEQ ID NO: 108, and a LCDR3 comprising a sequence of SEQ ID NO: 109.

10. The bi-functional molecule of claim 6, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 112, a HCDR2 comprising a sequence of SEQ ID NO: 113, and a HCDR3 comprising a sequence of SEQ ID NO: 114, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 115, a LCDR2 comprising a sequence of SEQ ID NO: 116, and a LCDR3 comprising a sequence of SEQ ID NO: 117.

11. The bi-functional molecule of claim 6, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 102, SEQ

ID NO: 110, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 103, SEQ ID NO: 111, and a homologous sequence thereof having at least 80% sequence identity thereof.

12. The bi-functional molecule of claim 6, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 102, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 103, and a homologous sequence thereof having at least 80% sequence identity thereof.

13. The bi-functional molecule of claim 6, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 110, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 111, and a homologous sequence thereof having at least 80% sequence identity thereof.

14. The bi-functional molecule of claim 5, wherein the IL-1R-binding moiety comprises Interleukin-1 receptor antagonist or a fragment or variant thereof, or an antibody against IL-1R or an antigen-binding fragment thereof.

15. The bi-functional molecule of claim 14, wherein the antibody against IL-1R or an antigen-binding fragment thereof comprises a heavy chain variable region and/or a light variable region from an antibody selected from the group consisting of: spesolimab, astegolimab, imsidolimab, AMG 108, melrilimab, nidanilimab, MEDI8968, REGN6490, HB0034 and CSC012.

16. A bi-functional molecule comprising a first moiety that binds to PD-L1, and a second moiety that a) blocks activity of an immunosuppressive cytokine or b)

stimulates immunity, wherein the first moiety comprises an antibody against PD-L1 or an antigen-binding fragment thereof comprising a heavy chain variable (VH) region and/or a light chain variable (VL) region, wherein the heavy chain variable region comprises:

a) a HCDR1 comprising **DYYMN** (SEQ ID NO: 1) or a homologous sequence of at least 80% sequence identity thereof,

b) a HCDR2 comprising **DINPNNX$_1$X$_2$TX$_3$YNHKFKG** (SEQ ID NO: 19) or a homologous sequence of at least 80% sequence identity thereof, and

c) a HCDR3 comprising **WGDGPFAY** (SEQ ID NO: 3) or a homologous sequence of at least 80% sequence identity thereof, and/or

wherein the light chain variable region comprises:

d) a LCDR1 comprises a sequence selected from the group consisting of **KASQNVX$_4$X$_5$X$_6$VA** (SEQ ID NO: 20) or a homologous sequence of at least 80% sequence identity thereof,

e) a LCDR2 comprises a sequence selected from the group consisting of **SX$_7$SX$_8$RYT** (SEQ ID NO: 21) or a homologous sequence of at least 80% sequence identity thereof, and

f) a LCDR3 comprises a sequence selected from the group consisting of **QQYSNYPT** (SEQ ID NO: 6) or a homologous sequence of at least 80% sequence identity thereof;

wherein X_1 is G or A, X_2 is G or D or Q or E or L, X_3 is S or M or Q or L or V, X_4 is G or P or K, X_5 is A or G, X_6 is A or I, X_7 is A or N or R or V, and X_8 is N or H or V or D.

17. The bi-functional molecule of claim 16, wherein the heavy chain variable region comprises:

a) a HCDR1 comprises a sequence of SEQ ID NO: 1,

b) a HCDR2 comprises a sequence selected from group consisting of SEQ ID NO: 2, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 17, and SEQ ID NO: 18 and

c) a HCDR3 comprises a sequence of SEQ ID NO: 3,

and/or

a light chain variable region comprising:

d) a LCDR1 comprises a sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9,

e) a LCDR2 comprises a sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, and

f) a LCDR3 comprises a sequence of SEQ ID NO: 6.

18. The bi-functional molecule of claim 17, wherein the heavy chain variable region is selected from the group consisting of:

a) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 2, and a HCDR3 comprising the sequence of SEQ ID NO: 3;

b) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 13, and a HCDR3 comprising the sequence of SEQ ID NO: 3;

c) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 14, and a HCDR3 comprising the sequence of SEQ ID NO: 3;

d) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 15, and a HCDR3 comprising the sequence of SEQ ID NO: 3; and

e) a heavy chain variable region comprising a HCDR1 comprising the sequence of SEQ ID NO: 1, a HCDR2 comprising the sequence of SEQ ID NO: 17, and a HCDR3 comprising the sequence of SEQ ID NO: 3.

19. The bi-functional molecule of claim 17 or 18, wherein the light chain variable region is selected from the group consisting of:

a) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 4, a LCDR2 comprising the sequence of SEQ ID NO: 5, and

a LCDR3 comprising the sequence of SEQ ID NO: 6;

b) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 9, a LCDR2 comprising the sequence of SEQ ID NO: 5, and a LCDR3 comprising the sequence of SEQ ID NO: 6;

c) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 8, a LCDR2 comprising the sequence of SEQ ID NO: 5, and a LCDR3 comprising the sequence of SEQ ID NO: 6;

d) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 4, a LCDR2 comprising the sequence of SEQ ID NO: 12, and a LCDR3 comprising the sequence of SEQ ID NO: 6; and

e) a light chain variable region comprising a LCDR1 comprising the sequence of SEQ ID NO: 4, a LCDR2 comprising the sequence of SEQ ID NO: 11, and a LCDR3 comprising the sequence of SEQ ID NO: 6.

20. The bi-functional molecule of any of claims 16-19, wherein the antibody against PD-L1 or the antigen-binding fragment thereof further comprises one or more of heavy chain HFR1, HFR2, HFR3 and HFR4, and/or one or more of light chain LFR1, LFR2, LFR3 and LFR4, wherein:

a) the HFR1 comprises **QVQLVQSGAEVKKPGASVKVSCKASGYX$_9$FT** (SEQ ID NO: 40) or a homologous sequence of at least 80% sequence identity thereof,

b) the HFR2 comprises **WVRQAPGQX$_{10}$LEWMG** (SEQ ID NO: 41) or a homologous sequence of at least 80% sequence identity thereof,

c) the HFR3 sequence comprises **RVTX$_{16}$TVDX$_{11}$SISTAYMELSRLRSDDTAVYYCX$_{12}$X$_{13}$** (SEQ ID NO: 42) or a homologous sequence of at least 80% sequence identity thereof,

d) the HFR4 comprises **WGQGTLVTVSS** (SEQ ID NO: 25) or a homologous sequence of at least 80% sequence identity thereof,

e) the LFR1 comprises **DIQMTQSPSSLSASVGDRVTITC** (SEQ ID NO: 26) or a homologous sequence of at least 80% sequence identity thereof,

f) the LFR2 comprises **WYQQKPGKX$_{14}$PKLLIY** (SEQ ID NO: 43) or a

homologous sequence of at least 80% sequence identity thereof,

g) the LFR3 comprises **GVPX$_{15}$RFSGSGSGTDFTX$_{17}$TISSLQPEDIATYYC** (SEQ ID NO: 44) or a homologous sequence of at least 80% sequence identity thereof, and

h) the LFR4 comprises **FGQGTKLEIK** (SEQ ID NO: 29) or a homologous sequence of at least 80% sequence identity thereof,

wherein X_9 is T or V, X_{10} is G or S, X_{11} is T or K, X_{12} is A or V, X_{13} is R or K, X_{14} is A or S, X_{15} is S or D, X_{16} is M or V, and X_{17} is F or L.

21. The bi-functional molecule of claim 20, wherein:

the HFR1 comprises a sequence selected from the group consisting of SEQ ID NOs: 22 and 30,

the HFR2 comprises a sequence selected from the group consisting of SEQ ID NOs: 23 and 31,

the HFR3 comprises the sequence selected from the group consisting of SEQ ID NOs: 24 and 32-35,

the HFR4 comprises a sequence of SEQ ID NOs: 25,

the LFR1 comprises the sequence from the group consisting of SEQ ID NO: 26,

the LFR2 comprises a sequence selected from the group consisting of SEQ ID NOs: 27 and 36,

the LFR3 comprises a sequence selected from the group consisting of SEQ ID NOs: 28, and 37-38, 39, 45, and

the LFR4 comprises a sequence of SEQ ID NO: 29.

22. The bi-functional molecule of any of claims 16-21, wherein the heavy chain variable region comprises a sequence selected from the group consisting of SEQ ID NO: 46, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, and a homologous sequence thereof having at least 80% sequence identity thereof.

23. The bi-functional molecule of any of claims 16-22, wherein the light chain variable region comprises a sequence selected from the group consisting of SEQ ID

NO: 47, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, and a homologous sequence thereof having at least 80% sequence identity thereof.

24. The bi-functional molecule of claim 16, wherein the antibody against PD-L1 or antigen-binding fragment thereof comprises a pair of heavy chain variable region and light chain variable region sequences selected from the group consisting of: SEQ ID NOs: 49/54, 50/54, 51/54, 52/54, 49/55, 50/55, 51/55, 52/55, 58/62, 58/63, 58/64, 58/65, 59/62, 59/63, 59/64, 59/65, 60/62, 60/63, 60/64, and 60/65.

25. The bi-functional molecule of any of claims 16-24, wherein the antibody against PD-L1 or antigen-binding fragment thereof further comprises one or more amino acid residue substitutions or modifications yet retains specific binding specificity and/or affinity to PD-L1.

26. The bi-functional molecule of claim 25, wherein at least one of the substitutions or modifications is in one or more of the CDR sequences, and/or in one or more of the non-CDR regions of the VH or VL sequences.

27. The bi-functional molecule of any of claims 16-26, wherein the antibody against PD-L1 or antigen-binding fragment thereof further comprises an immunoglobulin constant region, optionally a constant region of human Ig, or optionally a constant region of human IgG.

28. The bi-functional molecule of claim 27, wherein the constant region comprises an Fc region of human IgG1, IgG2, IgG3, or IgG4.

29. The bi-functional molecule of claim 28, wherein the constant region comprises an Fc variant having reduced effector function relative to the corresponding wildtype Fc region.

30. The bi-functional molecule of claim 29, wherein the Fc variant comprises one or more amino acid residue substitutions selected from the group consisting of: 220S, 226S, 228P, 229S, 233P, 234V, 234G, 234A, 234F, 234A, 235A, 235G, 235E, 236E,

236R, 237A, 237K, 238S, 267R, 268A, 268Q, 269R, 297A, 297Q, 297G, 309L, 318A, 322A, 325L, 328R, 330S, 331S and any combination thereof, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat.

31. The bi-functional molecule of any of claims 29-30, wherein the Fc variant comprises a combination of mutations selected from the group consisting of: a) K322A, L234A, and L235A; b) P331S, L234F, and L235E; c) L234A and L235A; c) N297A; d) N297Q; e) N297G; f) L235E; g) L234A and L235A (IgG1); h) F234A and L235A (IgG4); i) H268Q, V309L, A330S and P331S (IgG2); j) V234A, G237A, P238S, H268A, V309L, A330S and P331S (IgG2), wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat.

32. The bi-functional molecule of any of claims 29 – 31, wherein the Fc variant comprises an amino acid sequence of SEQ ID NO: 81.

33. The bi-functional molecule of any of claims 16-32 wherein the antibody against PD-L1 or antigen-binding fragment thereof is humanized.

34. The bi-functional molecule of any of claims 16-33, wherein the antigen-binding fragment is a diabody, a Fab, a Fab', a $F(ab')_2$, a Fd, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multispecific antibody, a camelized single domain antibody, a nanobody, a domain antibody, and a bivalent domain antibody.

35. The bi-functional molecule of any of claims 16-34, wherein the antibody or antigen-binding fragment thereof is capable of binding to both human PD-L1 and cyno PD-L1.

36. The bi-functional molecule of any of claims 1-15, wherein the first moiety comprises an antibody or an antigen-binding fragment thereof that competes for binding to PD-L1 with the antibody or antigen-binding fragment thereof of any of claims 16-35.

37. The bi-functional molecule of any of claims 16-36, wherein the immunosuppressive cytokine comprises a cytokine in transforming growth factor beta (TGF-β) superfamily, IL-1, or Vascular endothelial growth factor (VEGF).

38. The bi-functional molecule of claim 37, wherein the immunosuppressive cytokine in TGF-β superfamily includes TGF-β, bone morphogenetic proteins (BMPs), activins, NODAL, and growth and differentiation factors (GDFs).

39. The bi-functional molecule of any one of claims 16-38, wherein the immunosuppressive cytokine is TGF-β.

40. The bi-functional molecule of claim 16-39, wherein the second moiety comprises a TGFβ-binding moiety.

41. The bi-functional molecule of claim 40, wherein the TGFβ-binding moiety comprises a soluble TGFβ Receptor (TGFβR) or a TGFβ-binding fragment or variant thereof, or an antibody against TGFβ and an antigen-binding fragment thereof.

42. The bi-functional molecule of claim 41, wherein the soluble TGFβR comprises an extracellular domain (ECD) of the TGFβR, or a TGFβ-binding fragment or a variant thereof.

43. The bi-functional molecule of claim 42, wherein the TGFβR is selected from the group consisting of TGFβ Receptor I (TGFβRI), TGFβ Receptor II (TGFβRII), TGFβ Receptor III (TGFβRIII), and any combination thereof.

44. The bi-functional molecule of claim 42, wherein the TGFβR is TGFβRII.

45. The bi-functional molecule of claim 44, wherein the TGFβRII selectively binds to TGFβ1 over TGFβ2 and TGFβ3.

46. The bi-functional molecule of claim 45, wherein the TGFβ1 is human TGFβ1 or mouse TGFβ1.

47. The bi-functional molecule of any of claims 42-46, wherein the ECD of TGFβR comprises an amino acid sequence of SEQ ID NO: 66, 79, 78, 77 or a sequence

having at least 80% sequence identity thereof yet retains specific binding specificity and/or affinity to TGF-β.

48. The bi-functional molecule of claim 36, wherein the second moiety comprises an IL-1-binding moiety or an IL-1 Receptor (IL-1R)-binding moiety.

49. The bi-functional molecule of claim 48, wherein the IL-1-binding moiety comprises a soluble IL-1R, an IL-1-binding fragment or variant of an IL-1R, or an antibody against IL-1 or an antigen-binding fragment thereof.

50. The bi-functional molecule of claim 49, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises a heavy chain variable region and/or a light variable region from an anti-IL-1α antibody selected from the group consisting of: XB2001, lutikizumab, LY2189102 and bermekimab, or from an anti-IL-1β antibody selected from the group consisting of: SSGJ-613, CDP484, canakinumab and gevokizumab.

51. The bi-functional molecule of claim 49, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 104 or SEQ ID NO: 112, a HCDR2 comprising a sequence of SEQ ID NO: 105 or SEQ ID NO: 113, and a HCDR3 comprising a sequence of SEQ ID NO: 106 or SEQ ID NO: 114, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 107 or SEQ ID NO: 115, a LCDR2 comprising a sequence of SEQ ID NO: 108 or SEQ ID NO: 116, and a LCDR3 comprising a sequence of SEQ ID NO: 109 or SEQ ID NO: 117.

52. The bi-functional molecule of claim 49, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 104, a HCDR2 comprising a sequence of SEQ ID NO: 105, and a HCDR3 comprising a sequence of SEQ ID NO: 106, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 107, a LCDR2 comprising a sequence of SEQ

ID NO: 108, and a LCDR3 comprising a sequence of SEQ ID NO: 109.

53. The bi-functional molecule of claim 49, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a HCDR1 comprising a sequence of SEQ ID NO: 112, a HCDR2 comprising a sequence of SEQ ID NO: 113, and a HCDR3 comprising a sequence of SEQ ID NO: 114, and/or a light chain variable region comprising a LCDR1 comprising a sequence of SEQ ID NO: 115, a LCDR2 comprising a sequence of SEQ ID NO: 116, and a LCDR3 comprising a sequence of SEQ ID NO: 117.

54. The bi-functional molecule of claim 49, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 102, SEQ ID NO: 110, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 103, SEQ ID NO: 111, and a homologous sequence thereof having at least 80% sequence identity thereof.

55. The bi-functional molecule of claim 49, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 102, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 103, and a homologous sequence thereof having at least 80% sequence identity thereof.

56. The bi-functional molecule of claim 49, wherein the antibody against IL-1 or an antigen-binding fragment thereof comprises: a heavy chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 110, and a homologous sequence thereof having at least 80% sequence identity thereof, and/or a light chain variable region comprising a sequence selected from the group consisting of SEQ ID NO: 111, and a homologous sequence thereof having at least 80%

sequence identity thereof.

57. The bi-functional molecule of claim 49, wherein the bi-functional molecule comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 118 or SEQ ID NO: 120, and/or a light chain comprising an amino acid sequence of SEQ ID NO: 119 or SEQ ID NO: 121.

58. The bi-functional molecule of claim 49, wherein the IL-1-binding moiety comprises an extracellular domain (ECD) of the IL-1RI, an IL-1-binding fragment or variant of any of IL-1RI, ECD of IL-1RI, IL-1RII, or ECD of IL-1RII, or IL-1RAP, or ECD of IL-1RAP, IL-1sRI or IL-1sRII.

59. The bi-functional molecule of claim 48, wherein the IL-1R-binding moiety comprises IL-1Ra or an IL-1-binding fragment or variant thereof, or an antibody against IL-1R or an antigen-binding fragment thereof.

60. The bi-functional molecule of claim 59, wherein the antibody against IL-1R or an antigen-binding fragment thereof comprises a heavy chain variable region and/or a light variable region from an antibody selected from the group consisting of: spesolimab, astegolimab, imsidolimab, AMG 108, melrilimab, nidanilimab, MEDI8968, REGN6490, HB0034 and CSC012.

61. The bi-functional molecule of claim 60, wherein the IL-1R-binding moiety comprises an amino acid sequence of SEQ ID NO: 67 or 76, or an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 67 or 76, or an IL-1 binding fragment or variant thereof.

62. The bi-functional molecule of any of claims 48-49, wherein the IL-1 is IL-1α or IL-1β.

63. The bi-functional molecule of claim 62, wherein the IL-1β is human IL-1β.

64. The bi-functional molecule of any of claims 16-36, wherein the second moiety stimulates anti-tumor immunity and comprises an immunostimulatory polypeptide.

65. The bi-functional molecule of claim 64, wherein the immunostimulatory

polypeptide comprises Interleukin (IL)-2 (IL-2), IL-15, IL-21, IL-10, IL-12, IL-23, IL-27, IL-35, granulocyte-macrophage colony-stimulating factor (GM-CSF), soluble CD4, soluble LAG-3, IFN-α, or a functional equivalent thereof.

66. The bi-functional molecule of claim 65, wherein the soluble LAG-3 comprises an extracellular domain (ECD) of the LAG-3 or a MHCII-binding variant thereof.

67. The bi-functional molecule of any of claims 16-36, wherein the second moiety stimulates anti-tumor immunity and comprises an antagonist of an immunoinhibitory receptor signaling.

68. The bi-functional molecule of claim 67, wherein the immunoinhibitory receptor is Signal-regulatory protein alpha (SIRPα).

69. The bi-functional molecule of claim 68, wherein the second moiety blocks interaction between CD47 and SIRPα.

70. The bi-functional molecule of claim 69, wherein the second moiety comprises a CD47 binding domain or a SIRPα binding domain.

71. The bi-functional molecule of claim 70, wherein the CD47 binding domain comprises a soluble SIRPα or a CD47 binding fragment or variant thereof, or an anti-CD47 antibody or an antigen-binding fragment thereof.

72. The bi-functional molecule of claim 71, wherein the soluble SIRPα comprises an extracellular domain (ECD) of the SIRPα, or a CD47-binding variant thereof.

73. The bi-functional molecule of claim 70, wherein the CD47 binding domain comprises an amino acid sequence of SEQ ID NO: 84 or an amino acid sequence having at least 80% sequence identity thereof yet retaining binding specificity to CD47.

74. The bi-functional molecule of claim 70, wherein the SIRPα binding domain comprises a soluble CD47 or a SIRPα binding fragment or variant thereof, or an anti-SIRPα antibody or an antigen-binding fragment thereof.

75. The bi-functional molecule of claim 74 wherein the soluble CD47 comprises an extracellular domain (ECD) of the CD47 or a SIRPα binding fragment or variant thereof, an anti-SIRPα antibody or an antigen-binding fragment thereof.

76. The bi-functional molecule of any of the preceding claims, further comprising a linker connecting the first moiety and the second moiety.

77. The bi-functional molecule of claim 76, wherein the linker is selected from the group consisting of a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, and a non-helical linker.

78. The bi-functional molecule of claim 77, wherein the linker comprising an amino acid sequence of ((G)nS)m, wherein m and n are independently an integer selected from 0 to 30.

79. The bi-functional molecule of any of claims 16-78, wherein the bi-functional molecule comprises one or more of the second moieties.

80. The bi-functional molecule of claim 79, wherein at least one of the second moieties is linked to an N terminus or a C terminus of a polypeptide chain of the first moiety.

81. The bi-functional molecule of claim 79, wherein at least one of the second moieties is linked to: a) an N terminus or a C terminus of a heavy chain of the first moiety, or b) an N terminus or a C terminus of a light chain of the first moiety.

82. The bi-functional molecule of claim 79, wherein at least one of the second moieties is linked to a C terminus of a heavy chain constant region of the first moiety.

83. The bi-functional molecule of claim 79, wherein each of the second moieties is linked respectively to the C terminus of each heavy chain constant region of the first moiety.

84. The bi-functional molecule of claim 79, wherein the bi-functional molecule comprises more than one of the second moieties that are linked respectively to: an N terminus of a heavy chain of the first moiety, a C terminus of a heavy chain of the

first moiety, an N terminus of a light chain of the first moiety, a C terminus of a light chain of the first moiety, or any combination thereof.

85. The bi-functional molecule of any one of claims 79-84, wherein the bi-functional molecule comprises homodimeric or heterodimeric heavy chains.

86. The bi-functional molecule of any one of claims 79-84, wherein the heavy chains are heterodimeric with respect to presence or position of the second moiety.

87. The bi-functional molecule of claim 86, wherein the heterodimeric heavy chains comprise one heavy chain having the second moiety but the other heavy chain having not.

88. The bi-functional molecule of claim 86, wherein the heterodimeric heavy chains further comprise heterodimeric Fc regions that associate in a way that discourages homodimerization and/or favors heterodimerization.

89. The bi-functional molecule of claim 86, wherein the heterodimeric Fc regions are capable of associating into heterodimers via knobs-into-holes, hydrophobic interaction, electrostatic interaction, hydrophilic interaction, or increased flexibility.

90. The bi-functional molecule of any of claims 88-89, wherein the heterodimeric Fc regions comprises Y349C, T366S, L368A or Y407V or any combination thereof in one Fc polypeptide chain, and S354C, or T366W or combination thereof in another Fc polypeptide chain, wherein the numbering of the residues in the Fc polypeptide chain is that of the EU index as in Kabat.

91. The bi-functional molecule of any of the preceding claims, further linked to one or more conjugate moieties.

92. The bi-functional molecule of claim 91, wherein the conjugate moiety comprises a clearance-modifying agent, a chemotherapeutic agent, a toxin, a radioactive isotope, a lanthanide, a luminescent label, a fluorescent label, an enzyme-substrate label, a DNA-alkylator, a topoisomerase inhibitor, a tubulin-binders, or other anticancer drugs such as androgen receptor inhibitor.

93. A pharmaceutical composition or kit comprising the bi-functional molecule of any of the preceding claims, and a pharmaceutically acceptable carrier.

94. An isolated polynucleotide encoding the bi-functional molecule of any one of claims 16-92.

95. A vector comprising the isolated polynucleotide of claim 94.

96. A host cell comprising the vector of claim 95.

97. A method of expressing the bi-functional molecule of any one of claims 16-92, comprising culturing the host cell of claim 96 under the condition at which the vector of claim 95 is expressed.

98. A method of treating, preventing or alleviating a PD-L1 related disease in a subject, comprising administering to the subject a therapeutically effective amount of the bi-functional molecule of any one of claims 16-92 and/or the pharmaceutical composition or kit of claim 93.

99. The method of claim 98, wherein the disease is immune related disease or disorder, cancers, or infectious disease.

100. The method of claim 99, wherein the cancer is selected from the group consisting of: lung cancer (e.g., non-small cell lung cancer), liver cancer, pancreatic cancer, breast cancer, bronchial cancer, bone cancer, liver and bile duct cancer, ovarian cancer, testicle cancer, kidney cancer, bladder cancer, head and neck cancer, spine cancer, brain cancer, cervix cancer, uterine cancer, endometrial cancer, colon cancer, colorectal cancer, prostate cancer, gastric-esophageal cancer, rectal cancer, anal cancer, gastrointestinal cancer, skin cancer, pituitary cancer, stomach cancer, vagina cancer, thyroid cancer, glioblastoma, astrocytoma, melanoma, myelodysplastic syndrome, sarcoma, teratoma, glioma, and adenocarcinoma.

101. The method of any of claims 98-100, wherein the subject has been identified as having a PD-L1-expressing cancer cell.

102. The method of any of claims 98-101, wherein the PD-L1 related disease is

resistant to PD-L1/PD-1 mono therapy.

103. The method of any of claims 98-102, wherein the subject is human.

104. The method of any of claims 98-103, further comprising administering a therapeutically effective amount of a second therapeutic agent.

105. The method of claim 104, wherein the second therapeutic agent is selected from a chemotherapeutic agent, an anti-cancer drug, radiation therapy, an immunotherapy agent, anti-angiogenesis agent, a targeted therapy agent, a cellular therapy agent, a gene therapy agent, a hormonal therapy agent, or cytokines.

106. Use of the bi-functional molecule of any of claims 16-92 in the manufacture of a medicament for treating a PD-L1 related disease or condition in a subject.

107. A method of treating, preventing or alleviating in a subject a disease or condition that would benefit from suppression of an immunosuppressive cytokine, from induction of sustained immune responses, or from stimulation of anti-tumor immunity, comprising administering an effective amount of the bi-functional molecule of any of claims 16-92.

108. The method of claim 107, wherein the immunosuppressive cytokine is TGFβ.

109. The method of claim 108, wherein the disease or condition is a TGFβ-related disease or condition.

110. The method of claim 109, wherein the TGFβ-related disease is cancer, fibrotic disease, or kidney disease.

111. The method of claim 107, wherein the immunosuppressive cytokine is IL-1.

112. The method of claim 111, wherein the disease or condition is an IL-1-related disease or condition.

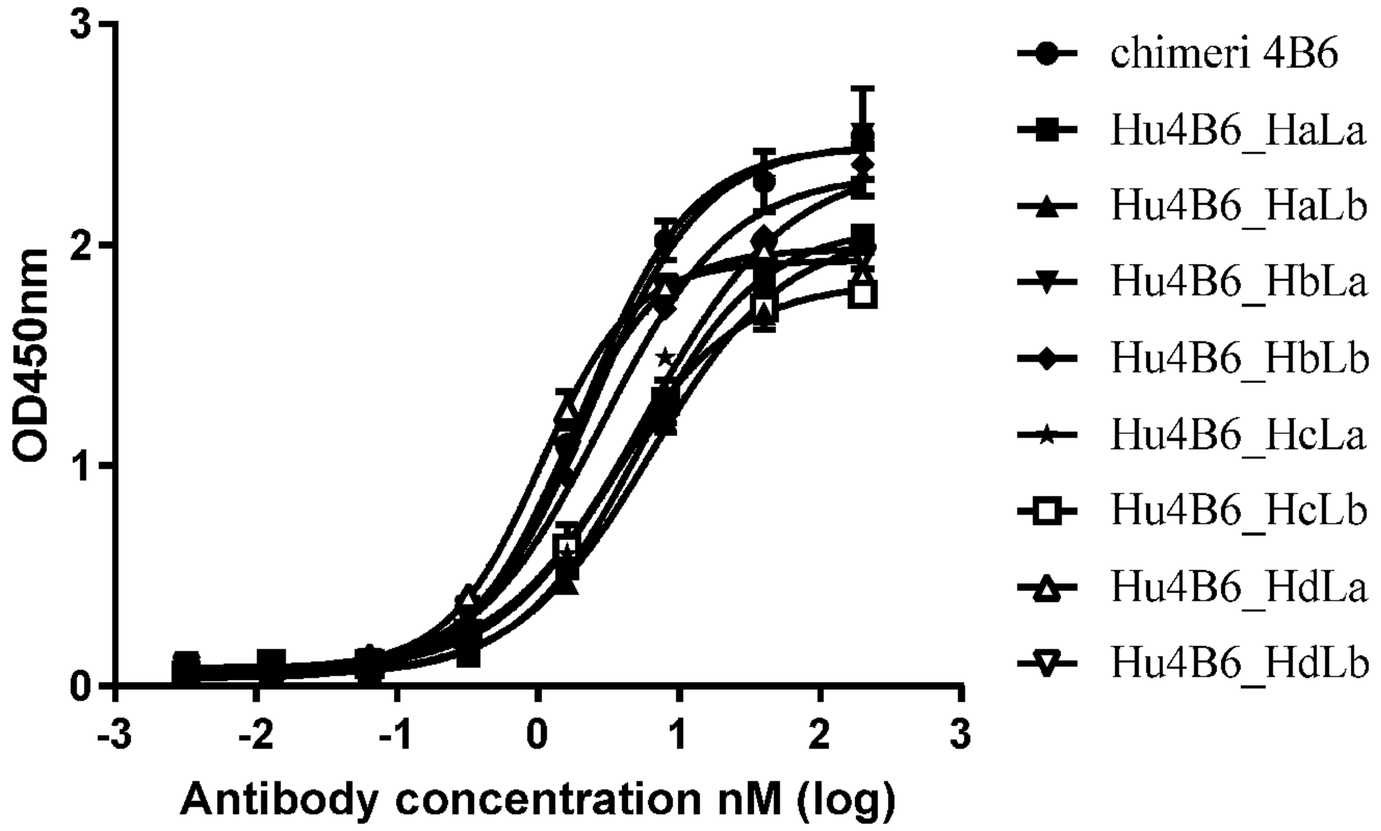
113. The method of claim 107, wherein the disease or condition would benefit from induction of sustained immune responses by stimulating MHCII signaling with

an immunostimulatory polypeptide.

114. The method of claim 113, wherein the immunostimulatory polypeptide is a soluble LAG-3.

115. The method of claim 107, wherein the disease or condition would benefit from stimulation of anti-tumor immunity by inhibiting an immunoinhibitory receptor signaling.

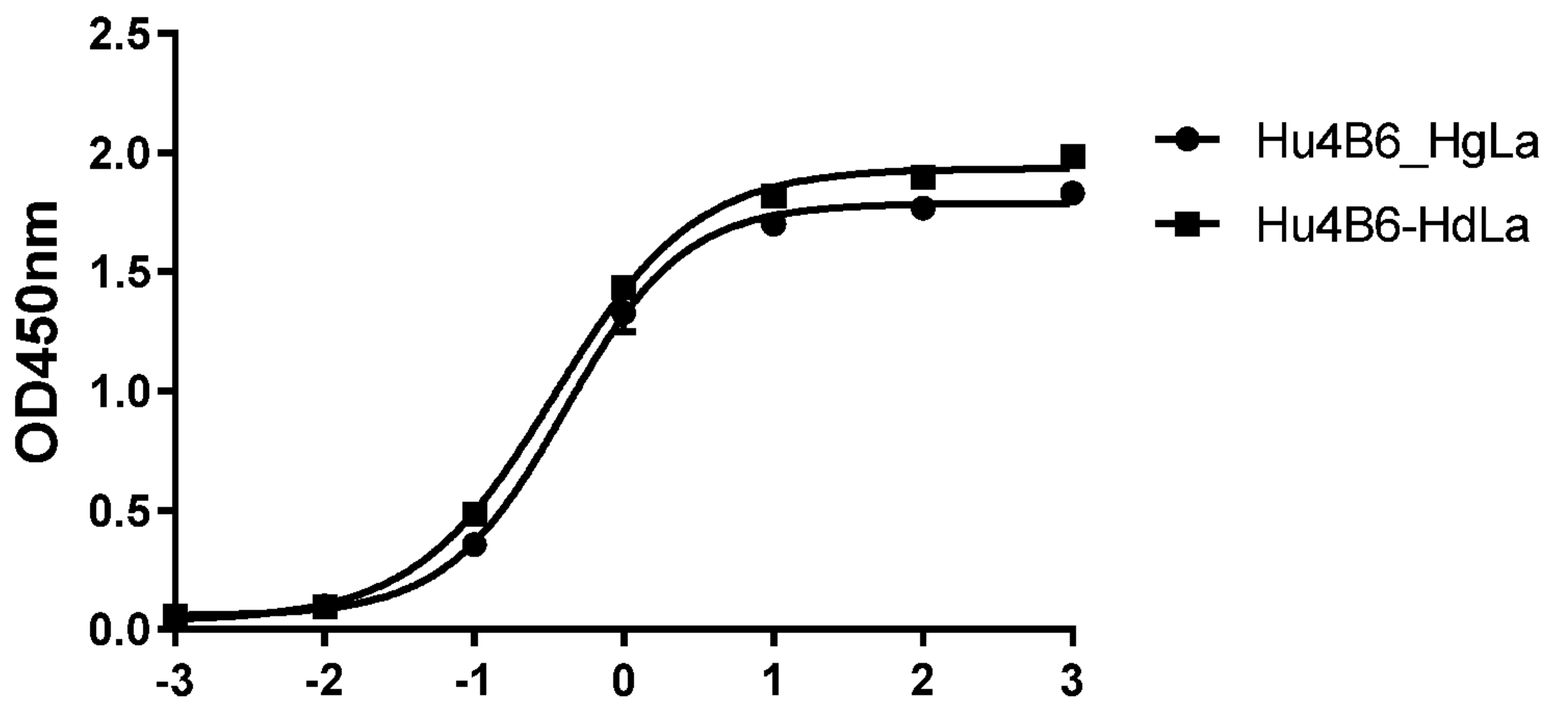
116. The method of claim 115, wherein the immunoinhibitory receptor is SIRPα.



	chimeri 4B6	Hu4B6_HaLa	Hu4B6_HaLb	Hu4B6_HbLa	Hu4B6_HbLb
EC50	2.047	5.158	6.263	2.375	2.805

Hu4B6_HcLa	Hu4B6_HcLb	Hu4B6_HdLa	Hu4B6_HdLb
5.738	3.437	1.042	1.465

Figure 1



	Hu4B6_HgLa	Hu4B6_HdLa
EC50	0.4069	0.3469

Figure 2

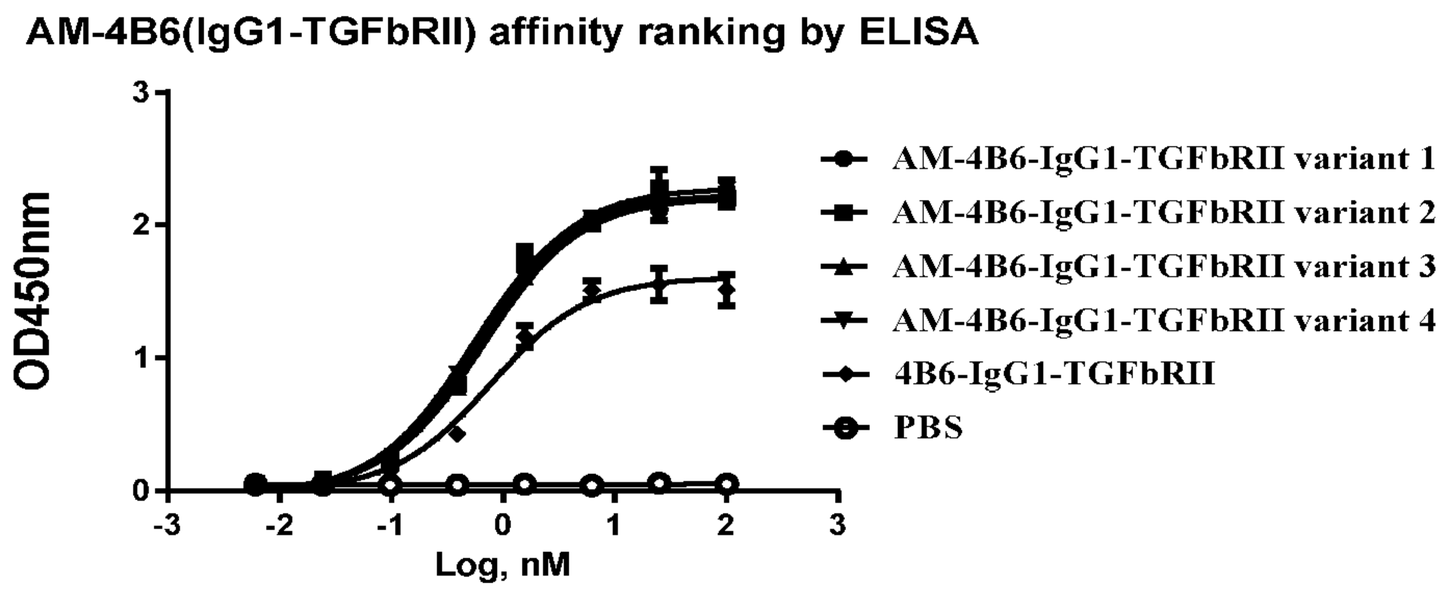


Figure 3A

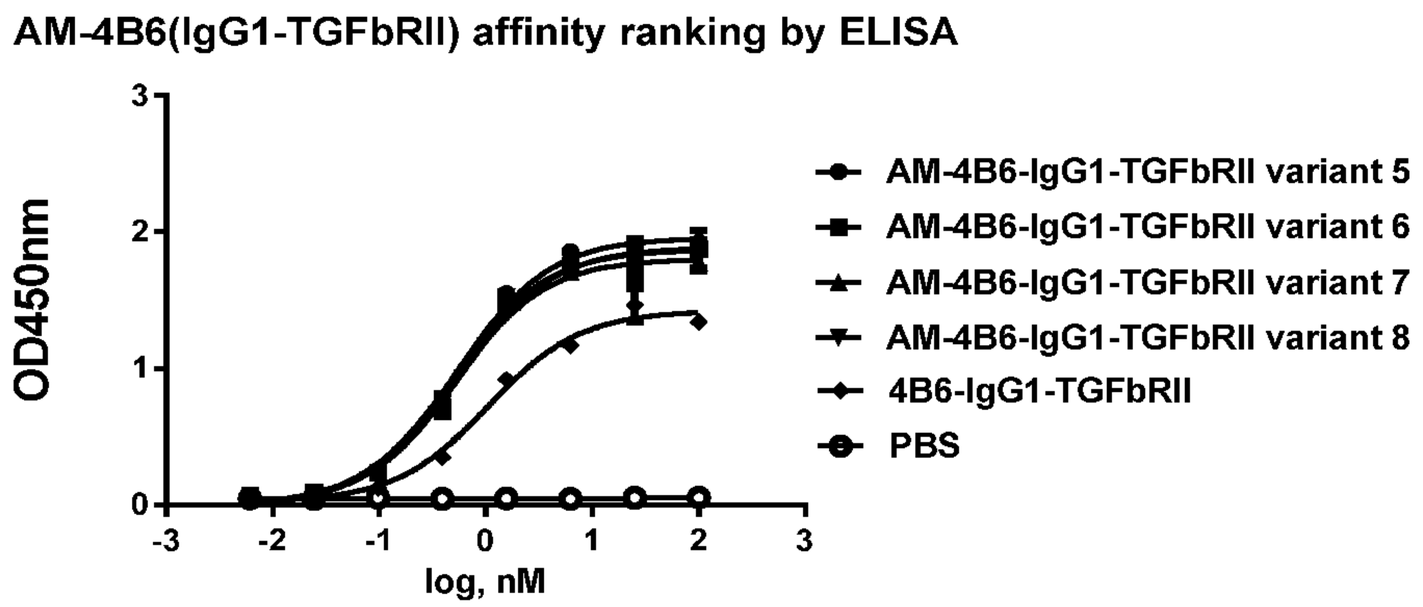


Figure 3B

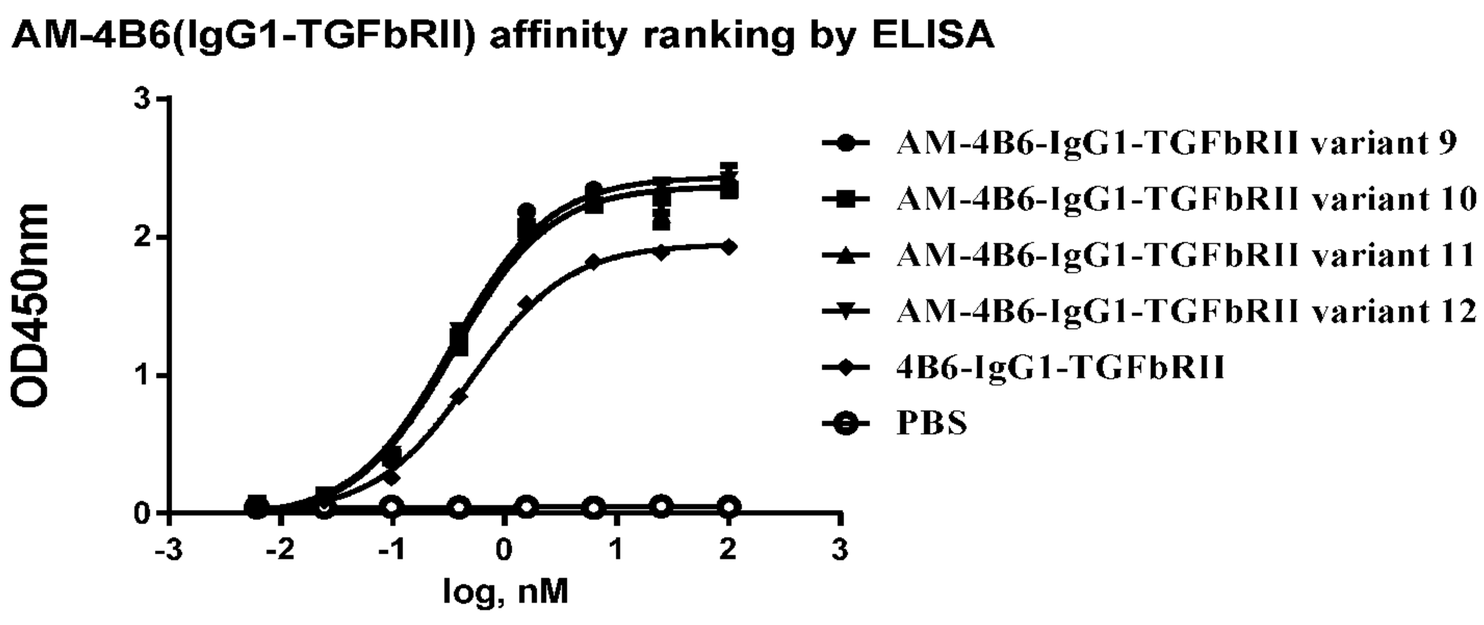
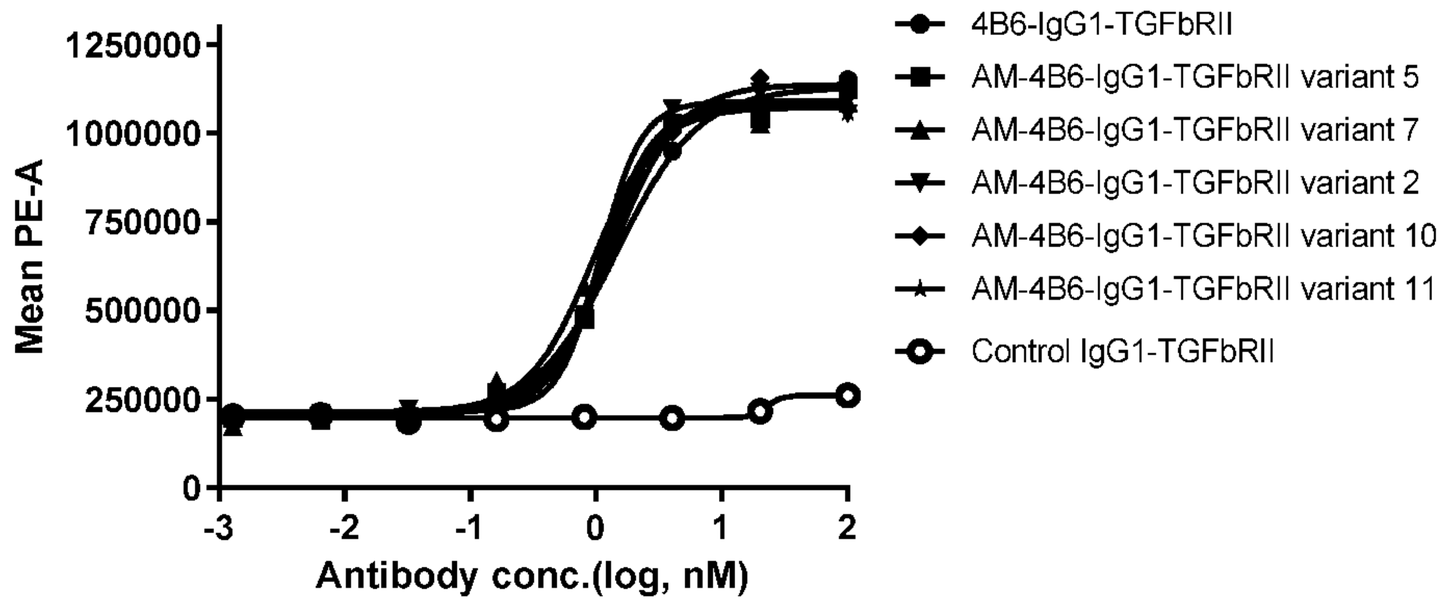


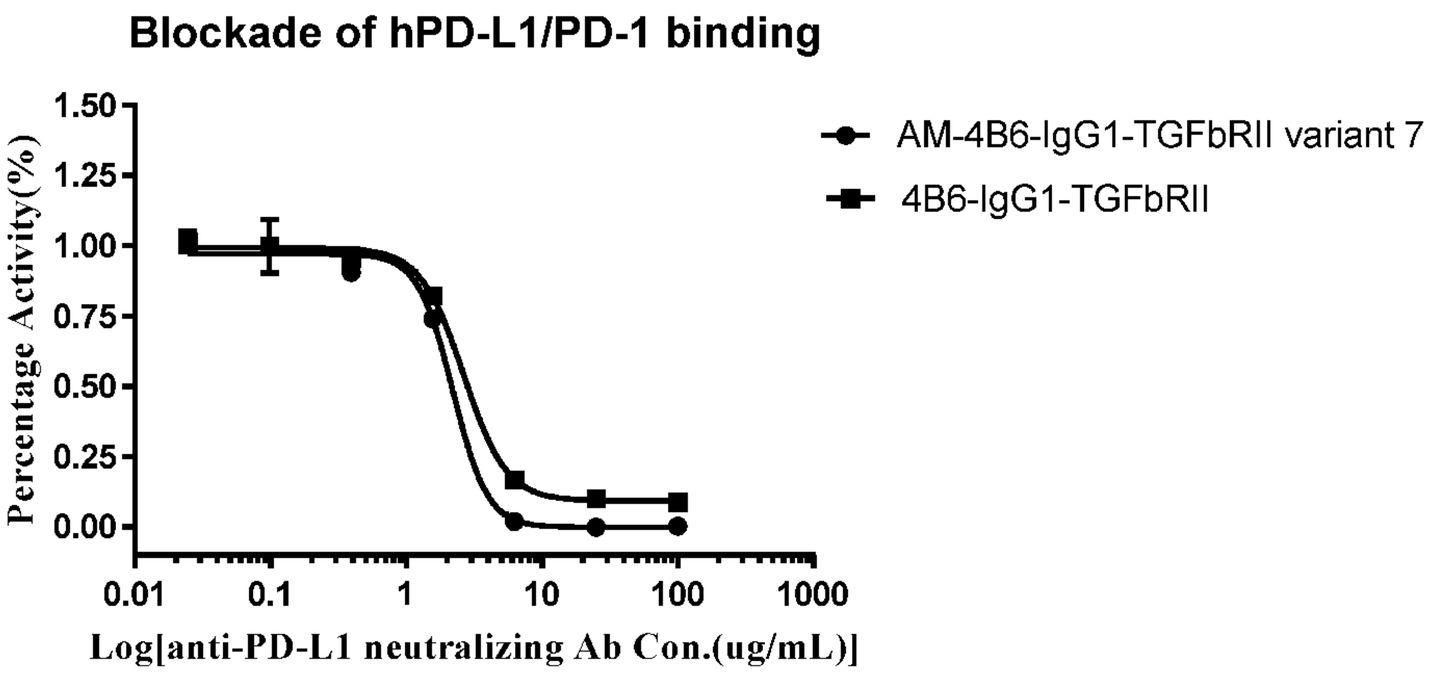
Figure 3C



	4B6-hIgG1-TGFbRII	AM-4B6-IgG1-TGFbRII variant 5	AM-4B6-IgG1-TGFbRII variant 7
EC50	1.436	1.196	0.9404

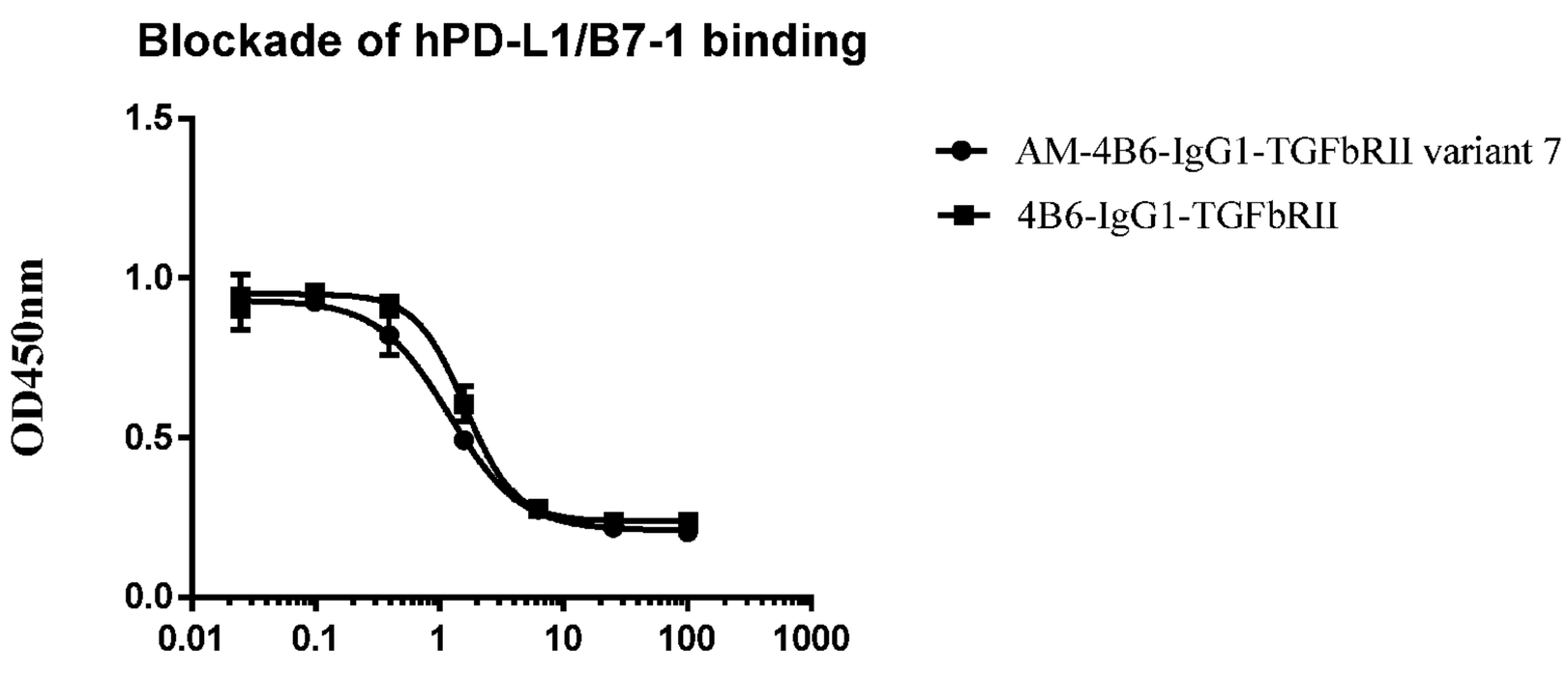
AM-4B6-IgG1-TGFbRII variant 2	AM-4B6-IgG1-TGFbRII variant 10	AM-4B6-IgG1-TGFbRII variant 11
1.074	1.363	1.11

Figure 4



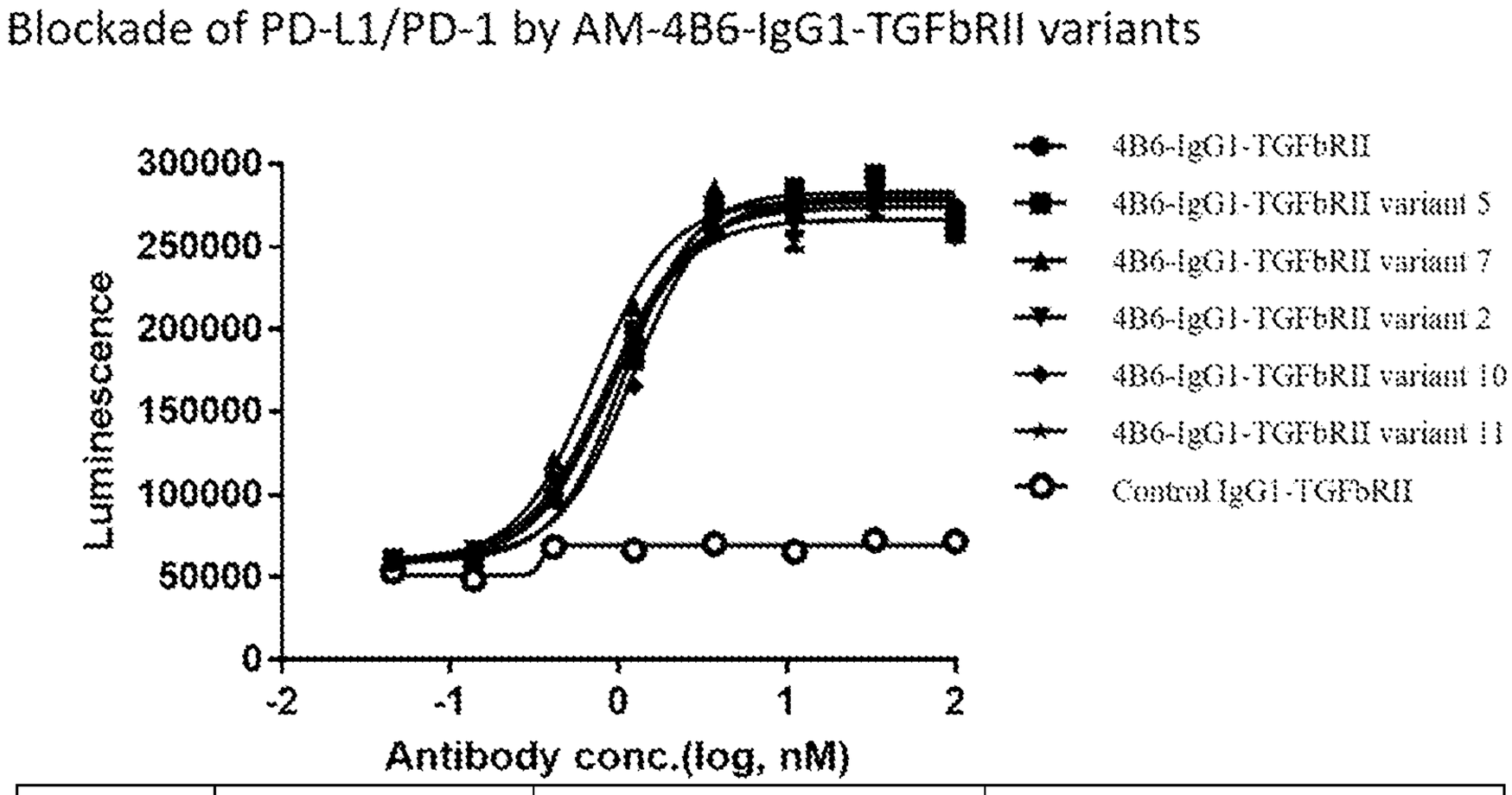
	AM-4B6-IgG1-TGFbRII variant 7	4B6-IgG1-TGFbRII
IC50	2.2	2.641

Figure 5A



	AM-4B6-IgG1-TGFbRII variant 7	4B6-IgG1-TGFbRII
IC50	1.194	1.608

Figure 5B

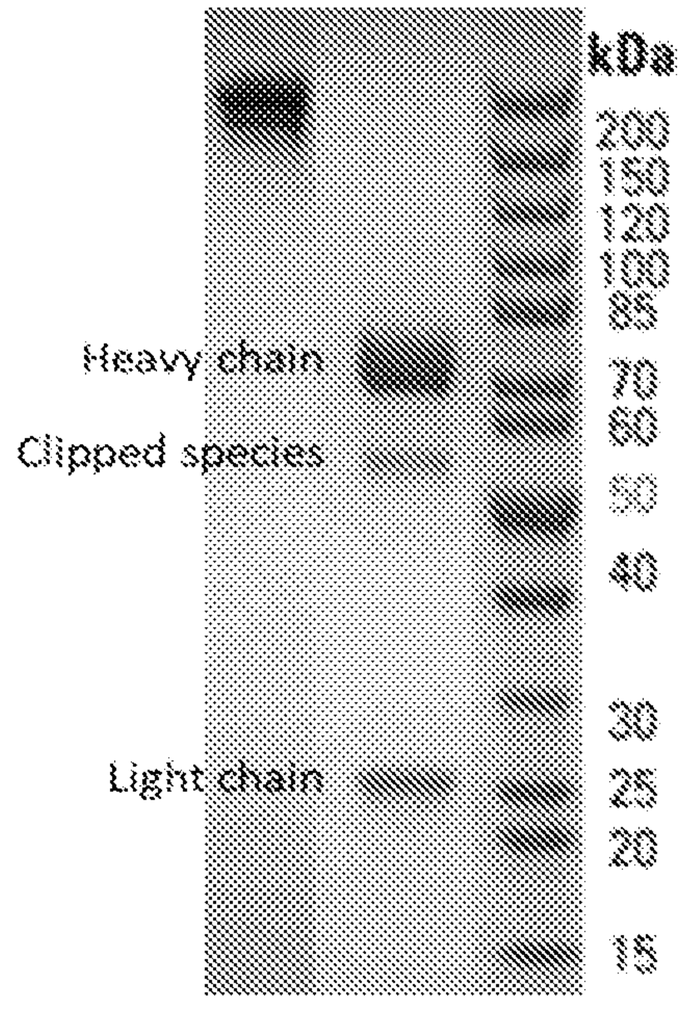


	4B6-IgG1-TGFbRII	4B6-IgG1-TGFbRII variant 5	4B6-IgG1-TGFbRII variant 7
EC50	0.9333	1.073	0.7112

4B6-IgG1-TGFbRII variant 2	4B6-IgG1-TGFbRII variant 10	4B6-IgG1-TGFbRII variant 11
0.8679	1.203	0.8934

Figure 6

Hu4B6_HgLa_hIgG1_TGFbRII (1-136)
Expressed with stable cell line



AM4B6_hIgG1_TGFbRII (20-136)
Expressed with stable cell line

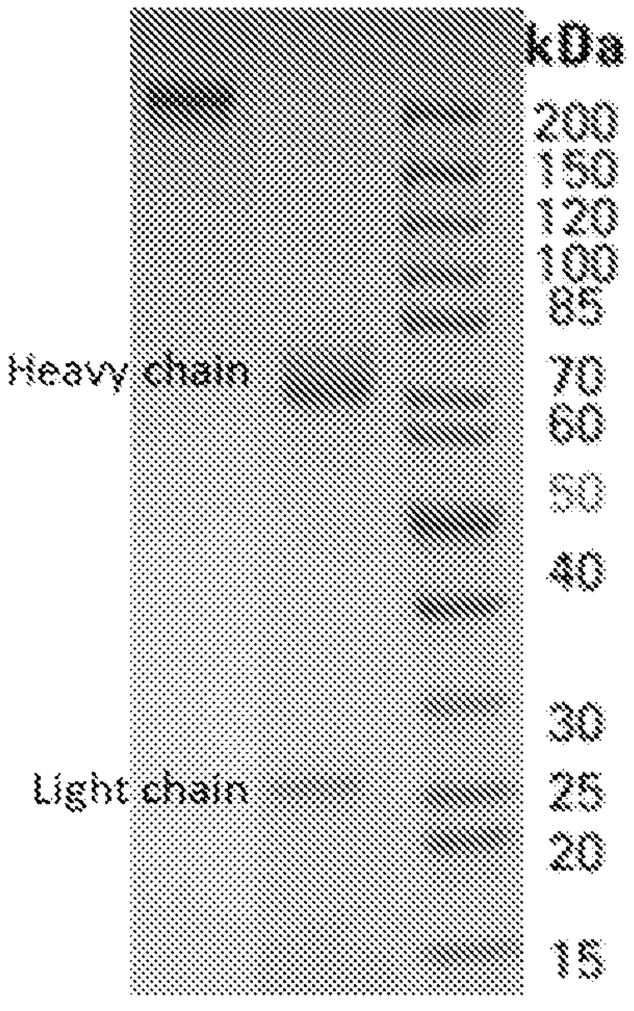
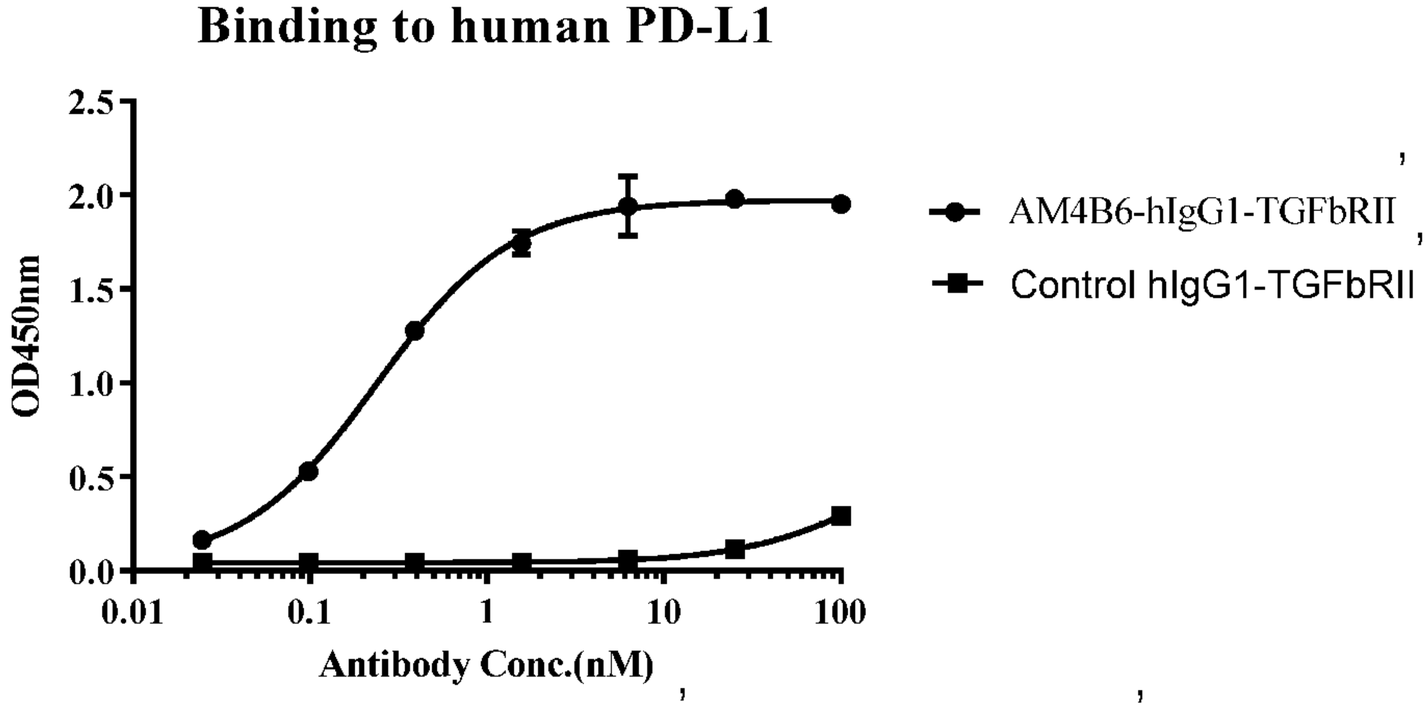
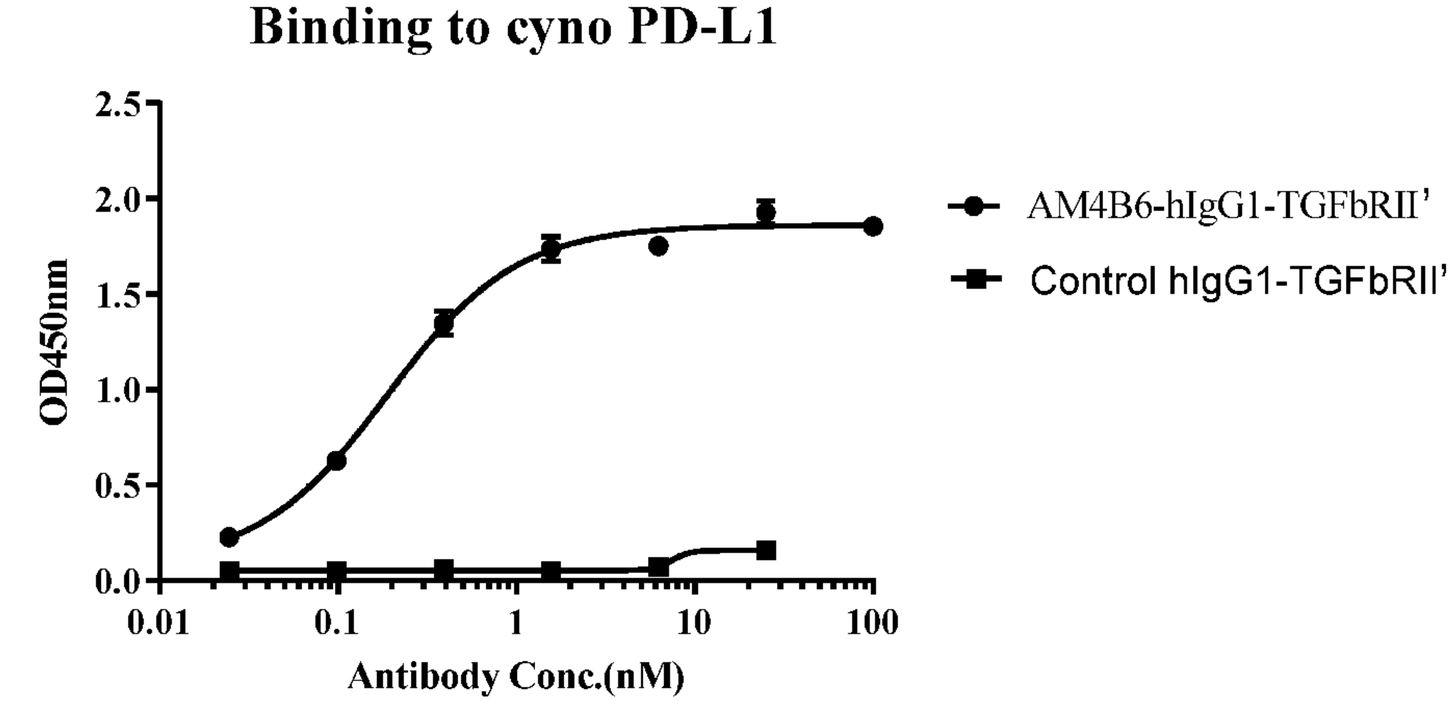


Figure 7



	AM4B6-hIgG1-TGFbRII	Control hIgG1-TGFbRII
EC50	0.2397	151.8

Figure 8A



	AM4B6-hIgG1-TGFbRII'	Control hIgG1-TGFbRII'
EC50	0.1891	~ 7.398

Figure 8B

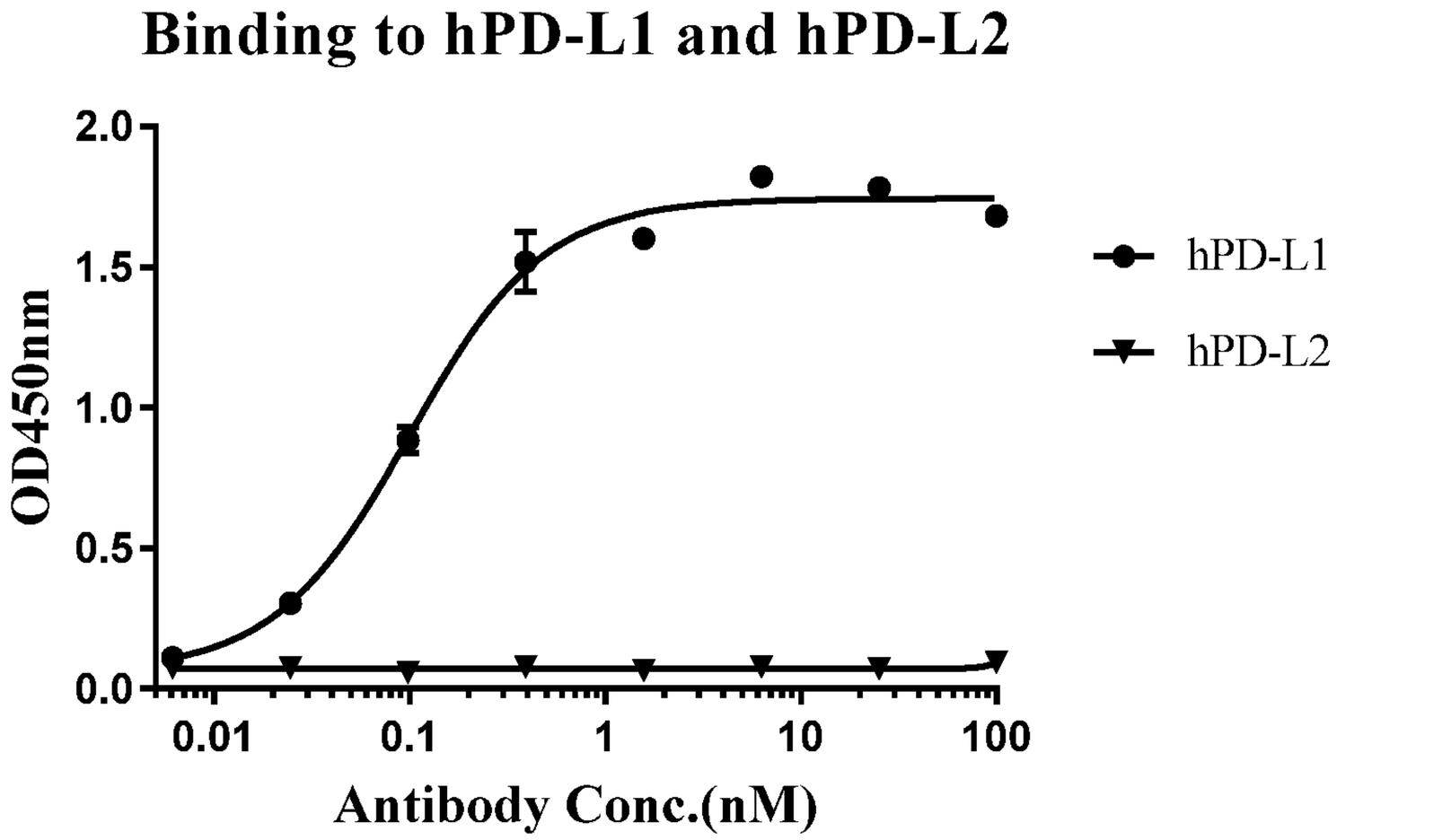


Figure 9A

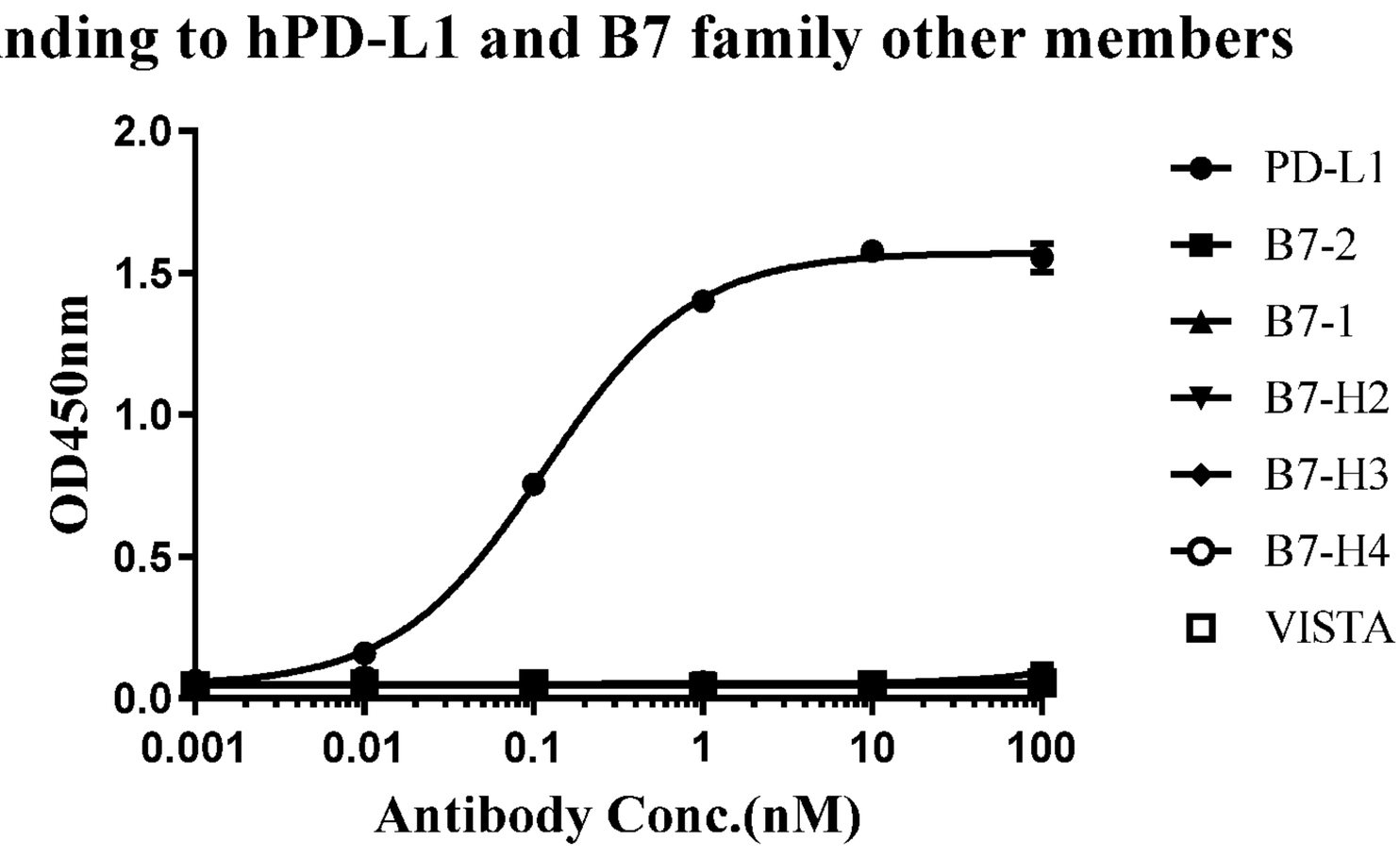


Figure 9B

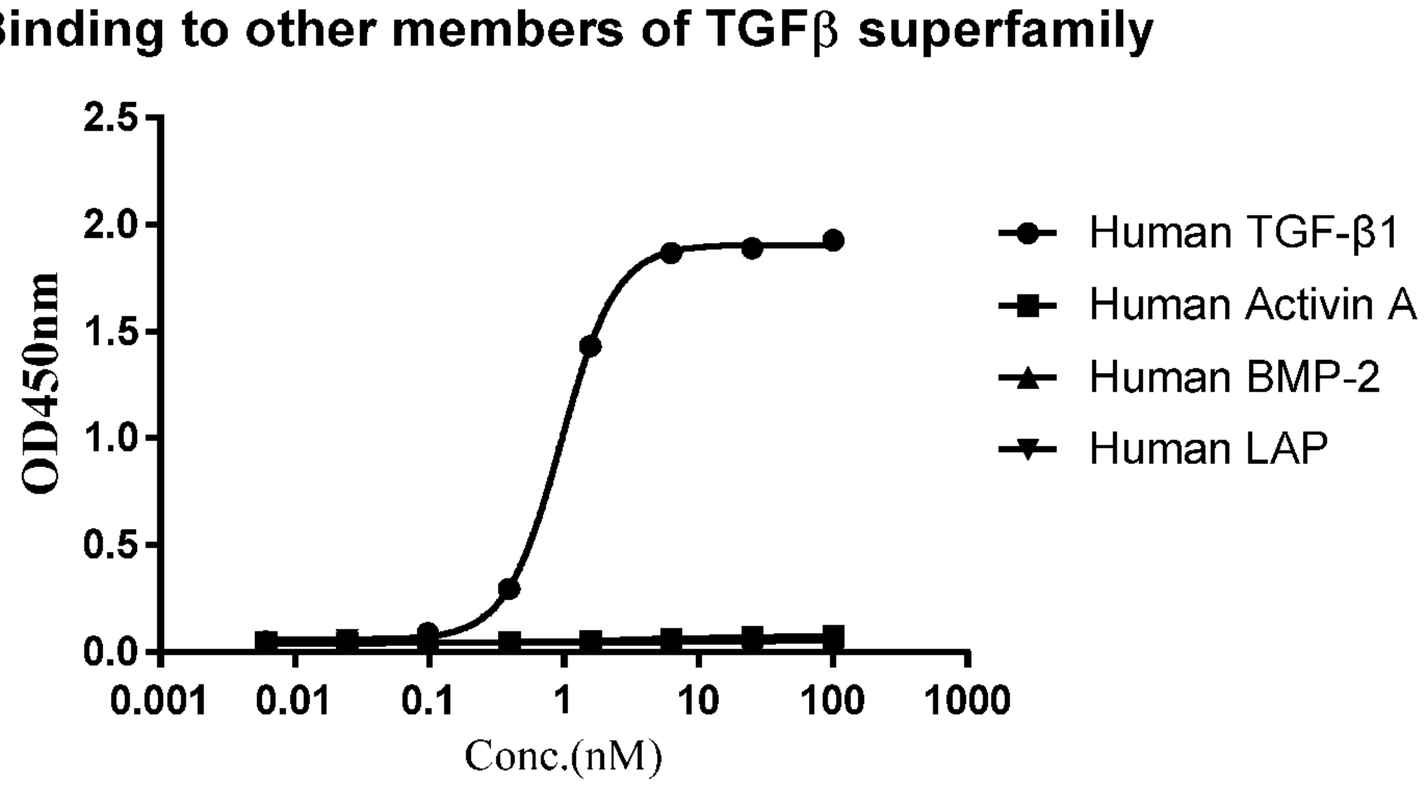
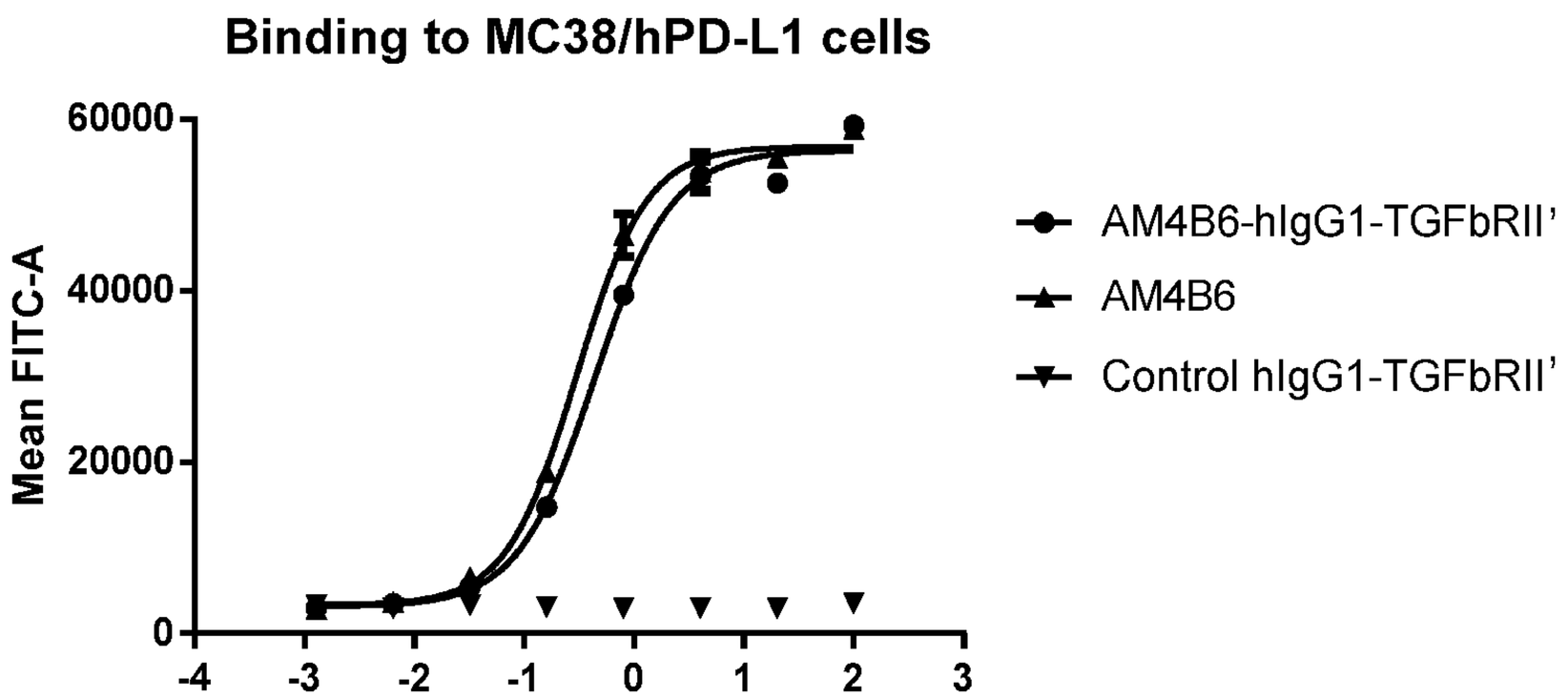
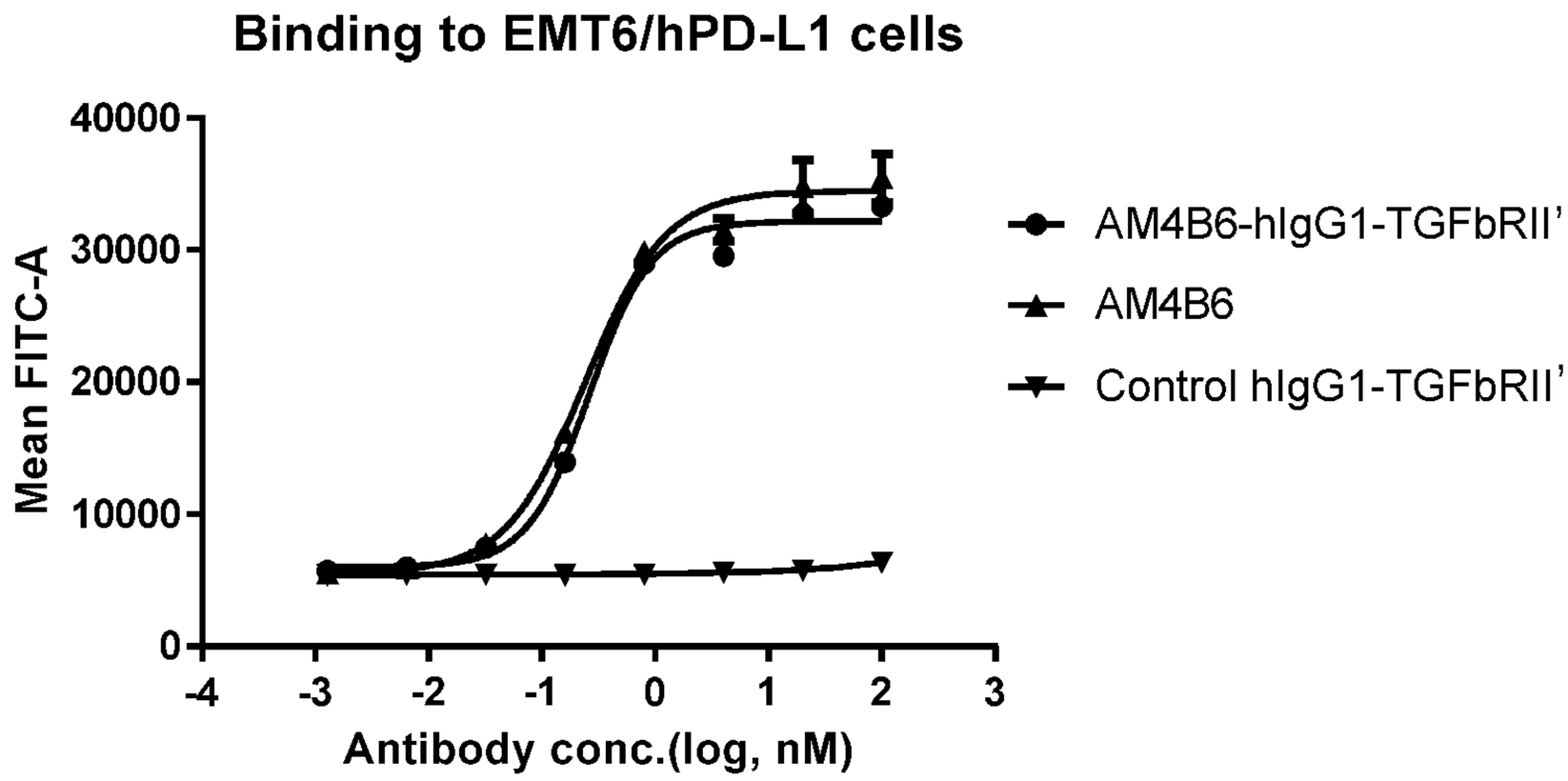

Binding to other members of TGFβ superfamily
OD450nm
2.5
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
100
1000
Conc.(nM)
Human TGF-β1
Human Activin A
Human BMP-2
Human LAP

Figure 9C



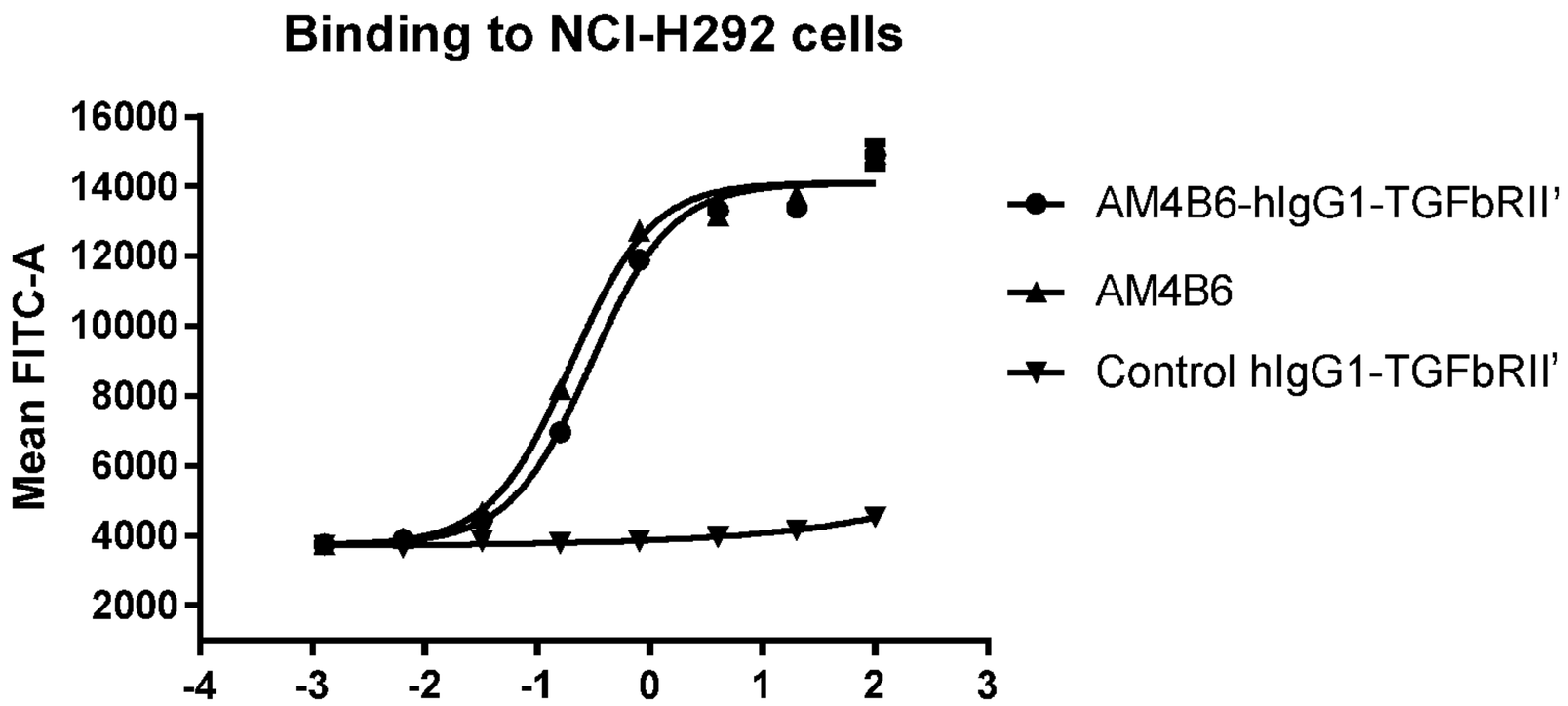
	AM4B6-hIgG1-TGFbRII'	AM4B6
EC50	0.4373	0.299

Figure 10A



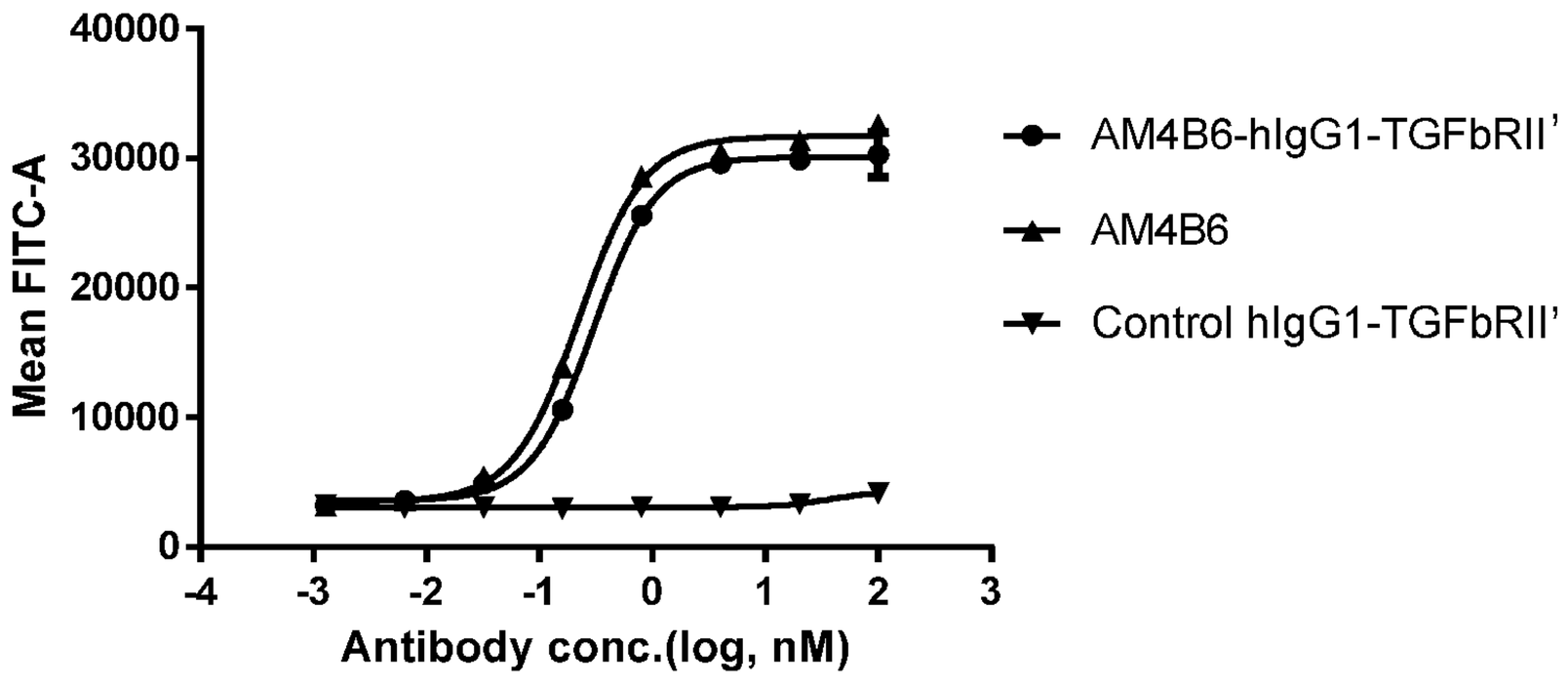
	AM4B6-hIgG1-TGFbRII'	AM4B6
EC50	0.2614	0.2403

Figure 10B



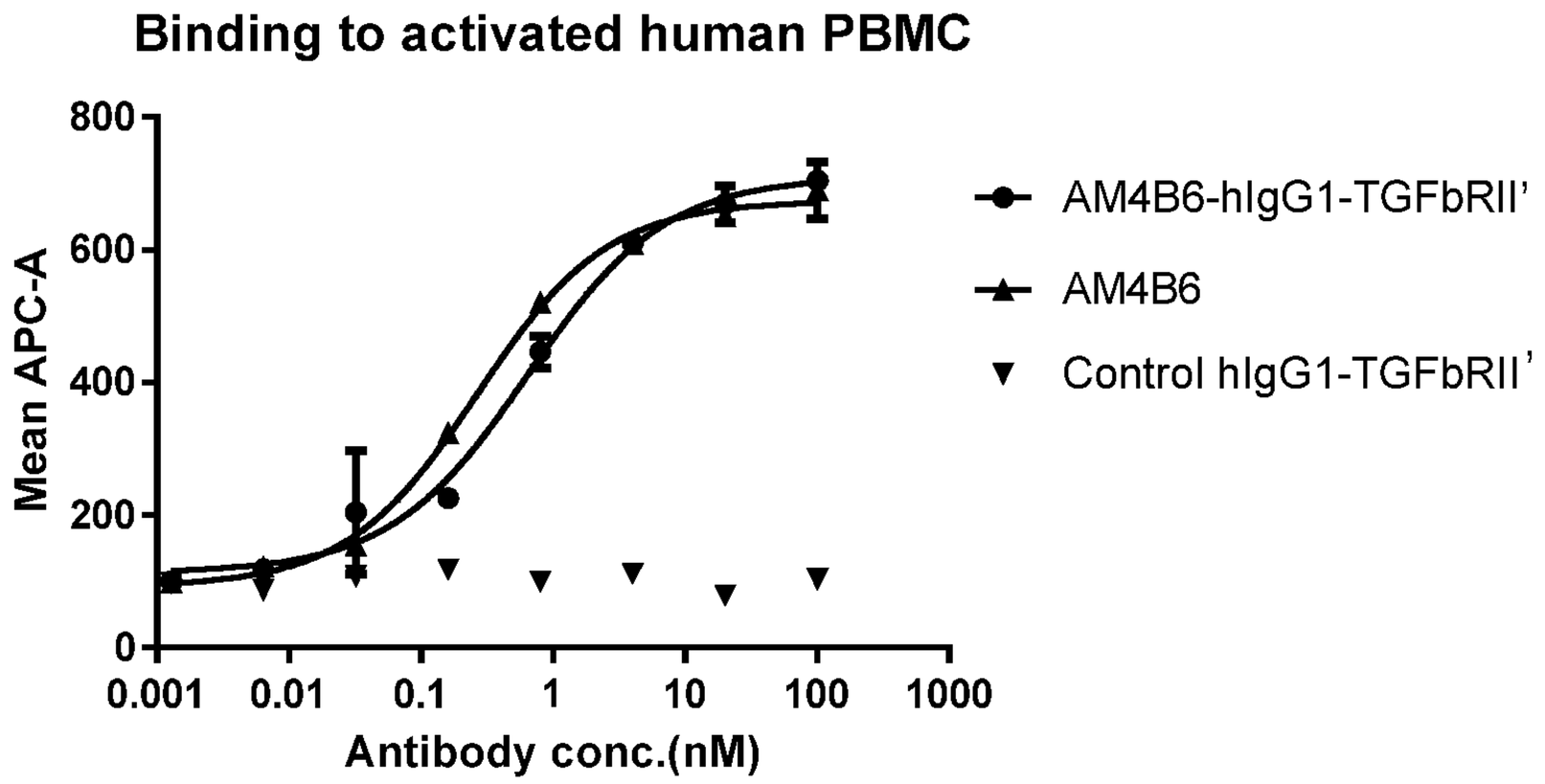
	AM4B6-hIgG1-TGFbRII'	AM4B6
EC50	0.2982	0.199

Figure 10C



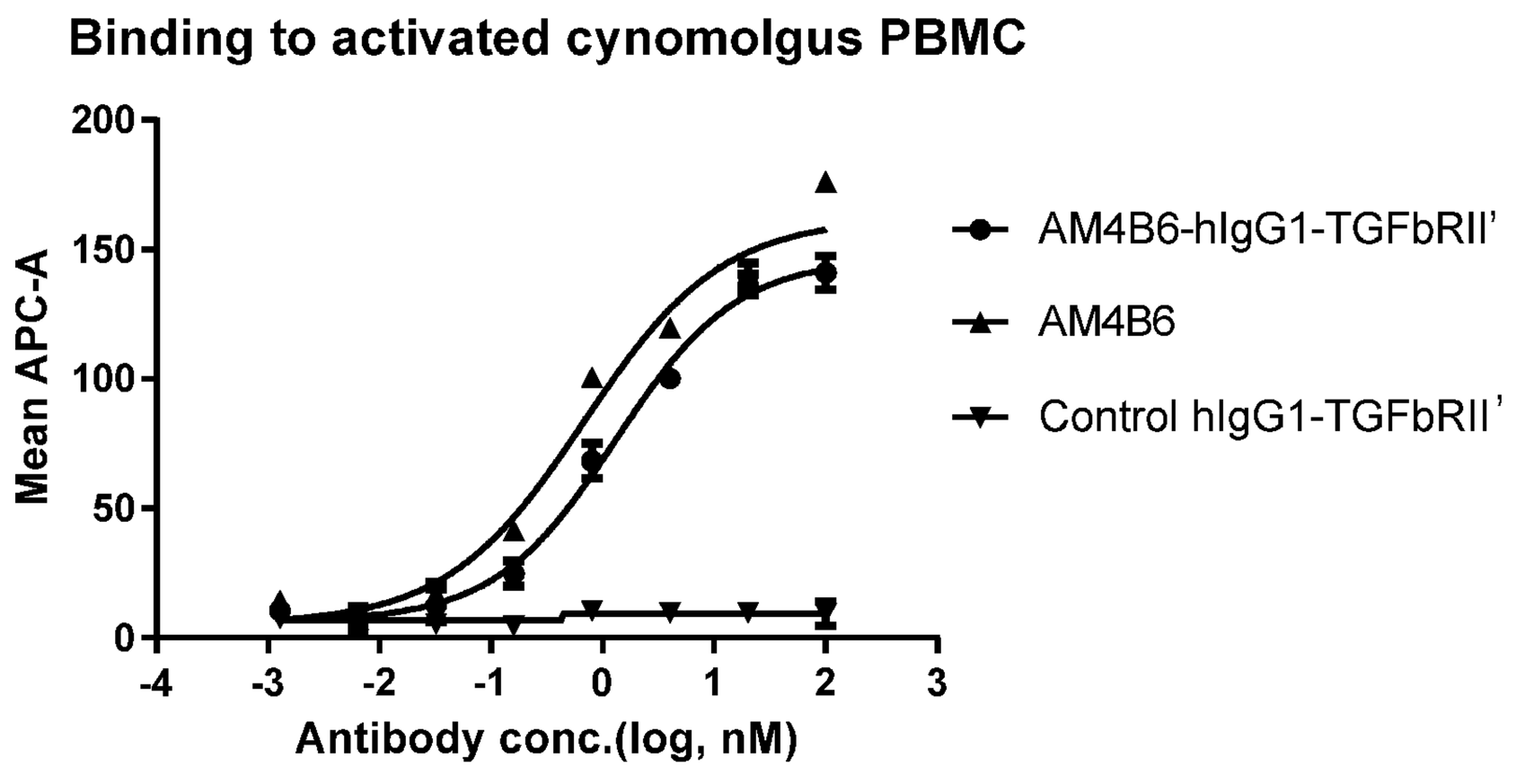
	AM4B6-hIgG1-TGFbRII'	AM4B6
EC50	0.2997	0.2224

Figure 10D



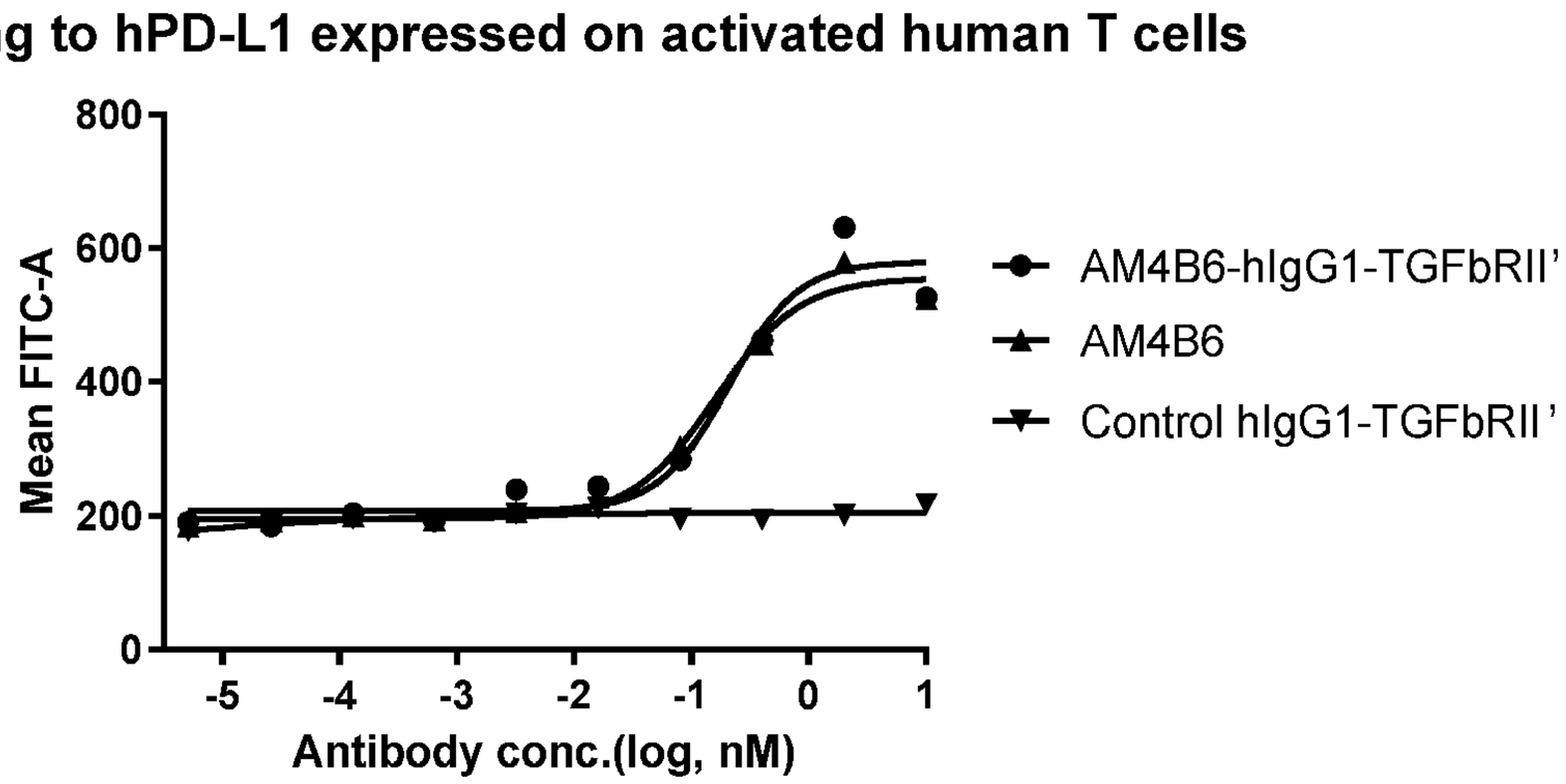
	AM4B6-hIgG1-TGFbRII'	AM4B6
EC50	0.6437	0.267

Figure 10E



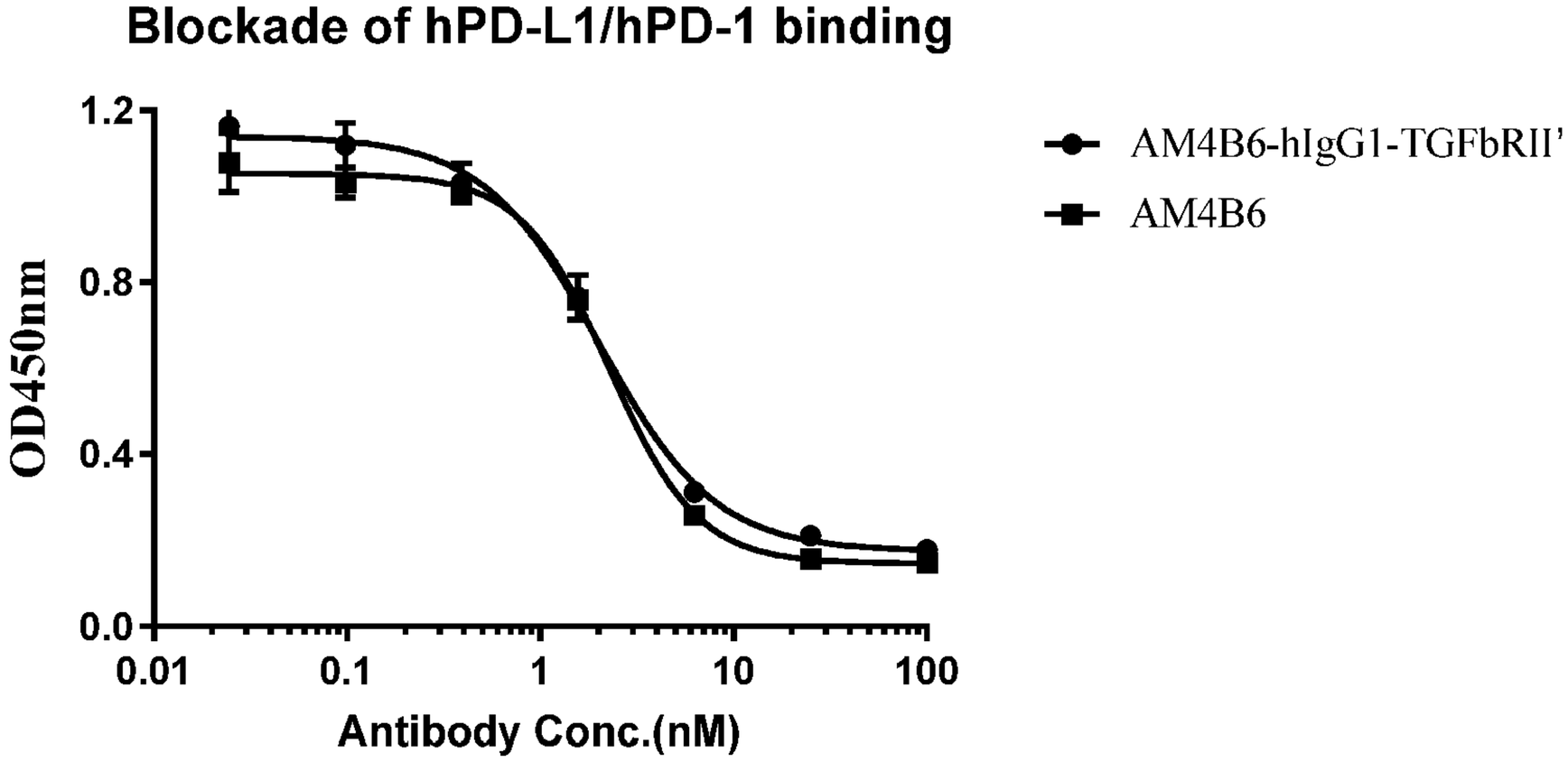
	AM4B6-hIgG1-TGFbRII'	AM4B6
EC50	1.306	0.684

Figure 10F



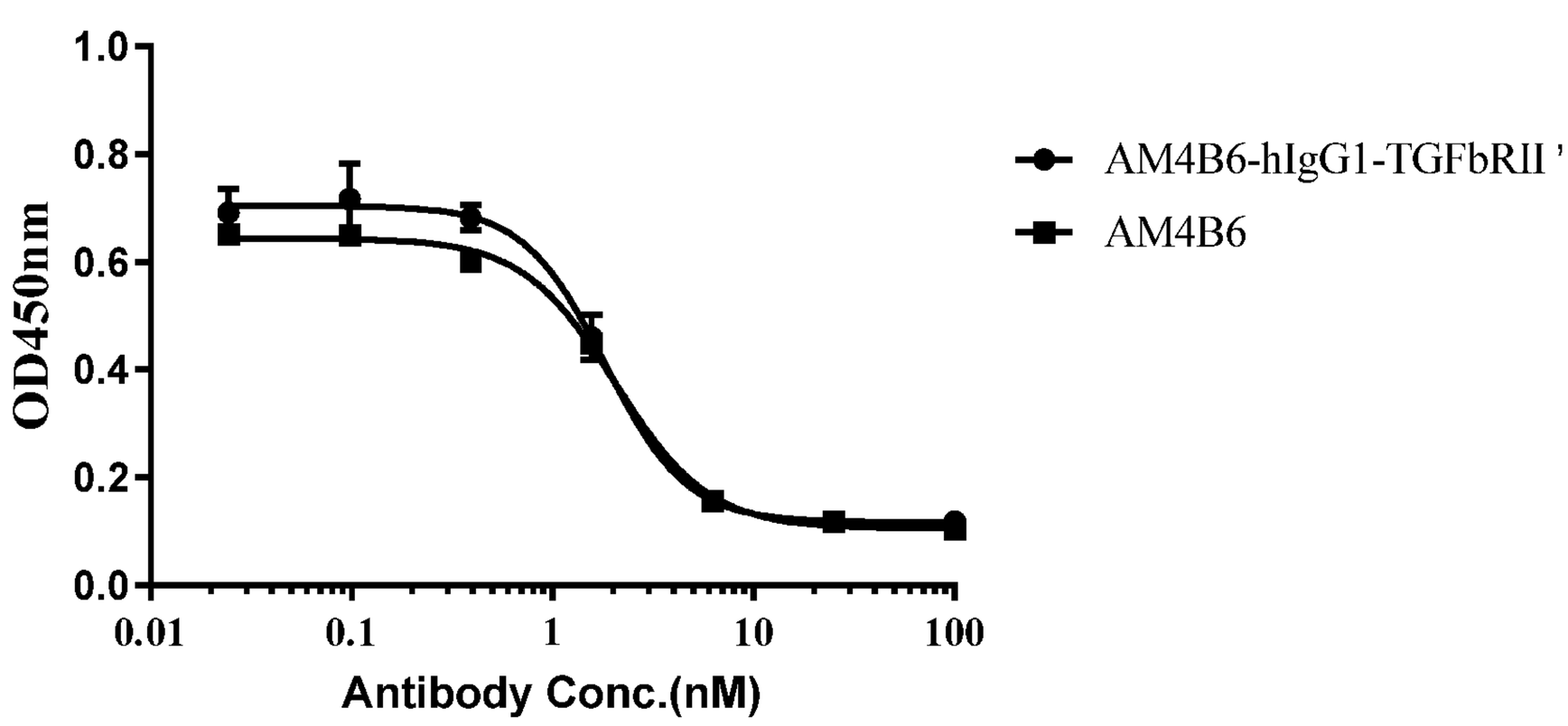
	AM4B6-hIgG1-TGFbRII'	AM4B6	Control hIgG1-TGFbRII'
EC50	0.2064	0.1604	~ 7.407e-013

Figure 11



	AM4B6-hIgG1-TGFbRII'	AM4B6
IC50	2.022	2.28

Figure 12A



	AM4B6-hIgG1-TGFbRII'	AM4B6
IC50	1.828	2.055

Figure 12B

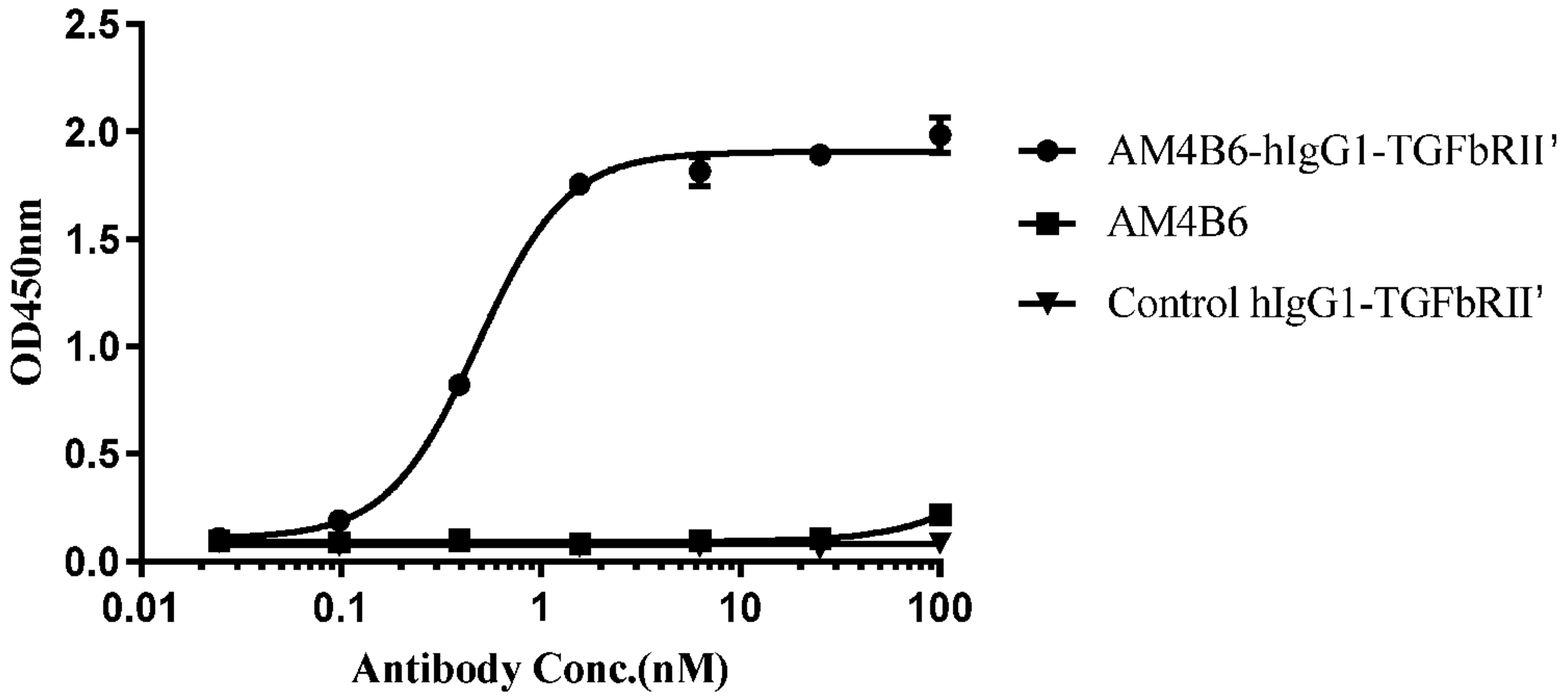
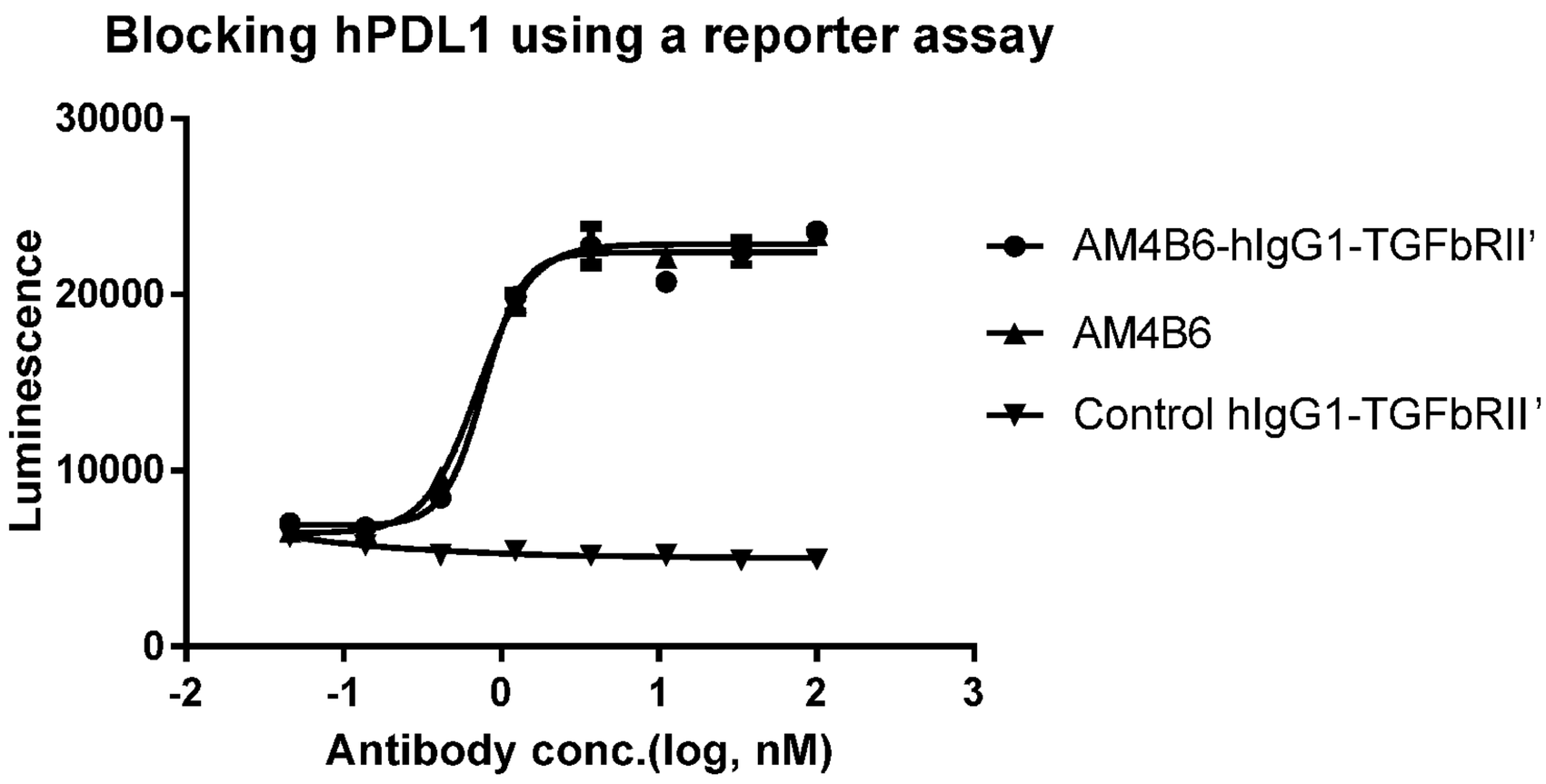


Figure 13



	AM4B6-hIgG1-TGFbRII'	AM4B6
IC50	0.7713	0.7144

Figure 14

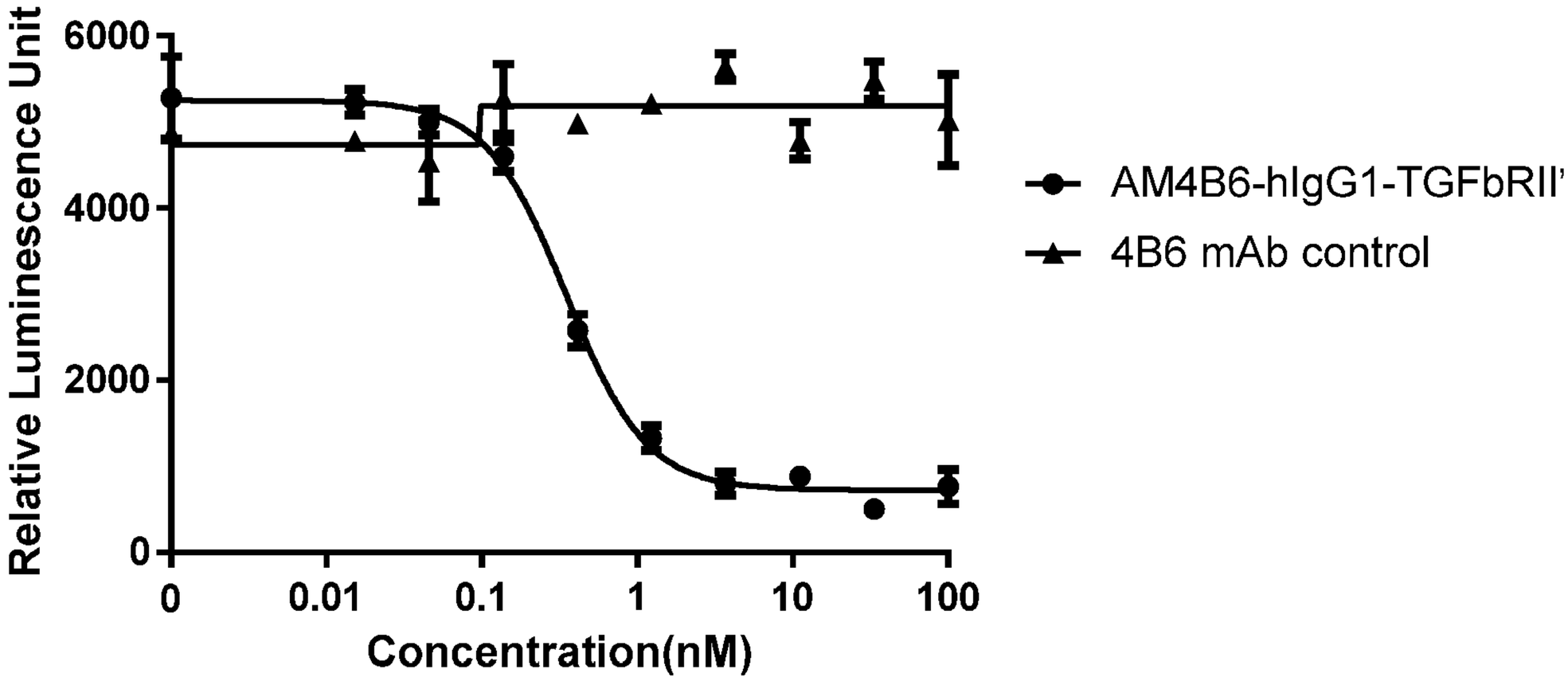


Figure 15.

Effect of AM4B6-hIgG1-TGFbRII'on tuberculin(TB) stimulated PBMC
(DC donor:TPCS, PB025C, A10K546006)

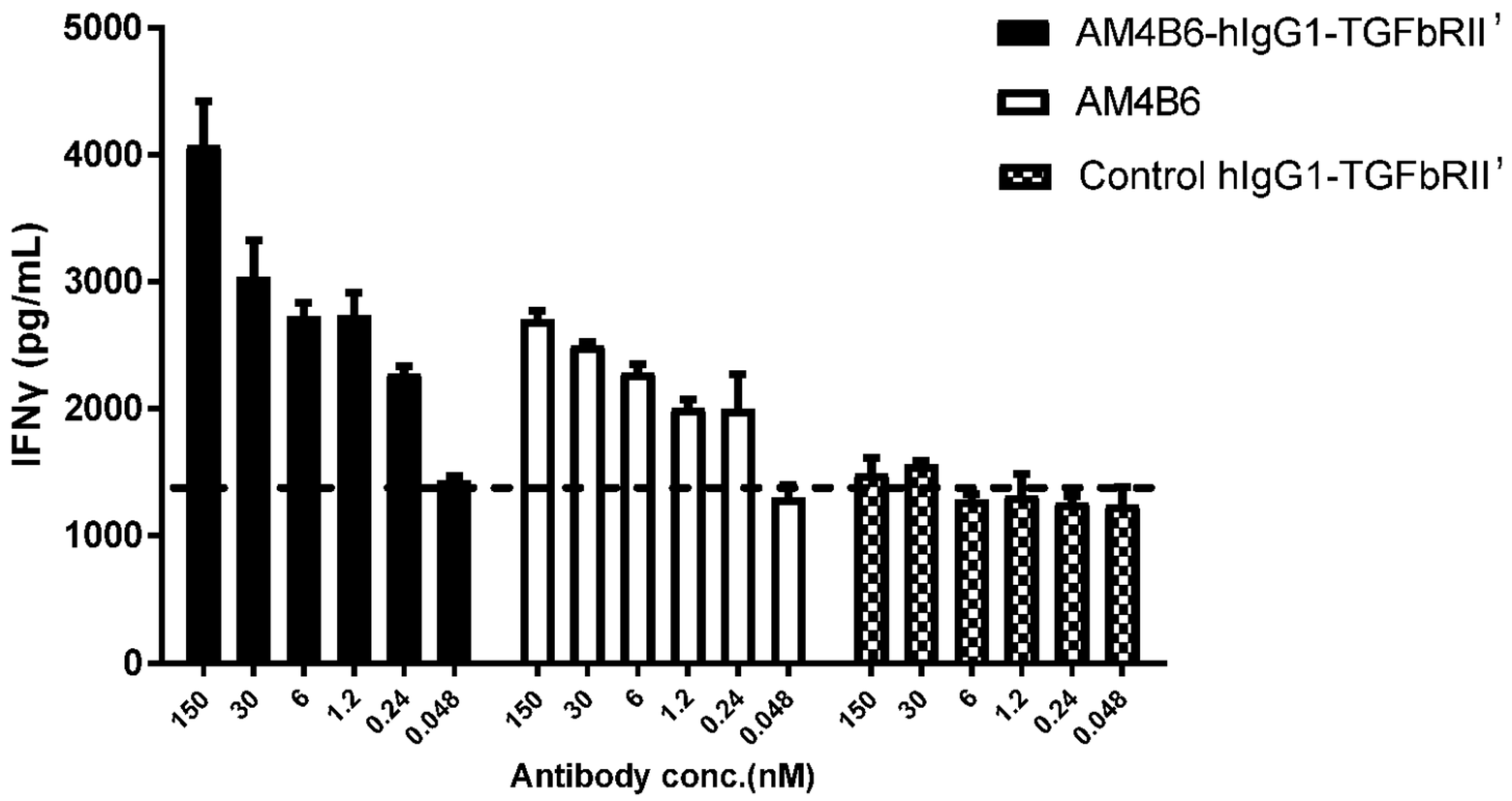


Figure 16

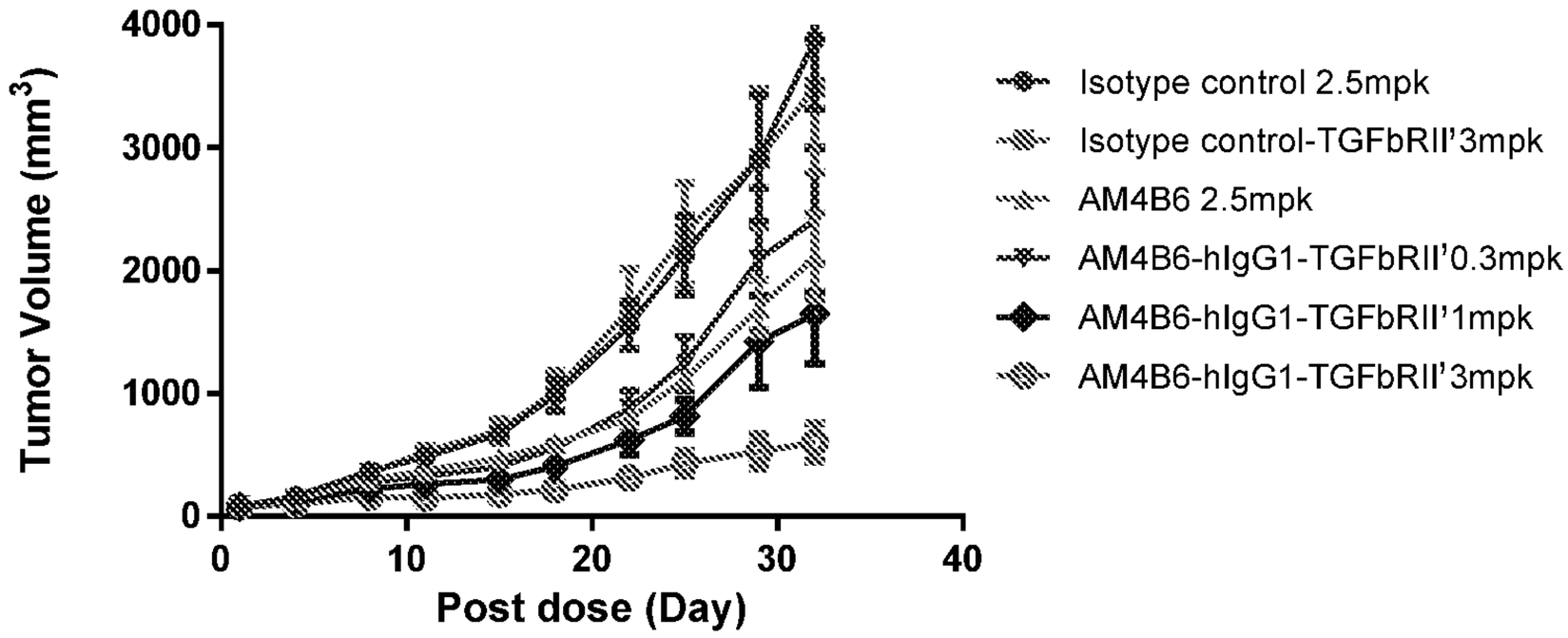
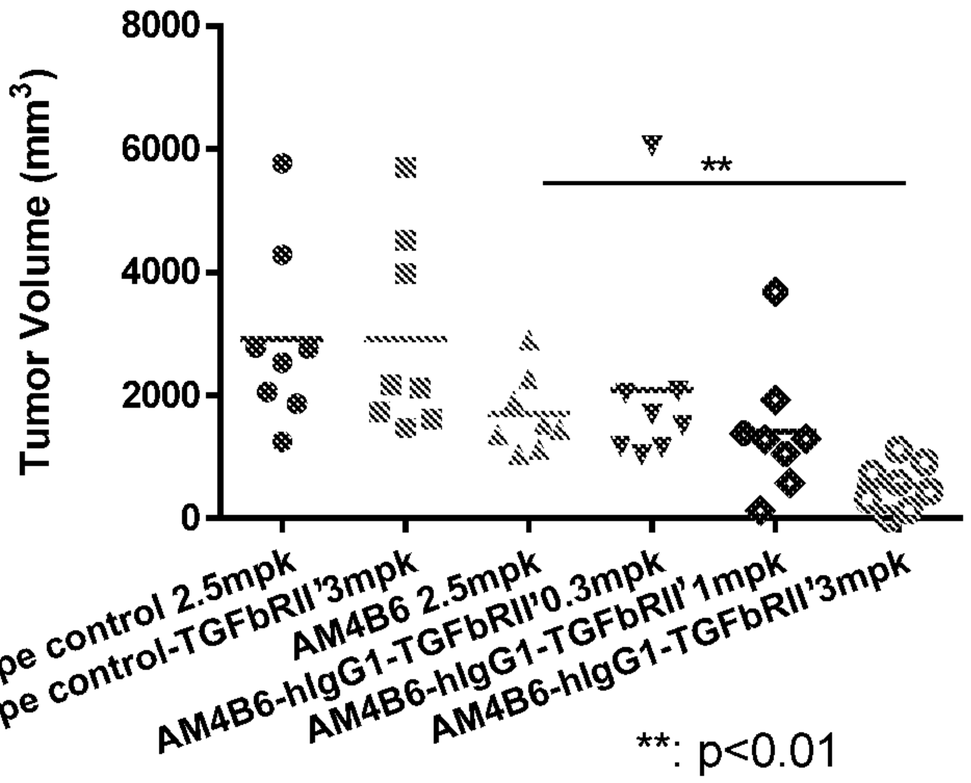


Figure 17A



**: p<0.01

Figure 17B

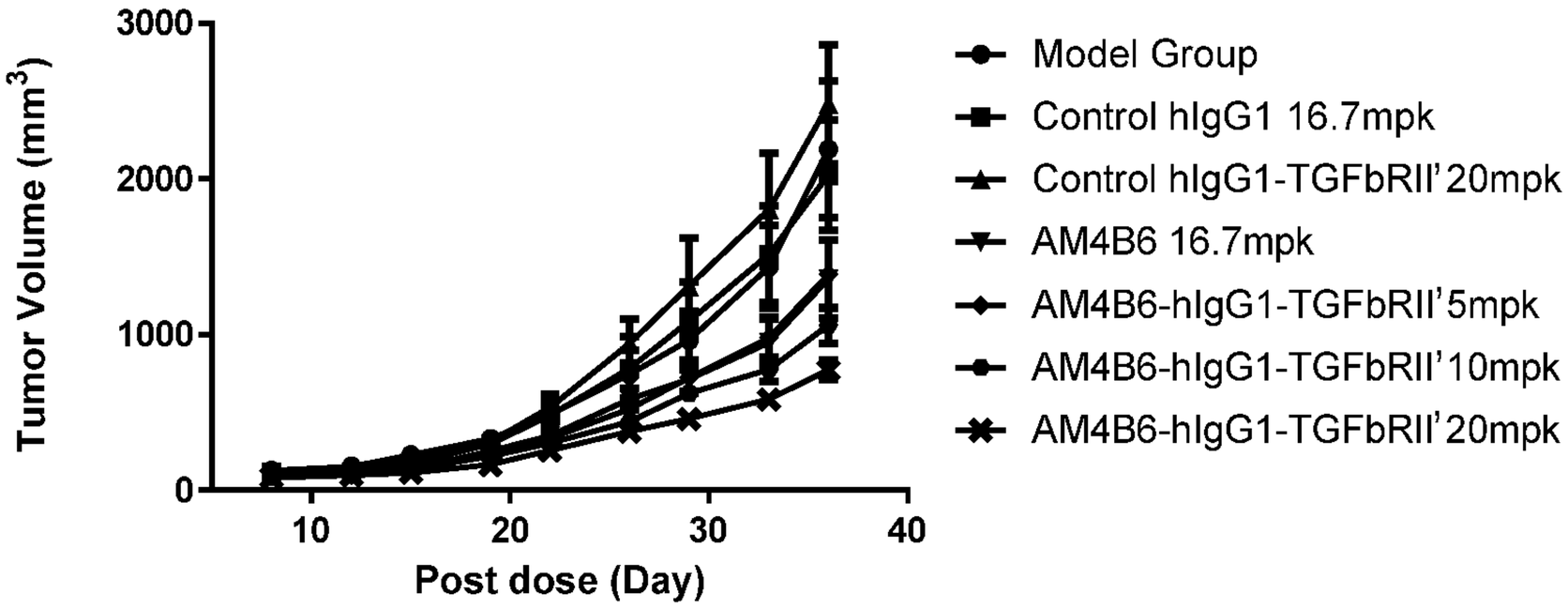


Figure 18A

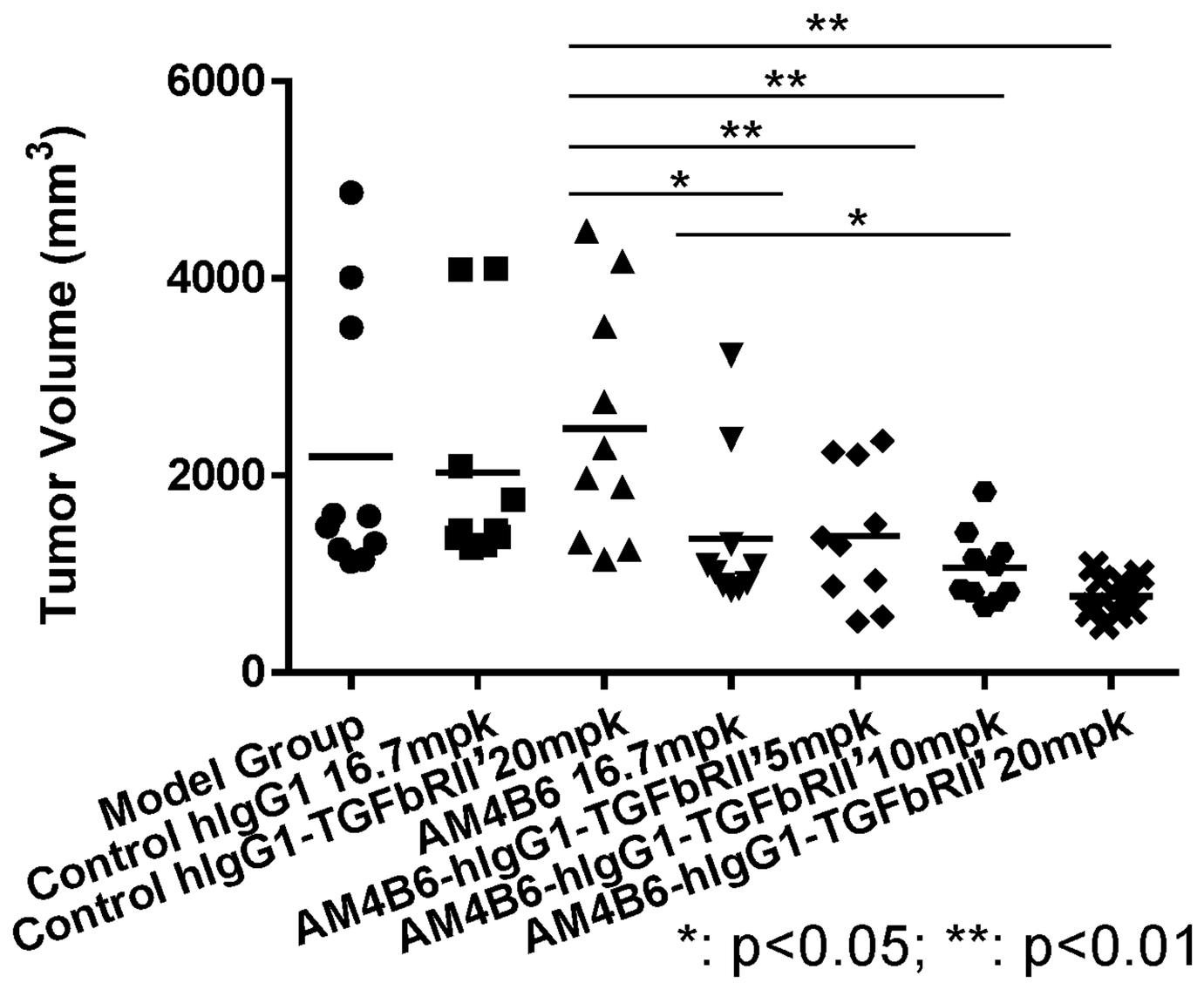


Figure 18B

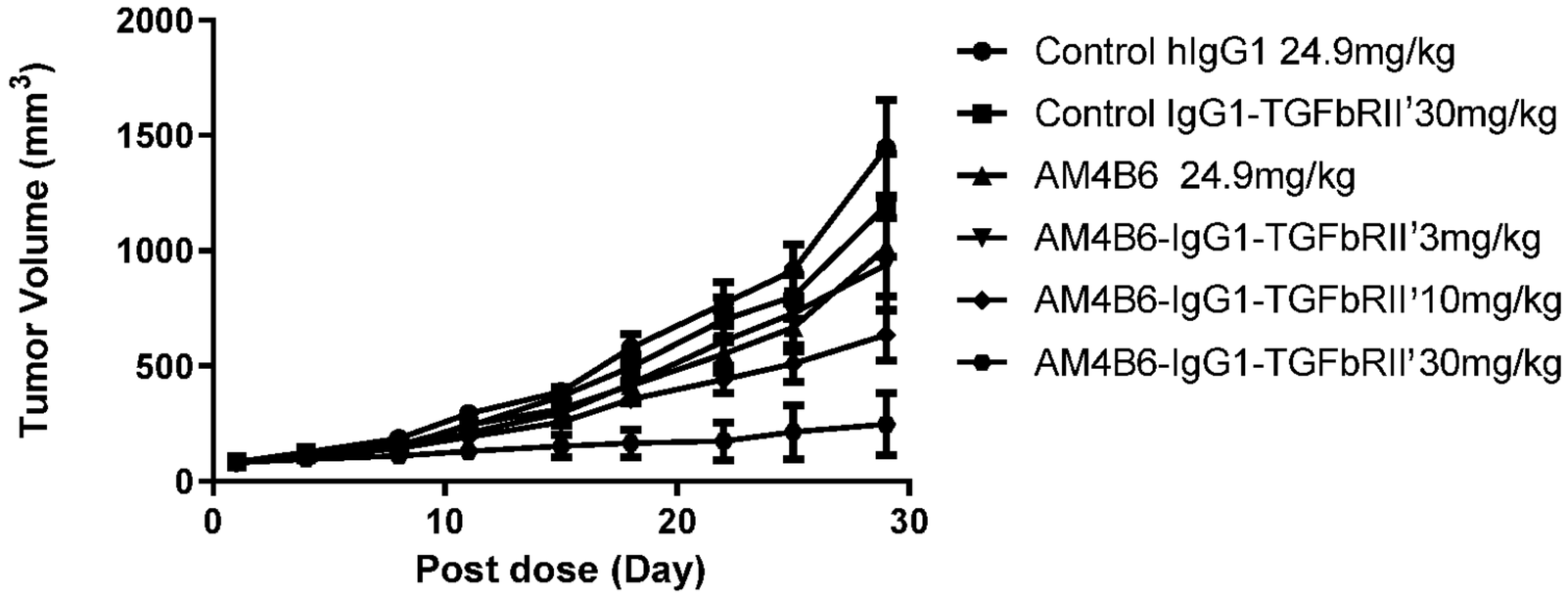
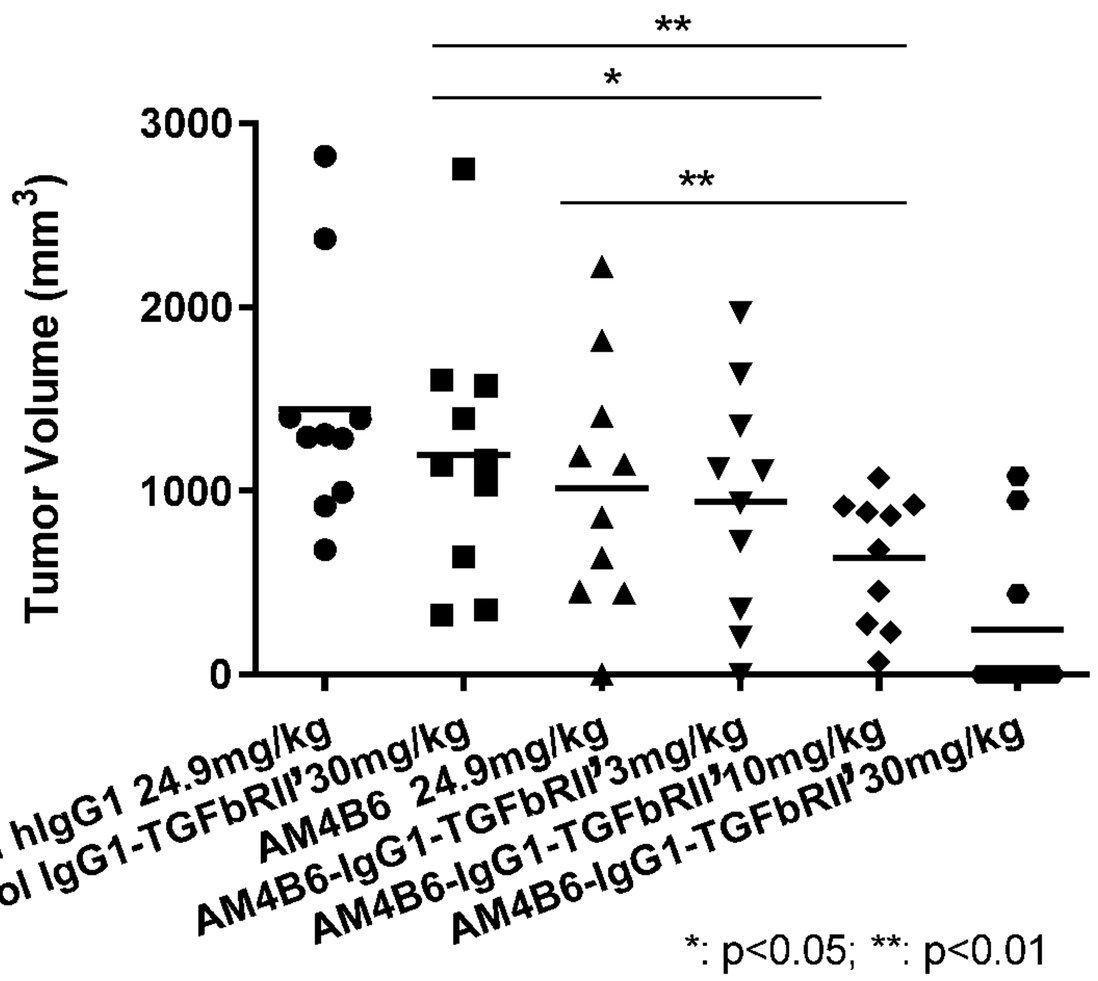


Figure 19A



*: p<0.05; **: p<0.01

Figure 19B

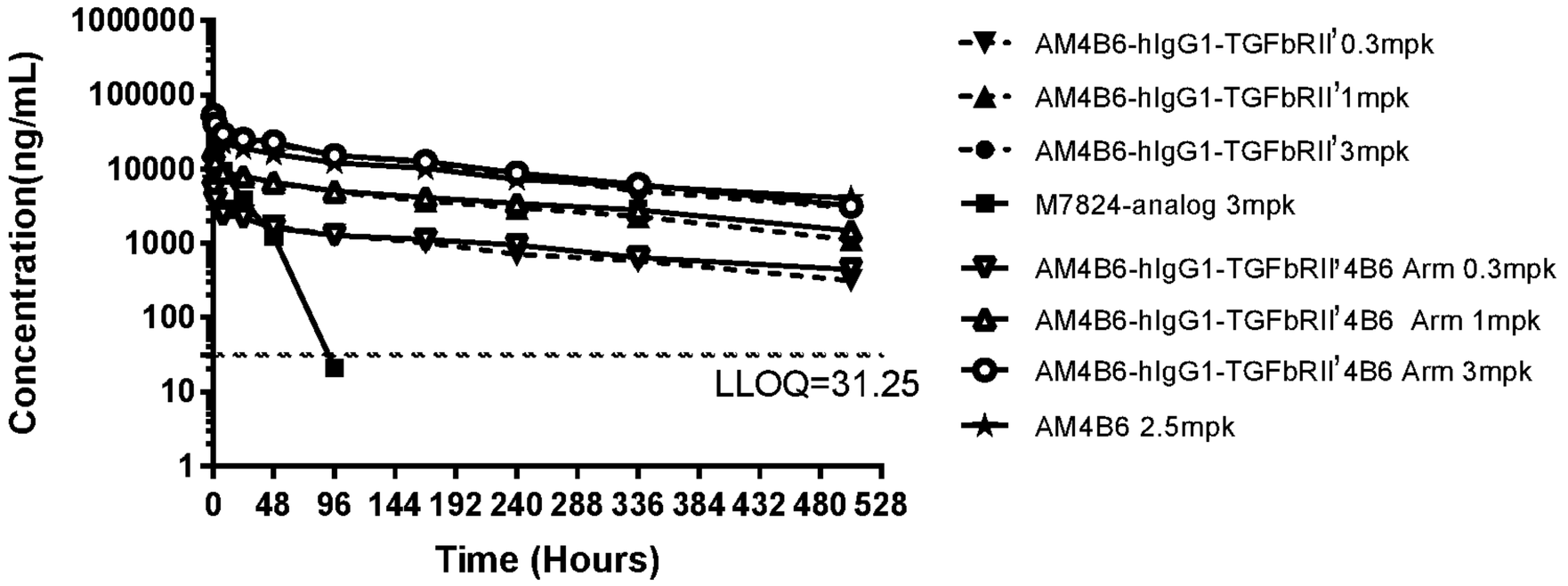


Figure 20A

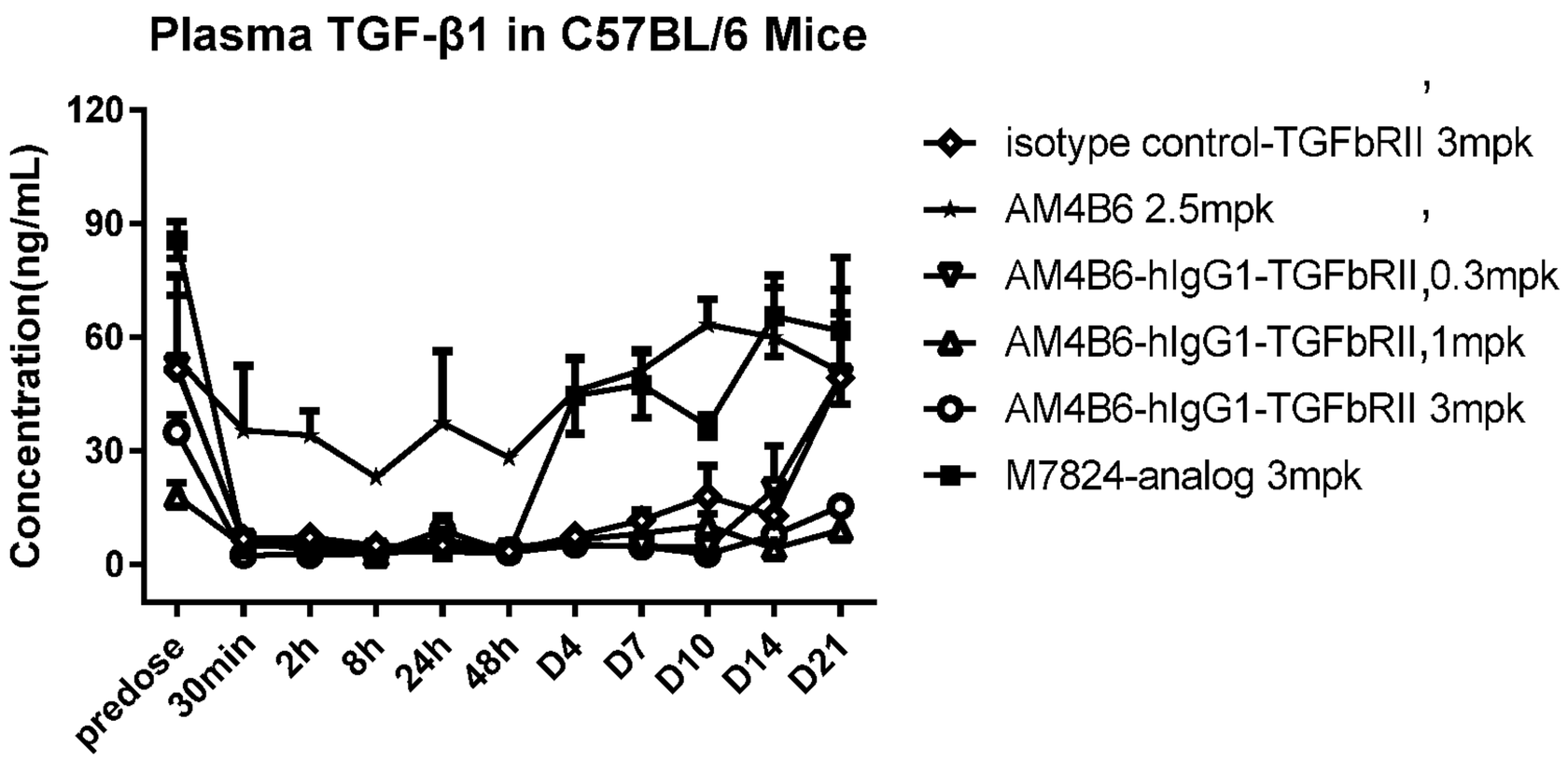


Figure 20B

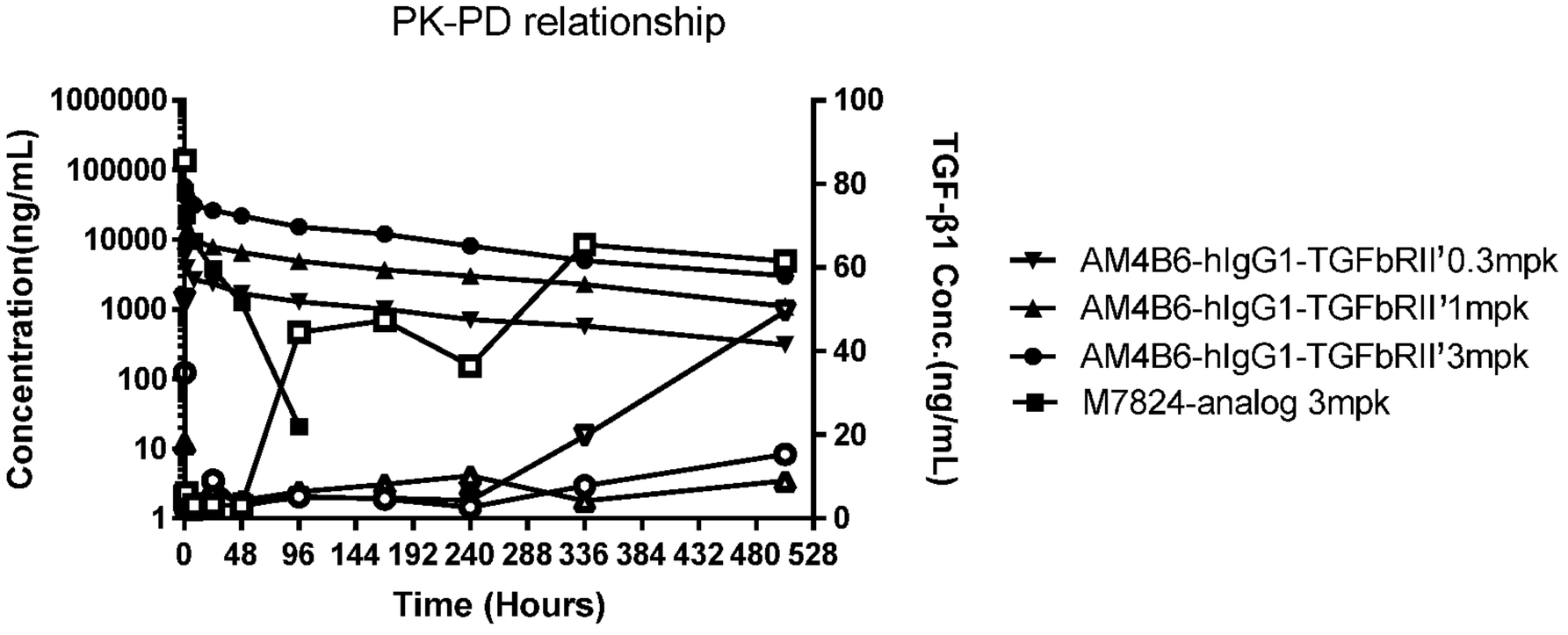
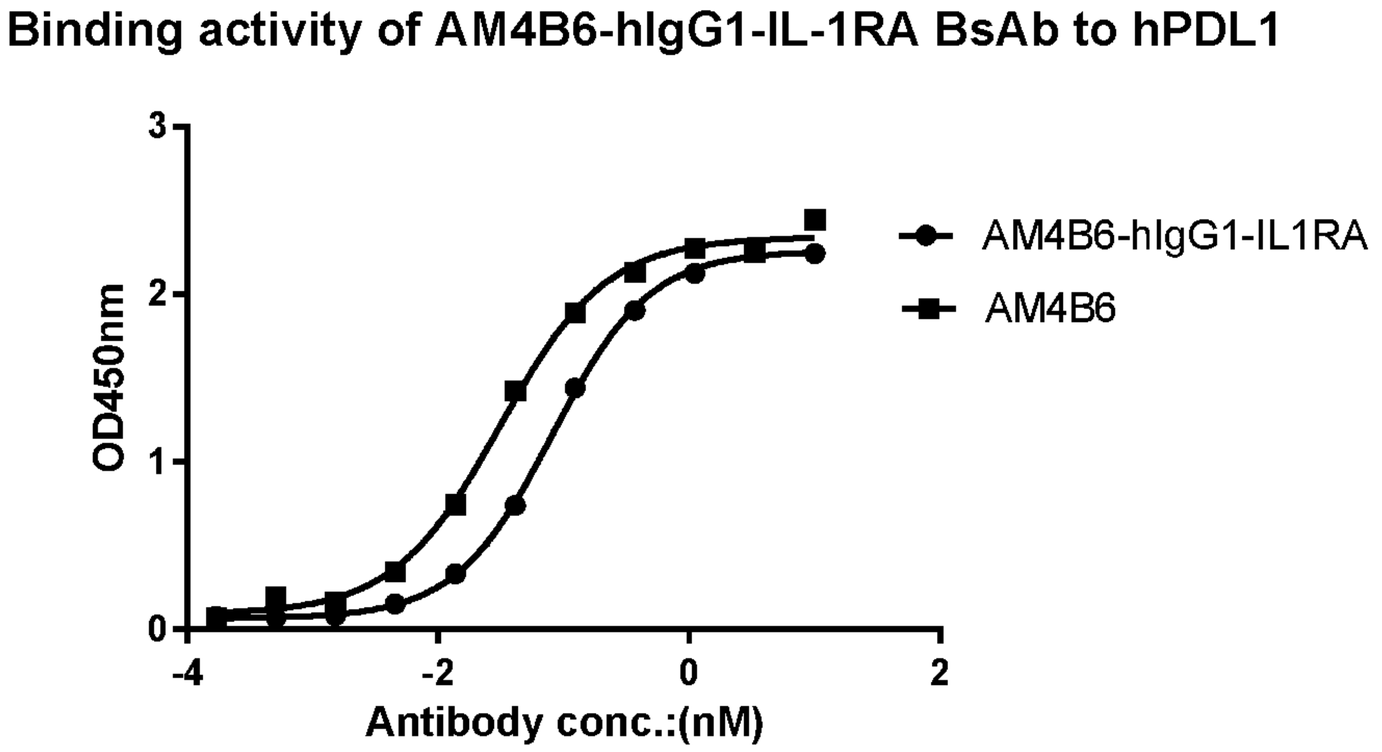
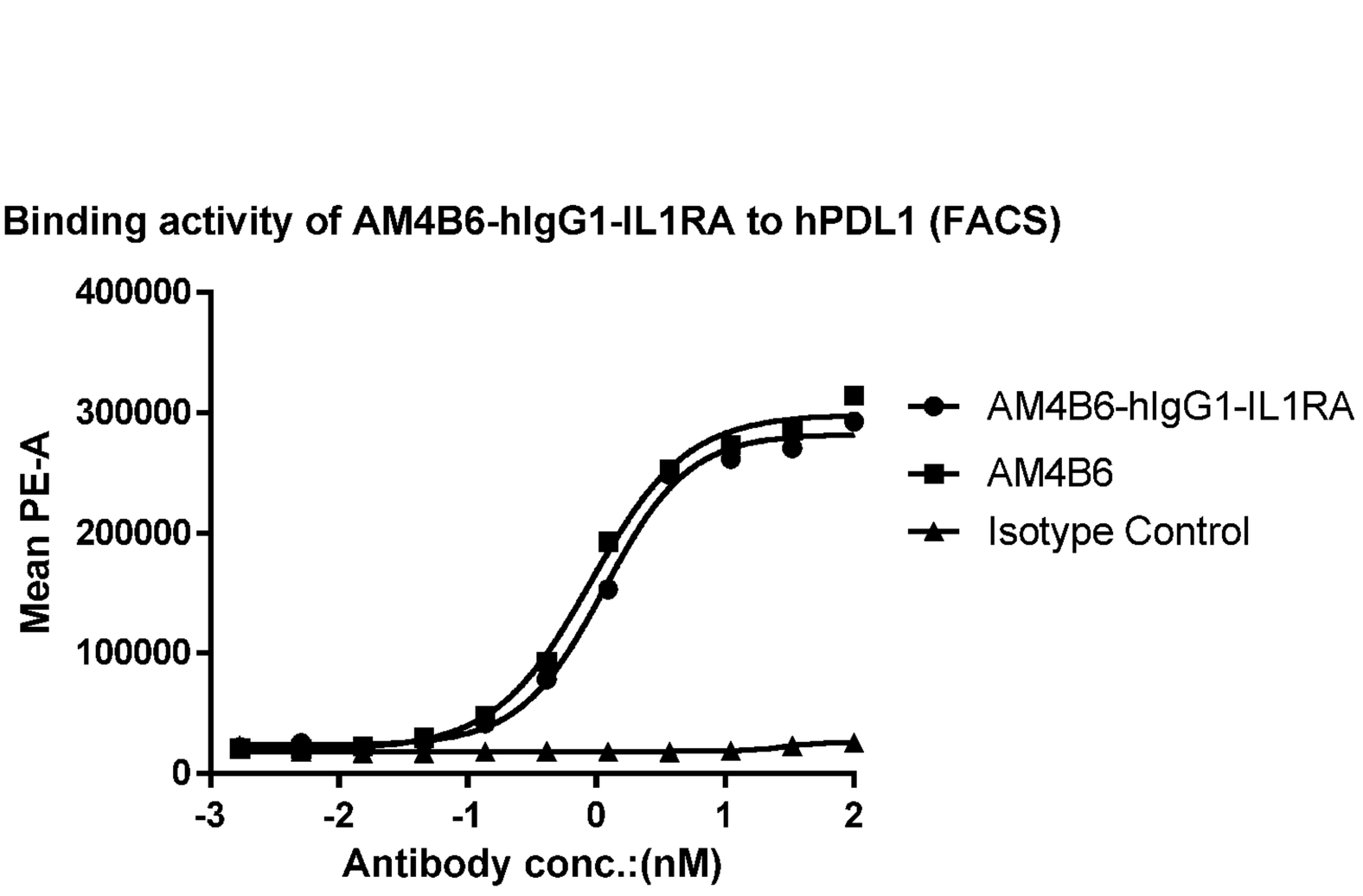


Figure 20C



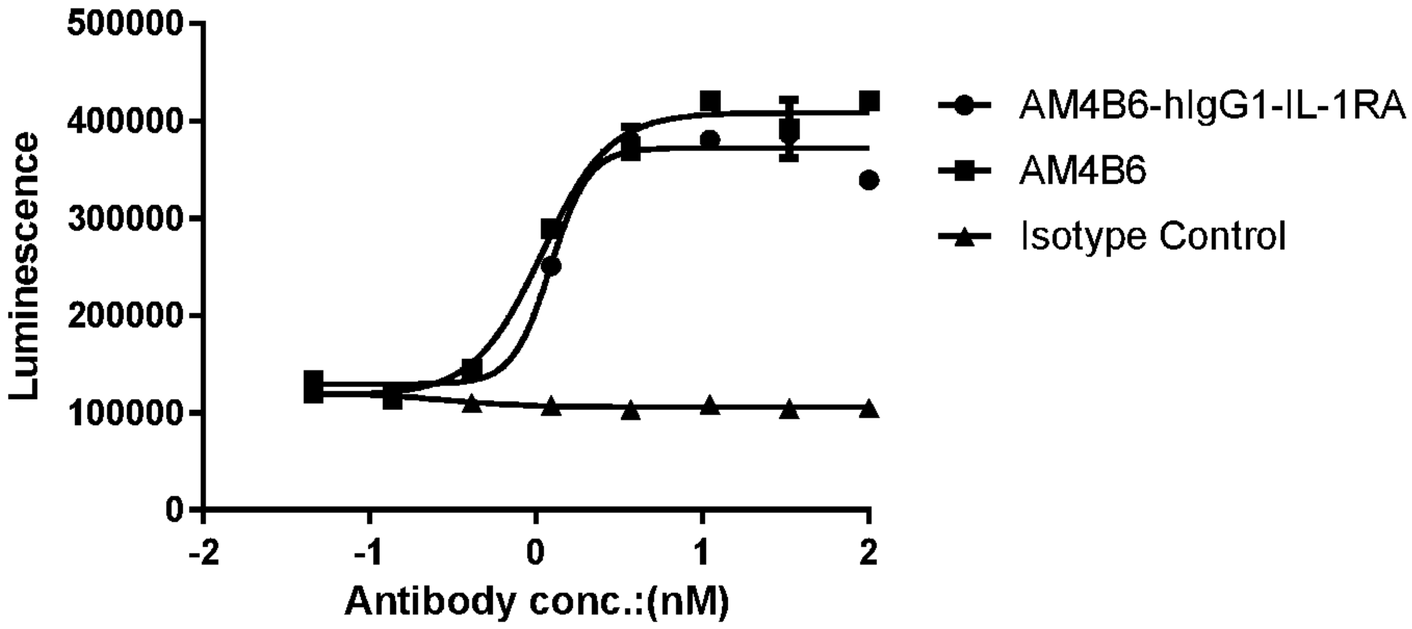
	AM4B6-hIgG1-IL1RA	AM4B6
EC50	0.08154	0.03128

Figure 21



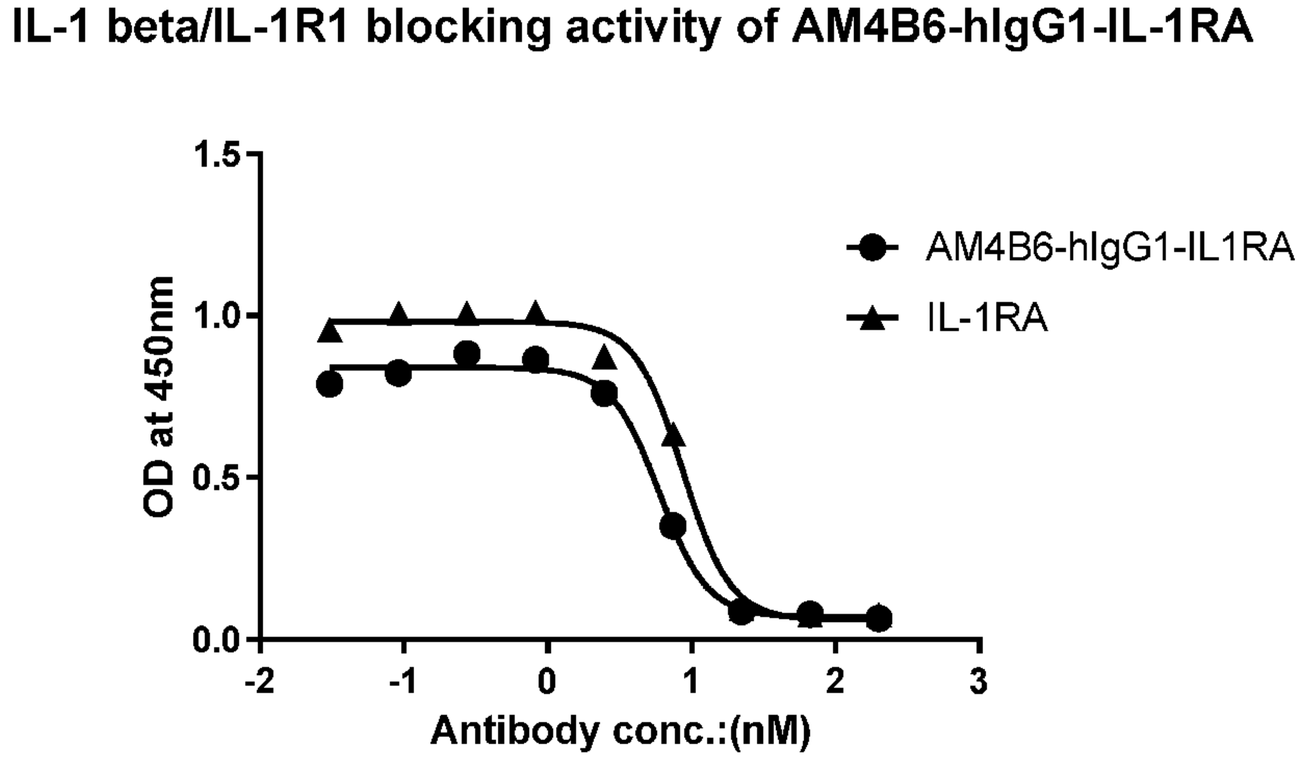
	AM4B6-hIgG1-IL1RA	AM4B6	Isotype Control
EC50	1.146	0.9033	29.17

Figure 22



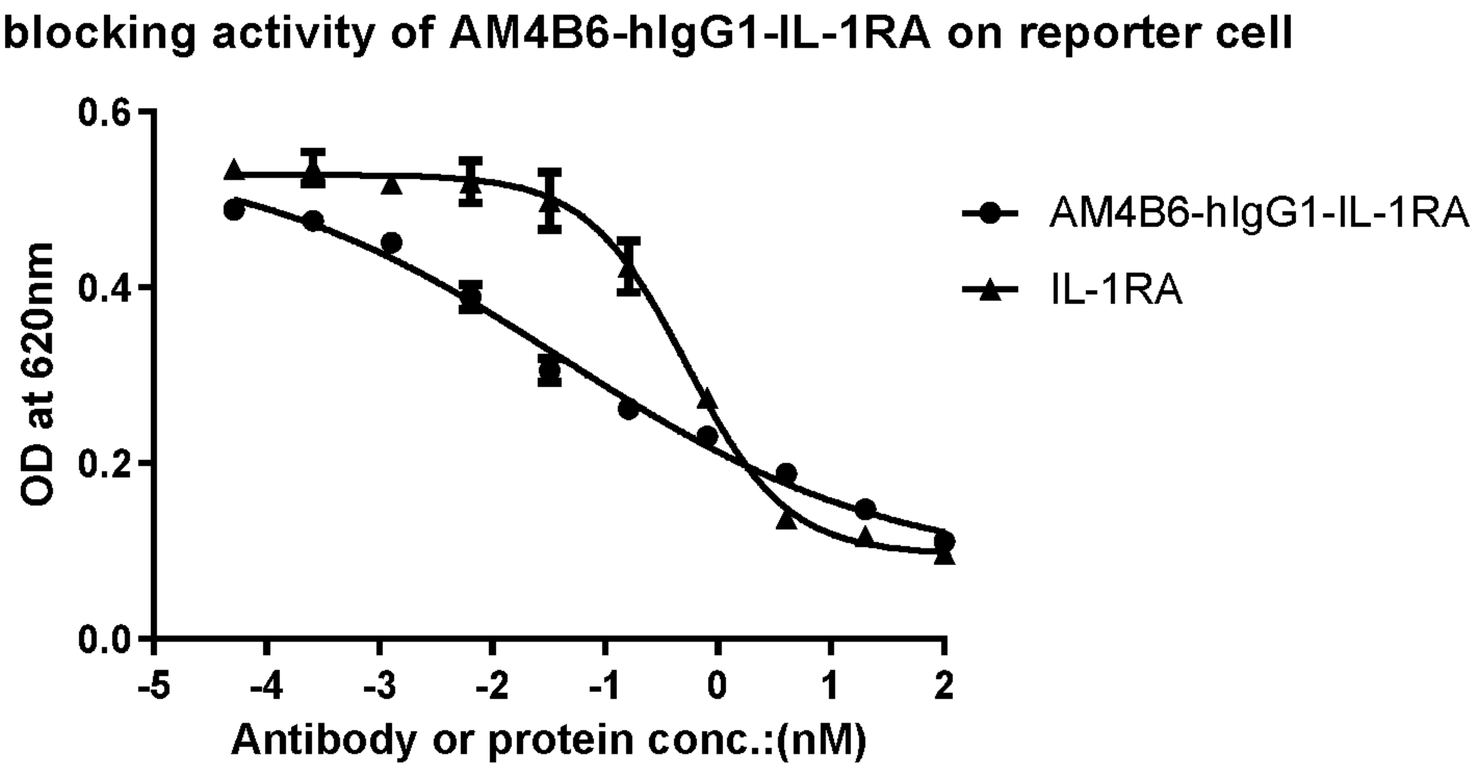
	AM4B6-hIgG1-IL-1RA	AM4B6	Isotype Control
IC50	1.227	1.083	0.275

Figure 23



	IL-1RA	AM4B6-hIgG1-IL1RA
IC50	8.681	5.947

Figure 24



	IL-1RA	AM4B6-hIgG1-IL-1RA
IC50	0.5238	0.04344

Figure 25

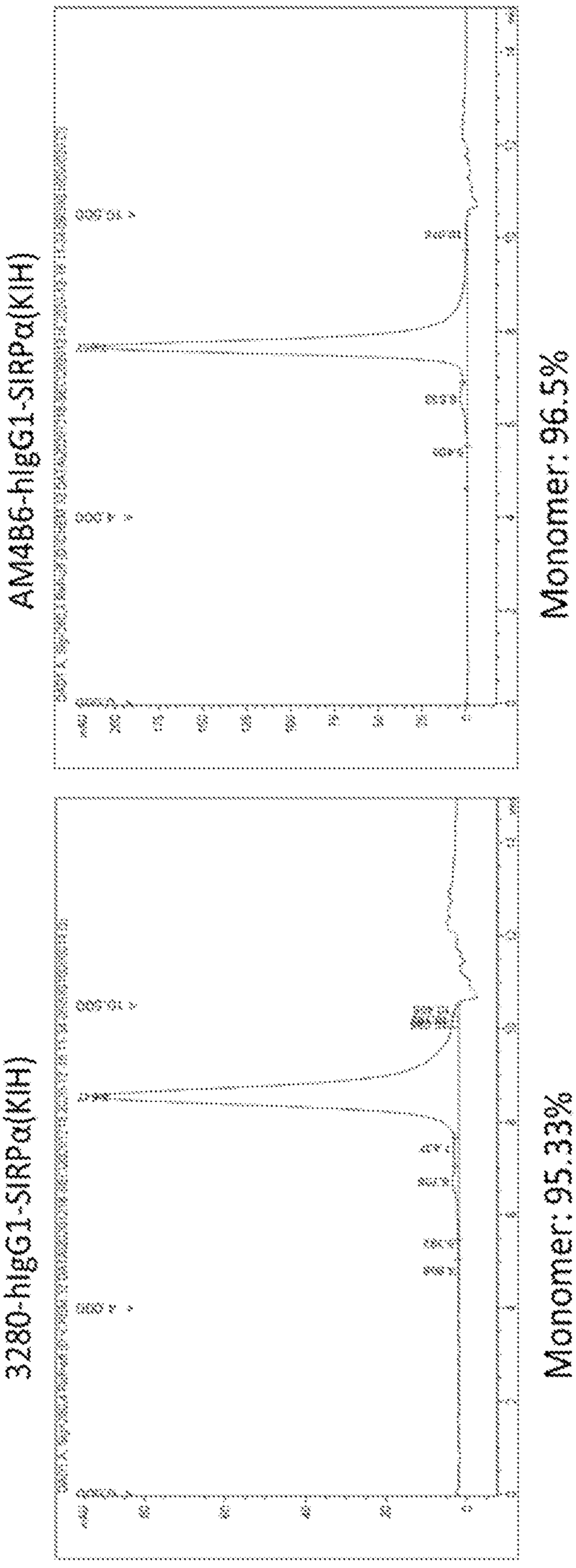
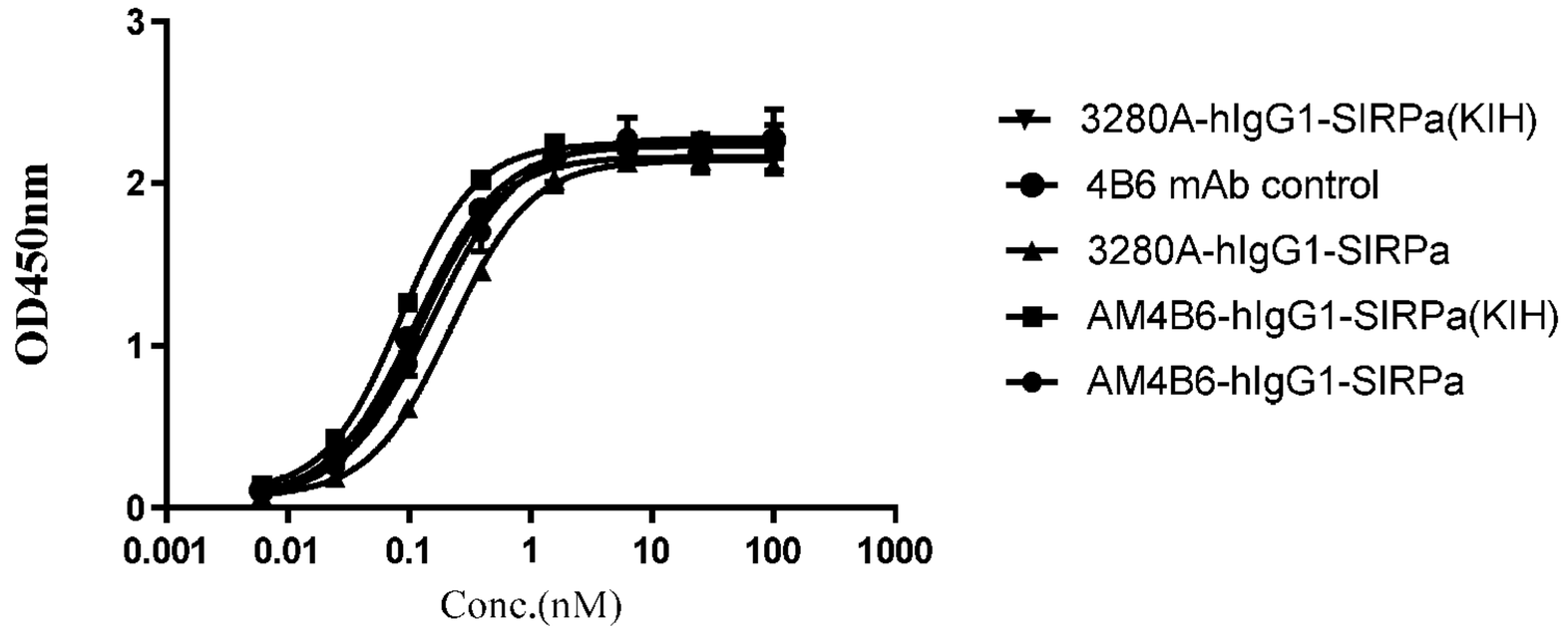

AM4B6-hIgG1-SIRPα(KIH)
Monomer: 96.5%
3280-hIgG1-SIRPα(KIH)
Monomer: 95.33%

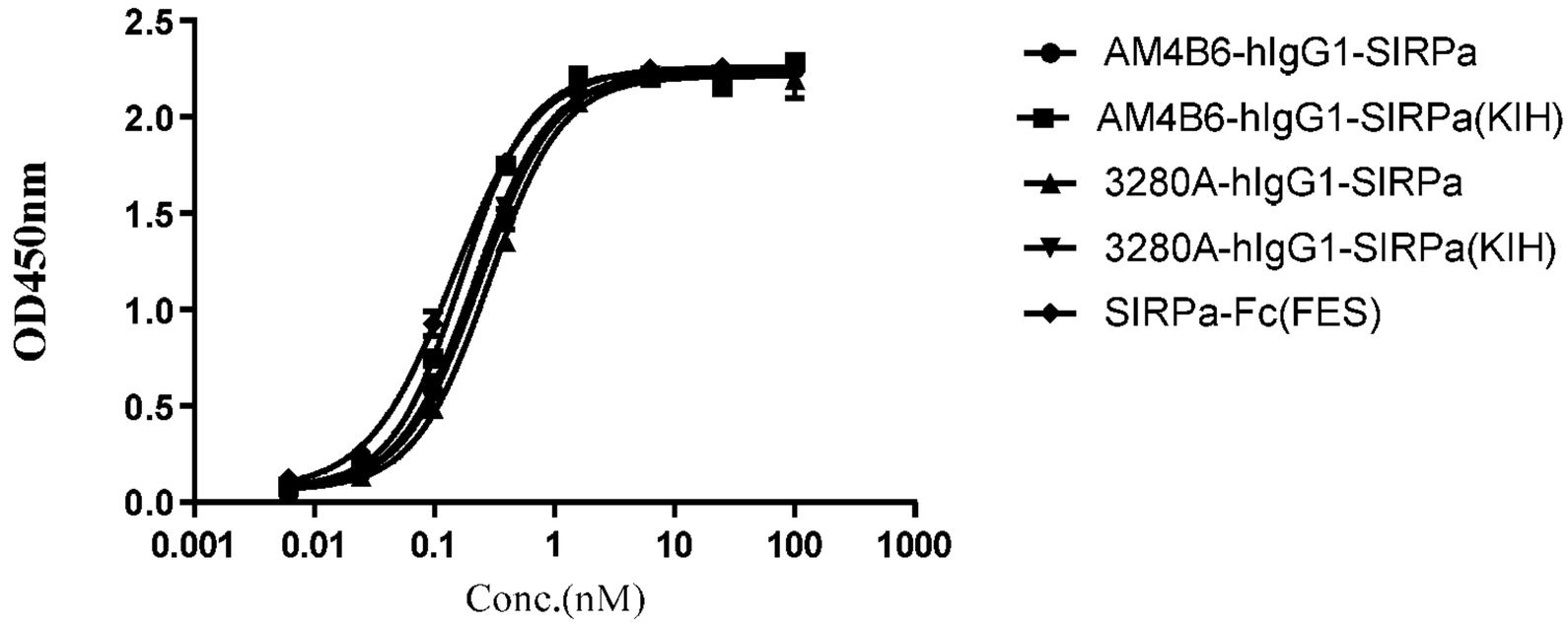
Figure 26



	AM4B6-hIgG1-SIRPa	AM4B6-hIgG1-SIRPa(KIH)	3280A-hIgG1-SIRPa
EC50	0.1537	0.08527	0.2194

	3280A-hIgG1-SIRPa(KIH)	4B6 mAb control
EC50	0.1221	0.1140

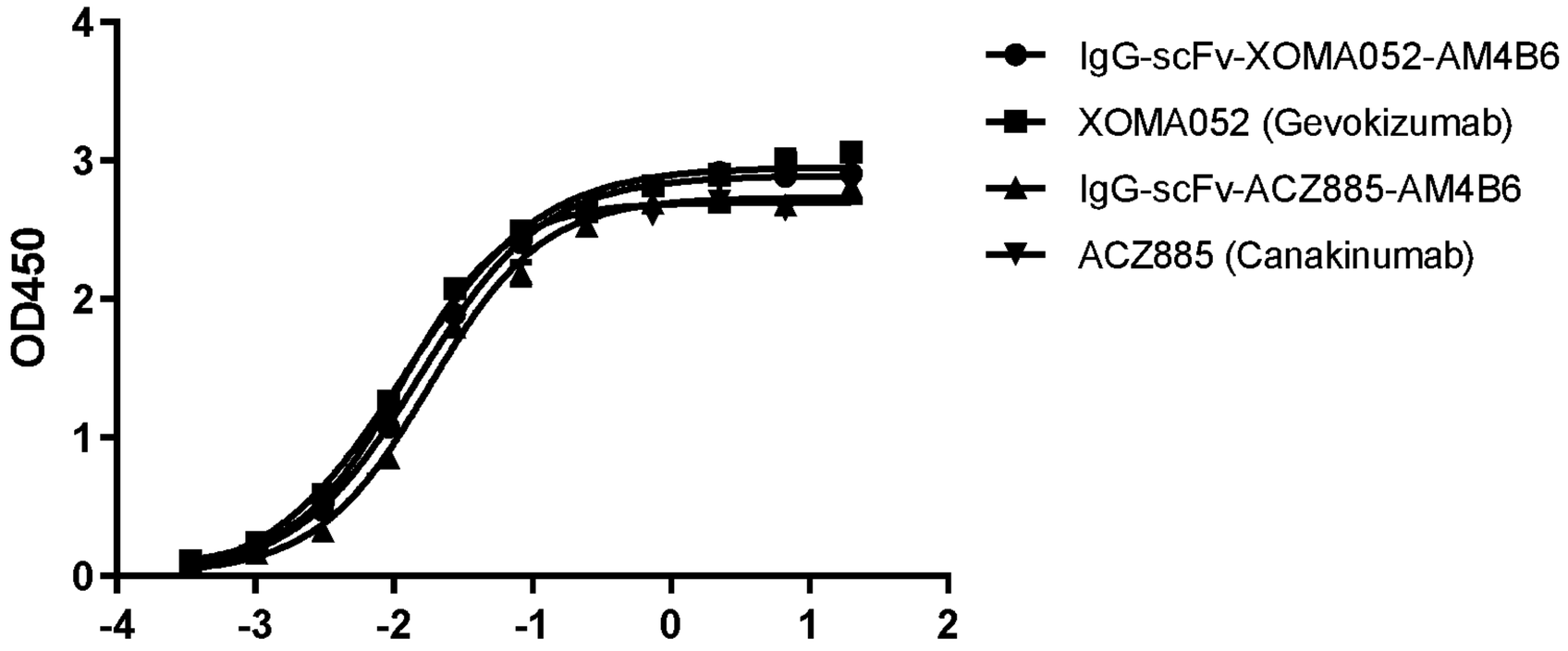
Figure 27



	AM4B6-hIgG1-SIRPa	AM4B6-hIgG1-SIRPa(KIH)	3280A-hIgG1-SIRPa
EC50	0.2403	0.1670	0.2820

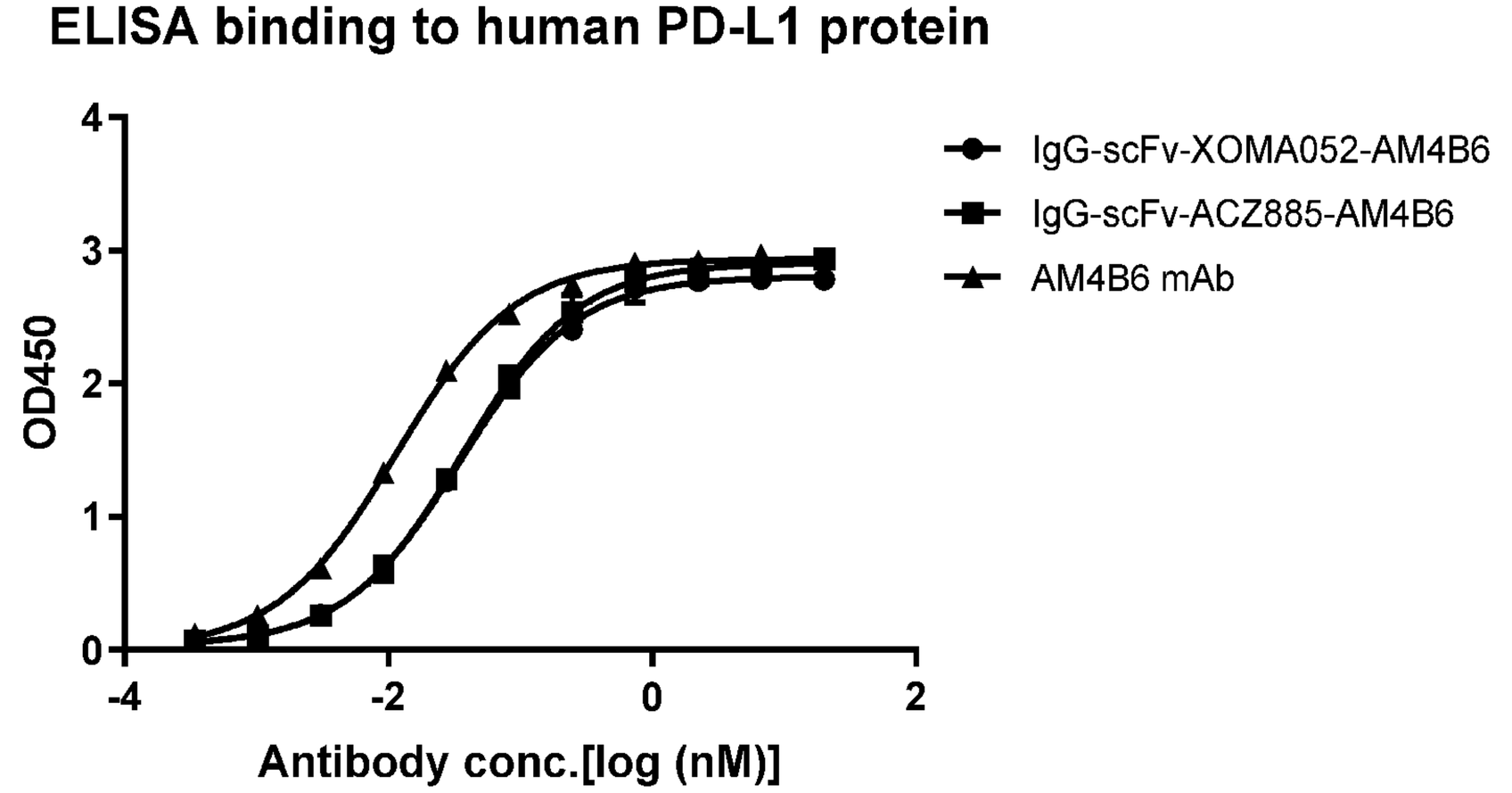
	3280A-hIgG1-SIRPa(KIH)	SIRPa-Fc(FES)
EC50	0.2162	0.1417

Figure 28



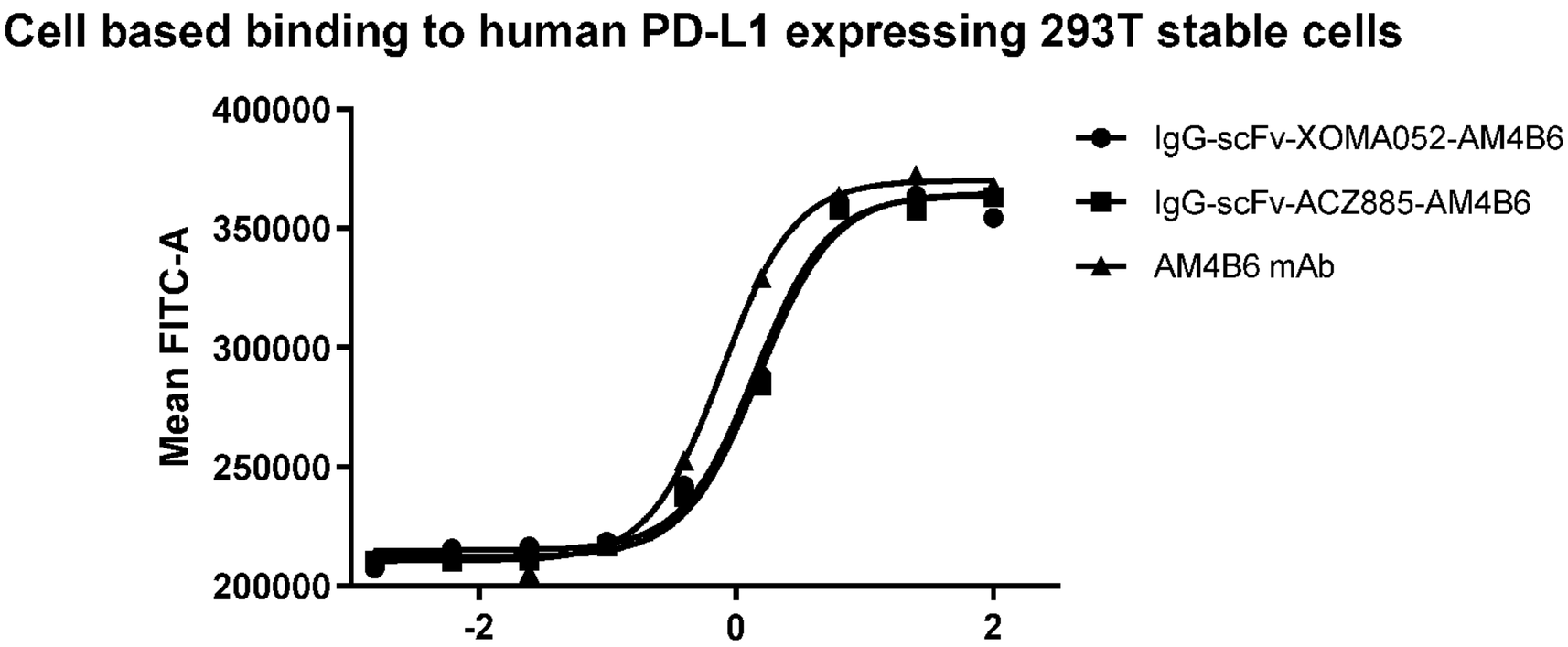
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XOMA052 (Gevokizumab)	0.01191
IgG-scFv-ACZ885-AM4B6	0.01816
ACZ885 (Canakinumab)	0.01129

Figure 29



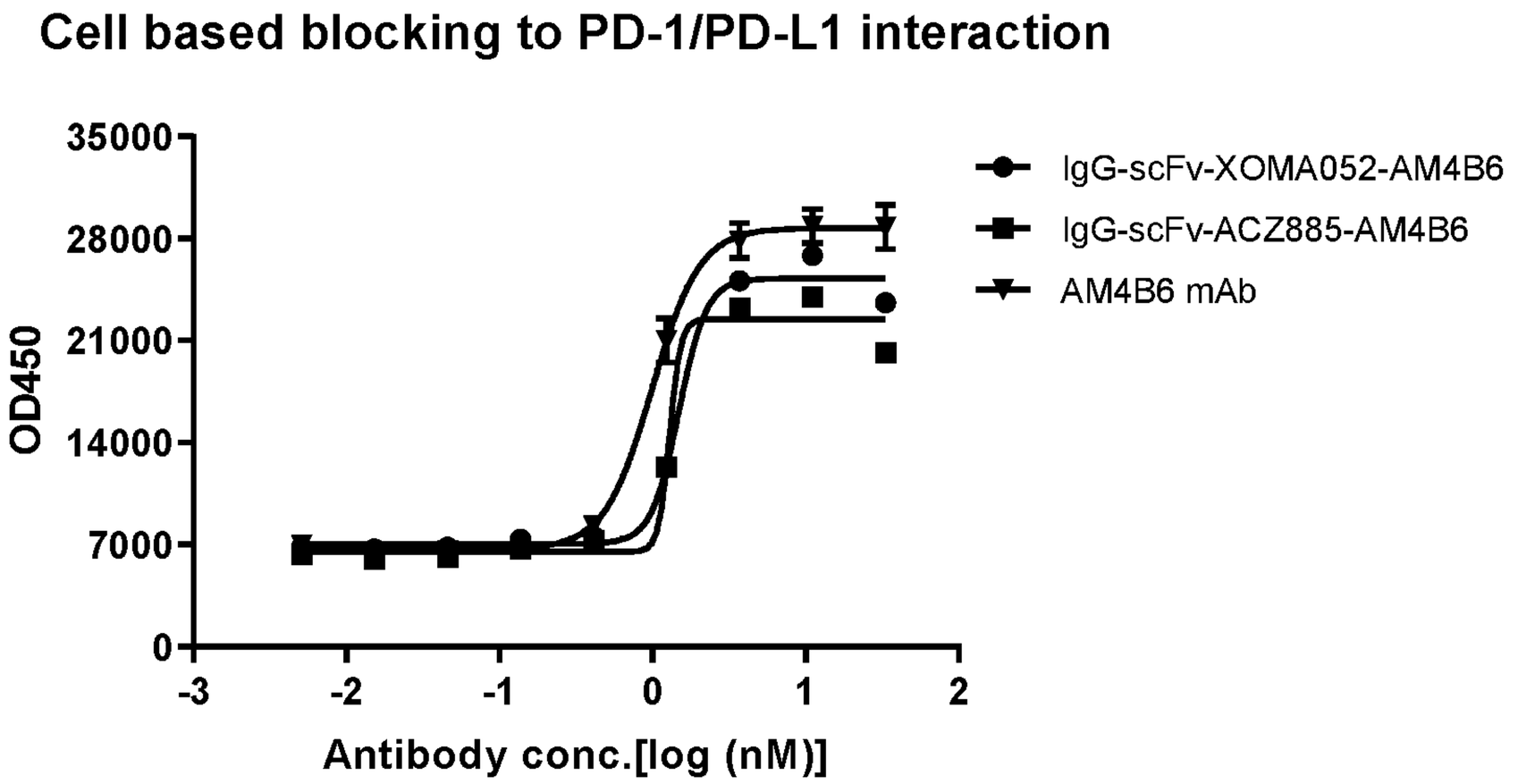
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IgG-scFv-XOMA052-AM4B6	0.03490
IgG-scFv-ACZ885-AM4B6	0.03628
AM4B6 mAb	0.01139

Figure 30



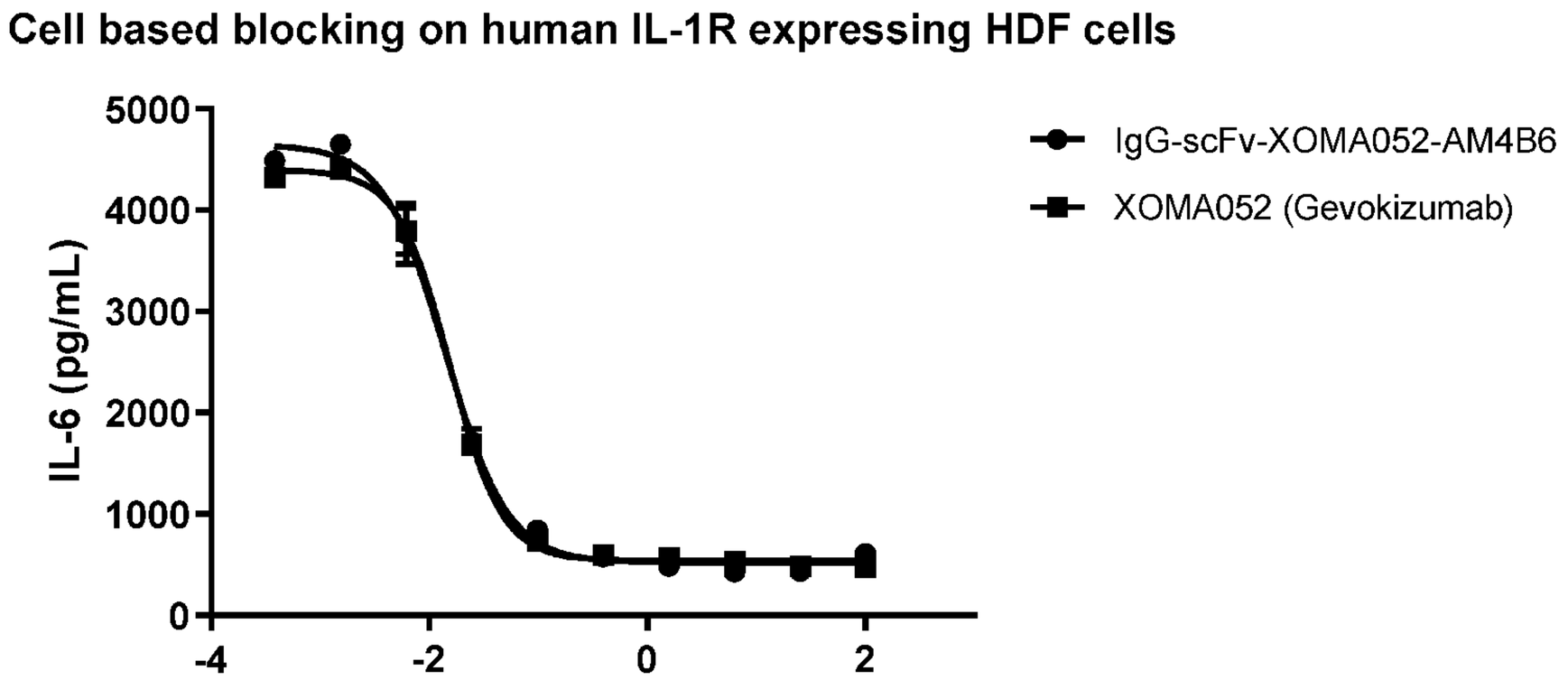
	EC50
IgG-scFv-XOMA052-AM4B6	1.401
IgG-scFv-ACZ885-AM4B6	1.506
AM4B6 mAb	0.7738

Figure 31



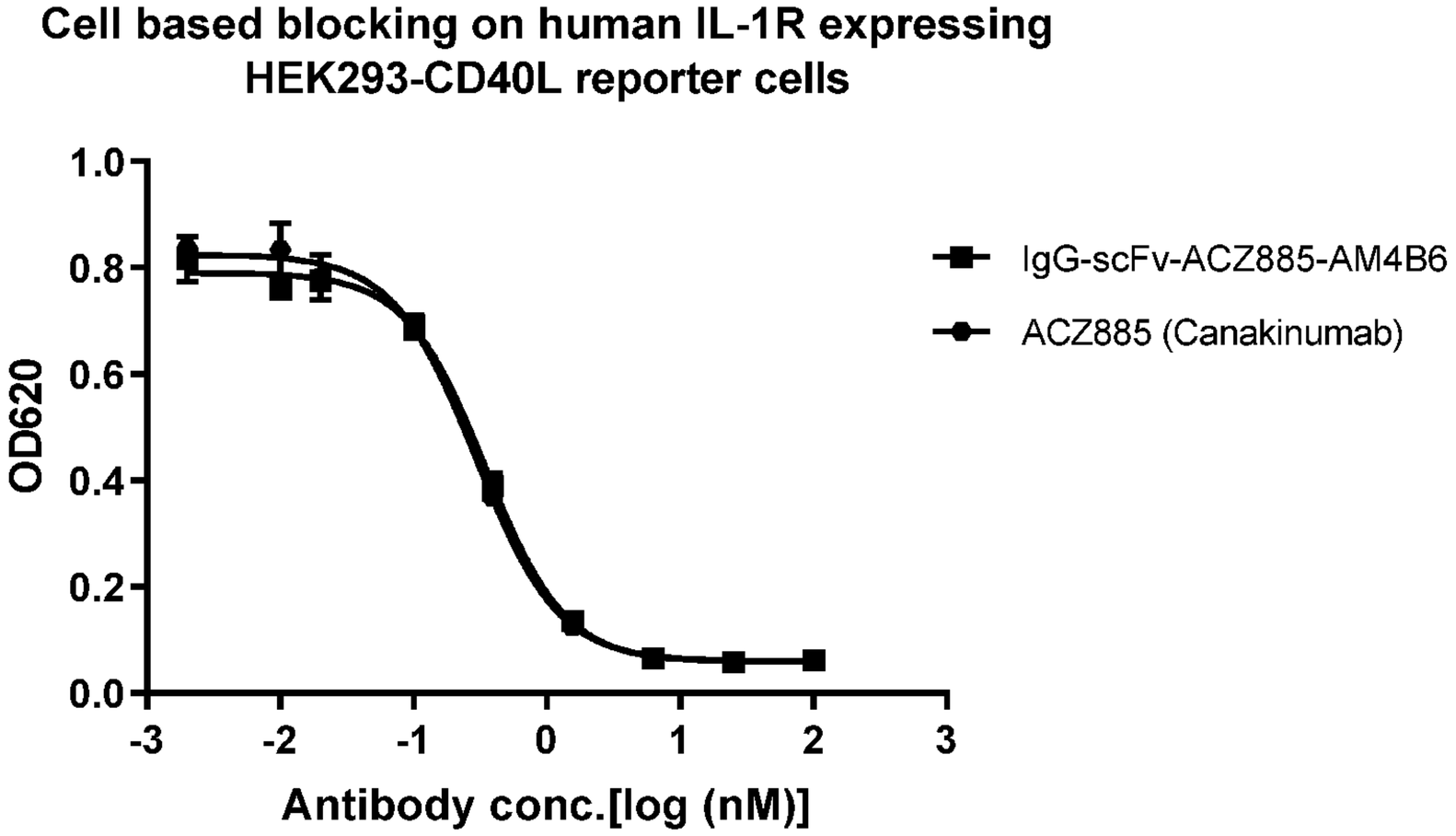
	IC50
IgG-scFv-XOMA052-AM4B6	1.487
IgG-scFv-ACZ885-AM4B6	~ 1.293
AM4B6 mAb	0.9941

Figure 32



	IC50
IgG-scFv-XOMA052-AM4B6	0.01412
XOMA052 (Gevokizumab)	0.01544

Figure 33



	IC50
IgG-scFv-ACZ885-AM4B6	0.3459
ACZ885 (Canakinumab)	0.2941

Figure 34

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<223> Synthetic

<220>
<221> misc_feature
<222> (9)..(9)
<223> Xaa can be any naturally occurring amino acid

<400> 41

Trp Val Arg Gln Ala Pro Gly Gln Xaa Leu Glu Trp Met Gly
1   5   10

<210> 42
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> misc_feature
<222> (4)..(4)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (8)..(8)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (31)..(32)
<223> Xaa can be any naturally occurring amino acid

<400> 42

Arg Val Thr Xaa Thr Val Asp Xaa Ser Ile Ser Thr Ala Tyr Met Glu
1   5   10   15

Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Xaa Xaa
            20   25   30

<210> 43
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> misc_feature
<222> (9)..(9)
<223> Xaa can be any naturally occurring amino acid

<400> 43

Trp Tyr Gln Gln Lys Pro Gly Lys Xaa Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> 44
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> misc_feature
<222> (4)..(4)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (17)..(17)
<223> Xaa can be any naturally occurring amino acid

<400> 44

Gly Val Pro Xaa Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Xaa Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys
20 25 30

<210> 45

<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 45

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys
20 25 30

<210> 46
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 46

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
20 25 30

Tyr Met Asn Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Asp Ile Asn Pro Asn Asn Gly Gly Thr Ser Tyr Asn His Lys Phe
50 55 60

Lys Gly Lys Ala Thr Val Thr Val Asp Lys Ser Ser Arg Thr Ala Tyr
65 70 75 80

Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

```
Val Lys Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ala
        115


<210>  47
<211>  106
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  47

Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
1               5                   10                  15


Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Ala Ala
            20                  25                  30


Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Ser Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Met Gln Ser
65                  70                  75                  80


Glu Asp Leu Ala Asp Tyr Phe Cys Gln Gln Tyr Ser Asn Tyr Pro Thr
                85                  90                  95


Phe Gly Ser Gly Thr Lys Leu Gly Ile Lys
            100                 105


<210>  48
<211>  98
<212>  PRT
```

<213> Homo sapiens

<400> 48

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
            20                  25                  30


Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
    50                  55                  60


Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg
```

<210> 49
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 49

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20                  25                  30
```

```
Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Asp Ile Asn Pro Asn Asn Gly Gly Thr Ser Tyr Asn His Lys Phe
    50                  55                  60


Lys Gly Arg Val Thr Val Thr Val Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser
        115


<210>  50
<211>  117
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  50

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20                  25                  30


Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Asp Ile Asn Pro Asn Asn Gly Gly Thr Ser Tyr Asn His Lys Phe
    50                  55                  60
```

```
Lys Gly Arg Val Thr Val Thr Val Asp Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Val Lys Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser
        115
```

<210> 51
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 51

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20                  25                  30


Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Asp Ile Asn Pro Asn Asn Gly Gly Thr Ser Tyr Asn His Lys Phe
    50                  55                  60


Lys Gly Arg Val Thr Val Thr Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
```

```
Ala Arg Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser
        115


<210>  52
<211>  117
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  52

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20                  25                  30


Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Ser Leu Glu Trp Met
        35                  40                  45


Gly Asp Ile Asn Pro Asn Asn Gly Gly Thr Ser Tyr Asn His Lys Phe
    50                  55                  60


Lys Gly Arg Val Thr Val Thr Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Val Lys Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser
        115
```

<210> 53
<211> 95
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 53

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Asp Asn Leu Pro
85 90 95

<210> 54
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 54

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Ala Ala
20 25 30

```
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Asn Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105
```

<210> 55
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 55

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Ala Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
```

```
Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Thr
                85                  90                  95


Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105


<210>  56
<211>  117
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  56

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20                  25                  30


Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Ser Leu Glu Trp Met
        35                  40                  45


Gly Asp Ile Asn Pro Asn Asn Ala Gly Thr Ser Tyr Asn His Lys Phe
    50                  55                  60


Lys Gly Arg Val Thr Val Thr Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Val Lys Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser
        115
```

<210> 57
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 57

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
20 25 30

Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Ser Leu Glu Trp Met
35 40 45

Gly Asp Ile Asn Pro Asn Asn Ala Gly Thr Ser Tyr Asn His Lys Phe
50 55 60

Lys Gly Arg Val Thr Val Thr Val Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Lys Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 58
<211> 117
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic

<400> 58

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1   5   10   15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20   25   30

Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Ser Leu Glu Trp Met
        35   40   45

Gly Asp Ile Asn Pro Asn Asn Ala Gln Thr Gln Tyr Asn His Lys Phe
    50   55   60

Lys Gly Arg Val Thr Val Thr Val Asp Lys Ser Ile Ser Thr Ala Tyr
65   70   75   80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Val Lys Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100   105   110

Val Thr Val Ser Ser
        115

<210> 59
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 59

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1   5   10   15

```
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20                  25                  30

Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Ser Leu Glu Trp Met
        35                  40                  45

Gly Asp Ile Asn Pro Asn Asn Ala Glu Thr Leu Tyr Asn His Lys Phe
    50                  55                  60

Lys Gly Arg Val Thr Val Thr Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Val Lys Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110

Val Thr Val Ser Ser
        115
```

<210> 60
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 60

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20                  25                  30

Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Ser Leu Glu Trp Met
        35                  40                  45
```

```
Gly Asp Ile Asn Pro Asn Asn Ala Gln Thr Val Tyr Asn His Lys Phe
    50                  55                  60

Lys Gly Arg Val Thr Val Thr Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Val Lys Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110

Val Thr Val Ser Ser
        115
```

<210> 61
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 61

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Ala Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Asn Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
```

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 62
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 62

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Lys Gly Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Asn Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 63
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 63

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Pro Ala Ala
            20   25   30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35   40   45

Tyr Ser Ala Ser Asn Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Thr
                85   90   95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100   105

<210> 64
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 64

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Ala Ala
            20   25   30

```
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Val Ser Asp Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105
```

<210> 65
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 65

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Ala Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Arg Ser Val Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
```

```
Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Thr
                85                  90                  95


Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105


<210>  66
<211>  117
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  66

Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe
1               5                   10                  15


Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr
            20                  25                  30


Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys
        35                  40                  45


Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu
    50                  55                  60


Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile
65                  70                  75                  80


Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys
                85                  90                  95


Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn
            100                 105                 110


Thr Ser Asn Pro Asp
        115
```

<210> 67
<211> 144
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 67

Lys Met Gln Ala Phe Arg Ile Trp Asp Val Asn Gln Lys Thr Phe Tyr
1 5 10 15

Leu Arg Asn Asn Gln Leu Val Ala Gly Tyr Leu Gln Gly Pro Asn Val
20 25 30

Asn Leu Glu Glu Lys Ile Asp Val Val Pro Ile Glu Pro His Ala Leu
35 40 45

Phe Leu Gly Ile His Gly Gly Lys Met Cys Leu Ser Cys Val Lys Ser
50 55 60

Gly Asp Glu Thr Arg Leu Gln Leu Glu Ala Val Asn Ile Thr Asp Leu
65 70 75 80

Ser Glu Asn Arg Lys Gln Asp Lys Arg Phe Ala Phe Ile Arg Ser Asp
85 90 95

Ser Gly Pro Thr Thr Ser Phe Glu Ser Ala Ala Cys Pro Gly Trp Phe
100 105 110

Leu Cys Thr Ala Met Glu Ala Asp Gln Pro Val Ser Leu Thr Asn Met
115 120 125

Pro Asp Glu Gly Val Met Val Thr Lys Phe Tyr Phe Gln Glu Asp Glu
130 135 140

<210> 68
<211> 21

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 68

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1   5   10   15

Gly Gly Gly Ser Gly
            20

<210> 69
<211> 470
<212> PRT
<213> Homo sapiens

<400> 69

Leu Gln Cys Phe Cys His Leu Cys Thr Lys Asp Asn Phe Thr Cys Val
1   5   10   15

Thr Asp Gly Leu Cys Phe Val Ser Val Thr Glu Thr Thr Asp Lys Val
            20   25   30

Ile His Asn Ser Met Cys Ile Ala Glu Ile Asp Leu Ile Pro Arg Asp
        35   40   45

Arg Pro Phe Val Cys Ala Pro Ser Ser Lys Thr Gly Ser Val Thr Thr
    50   55   60

Thr Tyr Cys Cys Asn Gln Asp His Cys Asn Lys Ile Glu Leu Pro Thr
65   70   75   80

Thr Val Lys Ser Ser Pro Gly Leu Gly Pro Val Glu Leu Ala Ala Val
                85   90   95

Ile Ala Gly Pro Val Cys Phe Val Cys Ile Ser Leu Met Leu Met Val
            100   105   110

```
Tyr Ile Cys His Asn Arg Thr Val Ile His His Arg Val Pro Asn Glu
        115                 120                 125

Glu Asp Pro Ser Leu Asp Arg Pro Phe Ile Ser Glu Gly Thr Thr Leu
    130                 135                 140

Lys Asp Leu Ile Tyr Asp Met Thr Thr Ser Gly Ser Gly Ser Gly Leu
145                 150                 155                 160

Pro Leu Leu Val Gln Arg Thr Ile Ala Arg Thr Ile Val Leu Gln Glu
                165                 170                 175

Ser Ile Gly Lys Gly Arg Phe Gly Glu Val Trp Arg Gly Lys Trp Arg
            180                 185                 190

Gly Glu Glu Val Ala Val Lys Ile Phe Ser Ser Arg Glu Glu Arg Ser
        195                 200                 205

Trp Phe Arg Glu Ala Glu Ile Tyr Gln Thr Val Met Leu Arg His Glu
    210                 215                 220

Asn Ile Leu Gly Phe Ile Ala Ala Asp Asn Lys Asp Asn Gly Thr Trp
225                 230                 235                 240

Thr Gln Leu Trp Leu Val Ser Asp Tyr His Glu His Gly Ser Leu Phe
                245                 250                 255

Asp Tyr Leu Asn Arg Tyr Thr Val Thr Val Glu Gly Met Ile Lys Leu
            260                 265                 270

Ala Leu Ser Thr Ala Ser Gly Leu Ala His Leu His Met Glu Ile Val
        275                 280                 285

Gly Thr Gln Gly Lys Pro Ala Ile Ala His Arg Asp Leu Lys Ser Lys
    290                 295                 300

Asn Ile Leu Val Lys Lys Asn Gly Thr Cys Cys Ile Ala Asp Leu Gly
```

```
305                 310                 315                 320

Leu Ala Val Arg His Asp Ser Ala Thr Asp Thr Ile Asp Ile Ala Pro
                325                 330                 335

Asn His Arg Val Gly Thr Lys Arg Tyr Met Ala Pro Glu Val Leu Asp
            340                 345                 350

Asp Ser Ile Asn Met Lys His Phe Glu Ser Phe Lys Arg Ala Asp Ile
        355                 360                 365

Tyr Ala Met Gly Leu Val Phe Trp Glu Ile Ala Arg Arg Cys Ser Ile
    370                 375                 380

Gly Gly Ile His Glu Asp Tyr Gln Leu Pro Tyr Tyr Asp Leu Val Pro
385                 390                 395                 400

Ser Asp Pro Ser Val Glu Glu Met Arg Lys Val Val Cys Glu Gln Lys
                405                 410                 415

Leu Arg Pro Asn Ile Pro Asn Arg Trp Gln Ser Cys Glu Ala Leu Arg
            420                 425                 430

Val Met Ala Lys Ile Met Arg Glu Cys Trp Tyr Ala Asn Gly Ala Ala
        435                 440                 445

Arg Leu Thr Ala Leu Arg Ile Lys Lys Thr Leu Ser Gln Leu Ser Gln
    450                 455                 460

Gln Glu Gly Ile Lys Met
465                 470
```

<210> 70
<211> 545
<212> PRT
<213> Homo sapiens

<400> 70

```
Thr Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val
1               5                  10                  15

Thr Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys
            20                  25                  30

Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn
        35                  40                  45

Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala
    50                  55                  60

Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His
65                  70                  75                  80

Asp Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser
                85                  90                  95

Pro Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe
            100                 105                 110

Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser
        115                 120                 125

Glu Glu Tyr Asn Thr Ser Asn Pro Asp Leu Leu Leu Val Ile Phe Gln
    130                 135                 140

Val Thr Gly Ile Ser Leu Leu Pro Pro Leu Gly Val Ala Ile Ser Val
145                 150                 155                 160

Ile Ile Ile Phe Tyr Cys Tyr Arg Val Asn Arg Gln Gln Lys Leu Ser
                165                 170                 175

Ser Thr Trp Glu Thr Gly Lys Thr Arg Lys Leu Met Glu Phe Ser Glu
            180                 185                 190

His Cys Ala Ile Ile Leu Glu Asp Asp Arg Ser Asp Ile Ser Ser Thr
        195                 200                 205
```

```
Cys Ala Asn Asn Ile Asn His Asn Thr Glu Leu Leu Pro Ile Glu Leu
    210                 215                 220

Asp Thr Leu Val Gly Lys Gly Arg Phe Ala Glu Val Tyr Lys Ala Lys
225                 230                 235                 240

Leu Lys Gln Asn Thr Ser Glu Gln Phe Glu Thr Val Ala Val Lys Ile
                245                 250                 255

Phe Pro Tyr Glu Glu Tyr Ala Ser Trp Lys Thr Glu Lys Asp Ile Phe
            260                 265                 270

Ser Asp Ile Asn Leu Lys His Glu Asn Ile Leu Gln Phe Leu Thr Ala
        275                 280                 285

Glu Glu Arg Lys Thr Glu Leu Gly Lys Gln Tyr Trp Leu Ile Thr Ala
    290                 295                 300

Phe His Ala Lys Gly Asn Leu Gln Glu Tyr Leu Thr Arg His Val Ile
305                 310                 315                 320

Ser Trp Glu Asp Leu Arg Lys Leu Gly Ser Ser Leu Ala Arg Gly Ile
                325                 330                 335

Ala His Leu His Ser Asp His Thr Pro Cys Gly Arg Pro Lys Met Pro
            340                 345                 350

Ile Val His Arg Asp Leu Lys Ser Ser Asn Ile Leu Val Lys Asn Asp
        355                 360                 365

Leu Thr Cys Cys Leu Cys Asp Phe Gly Leu Ser Leu Arg Leu Asp Pro
    370                 375                 380

Thr Leu Ser Val Asp Asp Leu Ala Asn Ser Gly Gln Val Gly Thr Ala
385                 390                 395                 400
```

```
Arg Tyr Met Ala Pro Glu Val Leu Glu Ser Arg Met Asn Leu Glu Asn
                405                 410                 415

Val Glu Ser Phe Lys Gln Thr Asp Val Tyr Ser Met Ala Leu Val Leu
            420                 425                 430

Trp Glu Met Thr Ser Arg Cys Asn Ala Val Gly Glu Val Lys Asp Tyr
        435                 440                 445

Glu Pro Pro Phe Gly Ser Lys Val Arg Glu His Pro Cys Val Glu Ser
    450                 455                 460

Met Lys Asp Asn Val Leu Arg Asp Arg Gly Arg Pro Glu Ile Pro Ser
465                 470                 475                 480

Phe Trp Leu Asn His Gln Gly Ile Gln Met Val Cys Glu Thr Leu Thr
                485                 490                 495

Glu Cys Trp Asp His Asp Pro Glu Ala Arg Leu Thr Ala Gln Cys Val
            500                 505                 510

Ala Glu Arg Phe Ser Glu Leu Glu His Leu Asp Arg Leu Ser Gly Arg
        515                 520                 525

Ser Cys Ser Glu Glu Lys Ile Pro Glu Asp Gly Ser Leu Asn Thr Thr
    530                 535                 540

Lys
545
```

<210> 71
<211> 570
<212> PRT
<213> Homo sapiens

<400> 71

```
Thr Ile Pro Pro His Val Gln Lys Ser Asp Val Glu Met Glu Ala Gln
1               5                   10                  15
```

```
Lys Asp Glu Ile Ile Cys Pro Ser Cys Asn Arg Thr Ala His Pro Leu
            20                  25                  30

Arg His Ile Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val
        35                  40                  45

Lys Phe Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys
    50                  55                  60

Asp Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys
65                  70                  75                  80

Glu Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu
                85                  90                  95

Asn Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Pro Tyr His
            100                 105                 110

Asp Phe Ile Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile Met Lys Glu
        115                 120                 125

Lys Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp
    130                 135                 140

Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn Thr Ser Asn
145                 150                 155                 160

Pro Asp Leu Leu Leu Val Ile Phe Gln Val Thr Gly Ile Ser Leu Leu
                165                 170                 175

Pro Pro Leu Gly Val Ala Ile Ser Val Ile Ile Ile Phe Tyr Cys Tyr
            180                 185                 190

Arg Val Asn Arg Gln Gln Lys Leu Ser Ser Thr Trp Glu Thr Gly Lys
        195                 200                 205

Thr Arg Lys Leu Met Glu Phe Ser Glu His Cys Ala Ile Ile Leu Glu
```

```
    210                 215                 220

Asp Asp Arg Ser Asp Ile Ser Ser Thr Cys Ala Asn Asn Ile Asn His
225                 230                 235                 240

Asn Thr Glu Leu Leu Pro Ile Glu Leu Asp Thr Leu Val Gly Lys Gly
                245                 250                 255

Arg Phe Ala Glu Val Tyr Lys Ala Lys Leu Lys Gln Asn Thr Ser Glu
            260                 265                 270

Gln Phe Glu Thr Val Ala Val Lys Ile Phe Pro Tyr Glu Glu Tyr Ala
        275                 280                 285

Ser Trp Lys Thr Glu Lys Asp Ile Phe Ser Asp Ile Asn Leu Lys His
    290                 295                 300

Glu Asn Ile Leu Gln Phe Leu Thr Ala Glu Glu Arg Lys Thr Glu Leu
305                 310                 315                 320

Gly Lys Gln Tyr Trp Leu Ile Thr Ala Phe His Ala Lys Gly Asn Leu
                325                 330                 335

Gln Glu Tyr Leu Thr Arg His Val Ile Ser Trp Glu Asp Leu Arg Lys
            340                 345                 350

Leu Gly Ser Ser Leu Ala Arg Gly Ile Ala His Leu His Ser Asp His
        355                 360                 365

Thr Pro Cys Gly Arg Pro Lys Met Pro Ile Val His Arg Asp Leu Lys
    370                 375                 380

Ser Ser Asn Ile Leu Val Lys Asn Asp Leu Thr Cys Cys Leu Cys Asp
385                 390                 395                 400

Phe Gly Leu Ser Leu Arg Leu Asp Pro Thr Leu Ser Val Asp Asp Leu
                405                 410                 415
```

```
Ala Asn Ser Gly Gln Val Gly Thr Ala Arg Tyr Met Ala Pro Glu Val
            420                 425                 430

Leu Glu Ser Arg Met Asn Leu Glu Asn Val Glu Ser Phe Lys Gln Thr
        435                 440                 445

Asp Val Tyr Ser Met Ala Leu Val Leu Trp Glu Met Thr Ser Arg Cys
    450                 455                 460

Asn Ala Val Gly Glu Val Lys Asp Tyr Glu Pro Pro Phe Gly Ser Lys
465                 470                 475                 480

Val Arg Glu His Pro Cys Val Glu Ser Met Lys Asp Asn Val Leu Arg
                485                 490                 495

Asp Arg Gly Arg Pro Glu Ile Pro Ser Phe Trp Leu Asn His Gln Gly
            500                 505                 510

Ile Gln Met Val Cys Glu Thr Leu Thr Glu Cys Trp Asp His Asp Pro
        515                 520                 525

Glu Ala Arg Leu Thr Ala Gln Cys Val Ala Glu Arg Phe Ser Glu Leu
    530                 535                 540

Glu His Leu Asp Arg Leu Ser Gly Arg Ser Cys Ser Glu Glu Lys Ile
545                 550                 555                 560

Pro Glu Asp Gly Ser Leu Asn Thr Thr Lys
                565                 570
```

<210> 72
<211> 831
<212> PRT
<213> Homo sapiens

<400> 72

```
Gly Pro Glu Pro Gly Ala Leu Cys Glu Leu Ser Pro Val Ser Ala Ser
1               5                   10                  15
```

```
His Pro Val Gln Ala Leu Met Glu Ser Phe Thr Val Leu Ser Gly Cys
            20                  25                  30

Ala Ser Arg Gly Thr Thr Gly Leu Pro Gln Glu Val His Val Leu Asn
        35                  40                  45

Leu Arg Thr Ala Gly Gln Gly Pro Gly Gln Leu Gln Arg Glu Val Thr
    50                  55                  60

Leu His Leu Asn Pro Ile Ser Ser Val His Ile His His Lys Ser Val
65                  70                  75                  80

Val Phe Leu Leu Asn Ser Pro His Pro Leu Val Trp His Leu Lys Thr
                85                  90                  95

Glu Arg Leu Ala Thr Gly Val Ser Arg Leu Phe Leu Val Ser Glu Gly
            100                 105                 110

Ser Val Val Gln Phe Ser Ser Ala Asn Phe Ser Leu Thr Ala Glu Thr
        115                 120                 125

Glu Glu Arg Asn Phe Pro His Gly Asn Glu His Leu Leu Asn Trp Ala
    130                 135                 140

Arg Lys Glu Tyr Gly Ala Val Thr Ser Phe Thr Glu Leu Lys Ile Ala
145                 150                 155                 160

Arg Asn Ile Tyr Ile Lys Val Gly Glu Asp Gln Val Phe Pro Pro Lys
                165                 170                 175

Cys Asn Ile Gly Lys Asn Phe Leu Ser Leu Asn Tyr Leu Ala Glu Tyr
            180                 185                 190

Leu Gln Pro Lys Ala Ala Glu Gly Cys Val Met Ser Ser Gln Pro Gln
        195                 200                 205
```

```
Asn Glu Glu Val His Ile Ile Glu Leu Ile Thr Pro Asn Ser Asn Pro
    210                 215                 220

Tyr Ser Ala Phe Gln Val Asp Ile Thr Ile Asp Ile Arg Pro Ser Gln
225                 230                 235                 240

Glu Asp Leu Glu Val Val Lys Asn Leu Ile Leu Ile Leu Lys Cys Lys
                245                 250                 255

Lys Ser Val Asn Trp Val Ile Lys Ser Phe Asp Val Lys Gly Ser Leu
            260                 265                 270

Lys Ile Ile Ala Pro Asn Ser Ile Gly Phe Gly Lys Glu Ser Glu Arg
        275                 280                 285

Ser Met Thr Met Thr Lys Ser Ile Arg Asp Asp Ile Pro Ser Thr Gln
    290                 295                 300

Gly Asn Leu Val Lys Trp Ala Leu Asp Asn Gly Tyr Ser Pro Ile Thr
305                 310                 315                 320

Ser Tyr Thr Met Ala Pro Val Ala Asn Arg Phe His Leu Arg Leu Glu
                325                 330                 335

Asn Asn Ala Glu Glu Met Gly Asp Glu Glu Val His Thr Ile Pro Pro
            340                 345                 350

Glu Leu Arg Ile Leu Leu Asp Pro Gly Ala Leu Pro Ala Leu Gln Asn
        355                 360                 365

Pro Pro Ile Arg Gly Gly Glu Gly Gln Asn Gly Gly Leu Pro Phe Pro
    370                 375                 380

Phe Pro Asp Ile Ser Arg Arg Val Trp Asn Glu Glu Gly Glu Asp Gly
385                 390                 395                 400

Leu Pro Arg Pro Lys Asp Pro Val Ile Pro Ser Ile Gln Leu Phe Pro
                405                 410                 415
```

```
Gly Leu Arg Glu Pro Glu Glu Val Gln Gly Ser Val Asp Ile Ala Leu
            420                 425                 430

Ser Val Lys Cys Asp Asn Glu Lys Met Ile Val Ala Val Glu Lys Asp
        435                 440                 445

Ser Phe Gln Ala Ser Gly Tyr Ser Gly Met Asp Val Thr Leu Leu Asp
    450                 455                 460

Pro Thr Cys Lys Ala Lys Met Asn Gly Thr His Phe Val Leu Glu Ser
465                 470                 475                 480

Pro Leu Asn Gly Cys Gly Thr Arg Pro Arg Trp Ser Ala Leu Asp Gly
                485                 490                 495

Val Val Tyr Tyr Asn Ser Ile Val Ile Gln Val Pro Ala Leu Gly Asp
            500                 505                 510

Ser Ser Gly Trp Pro Asp Gly Tyr Glu Asp Leu Glu Ser Gly Asp Asn
        515                 520                 525

Gly Phe Pro Gly Asp Met Asp Glu Gly Asp Ala Ser Leu Phe Thr Arg
    530                 535                 540

Pro Glu Ile Val Val Phe Asn Cys Ser Leu Gln Gln Val Arg Asn Pro
545                 550                 555                 560

Ser Ser Phe Gln Glu Gln Pro His Gly Asn Ile Thr Phe Asn Met Glu
                565                 570                 575

Leu Tyr Asn Thr Asp Leu Phe Leu Val Pro Ser Gln Gly Val Phe Ser
            580                 585                 590

Val Pro Glu Asn Gly His Val Tyr Val Glu Val Ser Val Thr Lys Ala
        595                 600                 605
```

```
Glu Gln Glu Leu Gly Phe Ala Ile Gln Thr Cys Phe Ile Ser Pro Tyr
    610                 615                 620

Ser Asn Pro Asp Arg Met Ser His Tyr Thr Ile Ile Glu Asn Ile Cys
625                 630                 635                 640

Pro Lys Asp Glu Ser Val Lys Phe Tyr Ser Pro Lys Arg Val His Phe
                645                 650                 655

Pro Ile Pro Gln Ala Asp Met Asp Lys Lys Arg Phe Ser Phe Val Phe
            660                 665                 670

Lys Pro Val Phe Asn Thr Ser Leu Leu Phe Leu Gln Cys Glu Leu Thr
        675                 680                 685

Leu Cys Thr Lys Met Glu Lys His Pro Gln Lys Leu Pro Lys Cys Val
    690                 695                 700

Pro Pro Asp Glu Ala Cys Thr Ser Leu Asp Ala Ser Ile Ile Trp Ala
705                 710                 715                 720

Met Met Gln Asn Lys Lys Thr Phe Thr Lys Pro Leu Ala Val Ile His
                725                 730                 735

His Glu Ala Glu Ser Lys Glu Lys Gly Pro Ser Met Lys Glu Pro Asn
            740                 745                 750

Pro Ile Ser Pro Pro Ile Phe His Gly Leu Asp Thr Leu Thr Val Met
        755                 760                 765

Gly Ile Ala Phe Ala Ala Phe Val Ile Gly Ala Leu Leu Thr Gly Ala
    770                 775                 780

Leu Trp Tyr Ile Tyr Ser His Thr Gly Glu Thr Ala Gly Arg Gln Gln
785                 790                 795                 800

Val Pro Thr Ser Pro Pro Ala Ser Glu Asn Ser Ser Ala Ala His Ser
                805                 810                 815
```

```
Ile Gly Ser Thr Gln Ser Thr Pro Cys Ser Ser Ser Ser Thr Ala
            820                 825                 830


<210>  73
<211>  552
<212>  PRT
<213>  Homo sapiens

<400>  73

Leu Glu Ala Asp Lys Cys Lys Glu Arg Glu Glu Lys Ile Ile Leu Val
1               5                  10                  15


Ser Ser Ala Asn Glu Ile Asp Val Arg Pro Cys Pro Leu Asn Pro Asn
            20                  25                  30


Glu His Lys Gly Thr Ile Thr Trp Tyr Lys Asp Asp Ser Lys Thr Pro
        35                  40                  45


Val Ser Thr Glu Gln Ala Ser Arg Ile His Gln His Lys Glu Lys Leu
    50                  55                  60


Trp Phe Val Pro Ala Lys Val Glu Asp Ser Gly His Tyr Tyr Cys Val
65                  70                  75                  80


Val Arg Asn Ser Ser Tyr Cys Leu Arg Ile Lys Ile Ser Ala Lys Phe
                85                  90                  95


Val Glu Asn Glu Pro Asn Leu Cys Tyr Asn Ala Gln Ala Ile Phe Lys
            100                 105                 110


Gln Lys Leu Pro Val Ala Gly Asp Gly Gly Leu Val Cys Pro Tyr Met
        115                 120                 125


Glu Phe Phe Lys Asn Glu Asn Asn Glu Leu Pro Lys Leu Gln Trp Tyr
    130                 135                 140


Lys Asp Cys Lys Pro Leu Leu Leu Asp Asn Ile His Phe Ser Gly Val
```

```
145                 150                 155                 160

Lys Asp Arg Leu Ile Val Met Asn Val Ala Glu Lys His Arg Gly Asn
                165                 170                 175

Tyr Thr Cys His Ala Ser Tyr Thr Tyr Leu Gly Lys Gln Tyr Pro Ile
            180                 185                 190

Thr Arg Val Ile Glu Phe Ile Thr Leu Glu Glu Asn Lys Pro Thr Arg
        195                 200                 205

Pro Val Ile Val Ser Pro Ala Asn Glu Thr Met Glu Val Asp Leu Gly
    210                 215                 220

Ser Gln Ile Gln Leu Ile Cys Asn Val Thr Gly Gln Leu Ser Asp Ile
225                 230                 235                 240

Ala Tyr Trp Lys Trp Asn Gly Ser Val Ile Asp Glu Asp Asp Pro Val
                245                 250                 255

Leu Gly Glu Asp Tyr Tyr Ser Val Glu Asn Pro Ala Asn Lys Arg Arg
            260                 265                 270

Ser Thr Leu Ile Thr Val Leu Asn Ile Ser Glu Ile Glu Ser Arg Phe
        275                 280                 285

Tyr Lys His Pro Phe Thr Cys Phe Ala Lys Asn Thr His Gly Ile Asp
    290                 295                 300

Ala Ala Tyr Ile Gln Leu Ile Tyr Pro Val Thr Asn Phe Gln Lys His
305                 310                 315                 320

Met Ile Gly Ile Cys Val Thr Leu Thr Val Ile Ile Val Cys Ser Val
                325                 330                 335

Phe Ile Tyr Lys Ile Phe Lys Ile Asp Ile Val Leu Trp Tyr Arg Asp
            340                 345                 350
```

```
Ser Cys Tyr Asp Phe Leu Pro Ile Lys Ala Ser Asp Gly Lys Thr Tyr
        355                 360                 365

Asp Ala Tyr Ile Leu Tyr Pro Lys Thr Val Gly Glu Gly Ser Thr Ser
    370                 375                 380

Asp Cys Asp Ile Phe Val Phe Lys Val Leu Pro Glu Val Leu Glu Lys
385                 390                 395                 400

Gln Cys Gly Tyr Lys Leu Phe Ile Tyr Gly Arg Asp Asp Tyr Val Gly
                405                 410                 415

Glu Asp Ile Val Glu Val Ile Asn Glu Asn Val Lys Lys Ser Arg Arg
            420                 425                 430

Leu Ile Ile Ile Leu Val Arg Glu Thr Ser Gly Phe Ser Trp Leu Gly
        435                 440                 445

Gly Ser Ser Glu Glu Gln Ile Ala Met Tyr Asn Ala Leu Val Gln Asp
    450                 455                 460

Gly Ile Lys Val Val Leu Leu Glu Leu Glu Lys Ile Gln Asp Tyr Glu
465                 470                 475                 480

Lys Met Pro Glu Ser Ile Lys Phe Ile Lys Gln Lys His Gly Ala Ile
                485                 490                 495

Arg Trp Ser Gly Asp Phe Thr Gln Gly Pro Gln Ser Ala Lys Thr Arg
            500                 505                 510

Phe Trp Lys Asn Val Arg Tyr His Met Pro Val Gln Arg Arg Ser Pro
        515                 520                 525

Ser Ser Lys His Gln Leu Leu Ser Pro Ala Thr Lys Glu Lys Leu Gln
    530                 535                 540

Arg Glu Ala His Val Pro Leu Gly
```

545 550

<210> 74
<211> 550
<212> PRT
<213> Homo sapiens

<400> 74

Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met Arg Gln Ile Gln
1 5 10 15

Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro Leu Phe Glu His
20 25 30

Phe Leu Lys Phe Asn Tyr Ser Thr Ala His Ser Ala Gly Leu Thr Leu
35 40 45

Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu Glu Pro Ile Asn
50 55 60

Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys Asp Val Leu Trp
65 70 75 80

Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr Thr Cys Met Leu
85 90 95

Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro Leu Glu Val Val
100 105 110

Gln Lys Asp Ser Cys Phe Asn Ser Pro Met Lys Leu Pro Val His Lys
115 120 125

Leu Tyr Ile Glu Tyr Gly Ile Gln Arg Ile Thr Cys Pro Asn Val Asp
130 135 140

Gly Tyr Phe Pro Ser Ser Val Lys Pro Thr Ile Thr Trp Tyr Met Gly
145 150 155 160

```
Cys Tyr Lys Ile Gln Asn Phe Asn Asn Val Ile Pro Glu Gly Met Asn
                165                 170                 175

Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn Asn Gly Asn Tyr Thr Cys
            180                 185                 190

Val Val Thr Tyr Pro Glu Asn Gly Arg Thr Phe His Leu Thr Arg Thr
        195                 200                 205

Leu Thr Val Lys Val Val Gly Ser Pro Lys Asn Ala Val Pro Pro Val
    210                 215                 220

Ile His Ser Pro Asn Asp His Val Val Tyr Glu Lys Glu Pro Gly Glu
225                 230                 235                 240

Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe Ser Phe Leu Met Asp Ser
                245                 250                 255

Arg Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys Pro Asp Asp Ile
            260                 265                 270

Thr Ile Asp Val Thr Ile Asn Glu Ser Ile Ser His Ser Arg Thr Glu
        275                 280                 285

Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys Val Thr Ser Glu
    290                 295                 300

Asp Leu Lys Arg Ser Tyr Val Cys His Ala Arg Ser Ala Lys Gly Glu
305                 310                 315                 320

Val Ala Lys Ala Ala Lys Val Lys Gln Lys Val Pro Ala Pro Arg Tyr
                325                 330                 335

Thr Val Glu Leu Ala Cys Gly Phe Gly Ala Thr Val Leu Leu Val Val
            340                 345                 350

Ile Leu Ile Val Val Tyr His Val Tyr Trp Leu Glu Met Val Leu Phe
        355                 360                 365
```

```
Tyr Arg Ala His Phe Gly Thr Asp Glu Thr Ile Leu Asp Gly Lys Glu
    370                 375                 380

Tyr Asp Ile Tyr Val Ser Tyr Ala Arg Asn Ala Glu Glu Glu Glu Phe
385                 390                 395                 400

Val Leu Leu Thr Leu Arg Gly Val Leu Glu Asn Glu Phe Gly Tyr Lys
                405                 410                 415

Leu Cys Ile Phe Asp Arg Asp Ser Leu Pro Gly Gly Ile Val Thr Asp
            420                 425                 430

Glu Thr Leu Ser Phe Ile Gln Lys Ser Arg Arg Leu Leu Val Val Leu
        435                 440                 445

Ser Pro Asn Tyr Val Leu Gln Gly Thr Gln Ala Leu Leu Glu Leu Lys
    450                 455                 460

Ala Gly Leu Glu Asn Met Ala Ser Arg Gly Asn Ile Asn Val Ile Leu
465                 470                 475                 480

Val Gln Tyr Lys Ala Val Lys Glu Thr Lys Val Lys Glu Leu Lys Arg
                485                 490                 495

Ala Lys Thr Val Leu Thr Val Ile Lys Trp Lys Gly Glu Lys Ser Lys
            500                 505                 510

Tyr Pro Gln Gly Arg Phe Trp Lys Gln Leu Gln Val Ala Met Pro Val
        515                 520                 525

Lys Lys Ser Pro Arg Arg Ser Ser Ser Asp Glu Gln Gly Leu Ser Tyr
    530                 535                 540

Ser Ser Leu Lys Asn Val
545                 550
```

<210> 75
<211> 385
<212> PRT
<213> Homo sapiens

<400> 75

```
Phe Thr Leu Gln Pro Ala Ala His Thr Gly Ala Ala Arg Ser Cys Arg
1               5                  10                  15


Phe Arg Gly Arg His Tyr Lys Arg Glu Phe Arg Leu Glu Gly Glu Pro
            20                  25                  30


Val Ala Leu Arg Cys Pro Gln Val Pro Tyr Trp Leu Trp Ala Ser Val
        35                  40                  45


Ser Pro Arg Ile Asn Leu Thr Trp His Lys Asn Asp Ser Ala Arg Thr
    50                  55                  60


Val Pro Gly Glu Glu Glu Thr Arg Met Trp Ala Gln Asp Gly Ala Leu
65                  70                  75                  80


Trp Leu Leu Pro Ala Leu Gln Glu Asp Ser Gly Thr Tyr Val Cys Thr
                85                  90                  95


Thr Arg Asn Ala Ser Tyr Cys Asp Lys Met Ser Ile Glu Leu Arg Val
            100                 105                 110


Phe Glu Asn Thr Asp Ala Phe Leu Pro Phe Ile Ser Tyr Pro Gln Ile
        115                 120                 125


Leu Thr Leu Ser Thr Ser Gly Val Leu Val Cys Pro Asp Leu Ser Glu
    130                 135                 140


Phe Thr Arg Asp Lys Thr Asp Val Lys Ile Gln Trp Tyr Lys Asp Ser
145                 150                 155                 160


Leu Leu Leu Asp Lys Asp Asn Glu Lys Phe Leu Ser Val Arg Gly Thr
                165                 170                 175
```

```
Thr His Leu Leu Val His Asp Val Ala Leu Glu Asp Ala Gly Tyr Tyr
            180                 185                 190

Arg Cys Val Leu Thr Phe Ala His Glu Gly Gln Gln Tyr Asn Ile Thr
        195                 200                 205

Arg Ser Ile Glu Leu Arg Ile Lys Lys Lys Lys Glu Glu Thr Ile Pro
    210                 215                 220

Val Ile Ile Ser Pro Leu Lys Thr Ile Ser Ala Ser Leu Gly Ser Arg
225                 230                 235                 240

Leu Thr Ile Pro Cys Lys Val Phe Leu Gly Thr Gly Thr Pro Leu Thr
                245                 250                 255

Thr Met Leu Trp Trp Thr Ala Asn Asp Thr His Ile Glu Ser Ala Tyr
            260                 265                 270

Pro Gly Gly Arg Val Thr Glu Gly Pro Arg Gln Glu Tyr Ser Glu Asn
        275                 280                 285

Asn Glu Asn Tyr Ile Glu Val Pro Leu Ile Phe Asp Pro Val Thr Arg
    290                 295                 300

Glu Asp Leu His Met Asp Phe Lys Cys Val Val His Asn Thr Leu Ser
305                 310                 315                 320

Phe Gln Thr Leu Arg Thr Thr Val Lys Glu Ala Ser Ser Thr Phe Ser
                325                 330                 335

Trp Gly Ile Val Leu Ala Pro Leu Ser Leu Ala Phe Leu Val Leu Gly
            340                 345                 350

Gly Ile Trp Met His Arg Arg Cys Lys His Arg Thr Gly Lys Ala Asp
        355                 360                 365

Gly Leu Thr Val Leu Trp Pro His His Gln Asp Phe Gln Ser Tyr Pro
```

370 375 380

Lys
385

<210> 76
<211> 152
<212> PRT
<213> Homo sapiens

<400> 76

Arg Pro Ser Gly Arg Lys Ser Ser Lys Met Gln Ala Phe Arg Ile Trp
1 5 10 15

Asp Val Asn Gln Lys Thr Phe Tyr Leu Arg Asn Asn Gln Leu Val Ala
20 25 30

Gly Tyr Leu Gln Gly Pro Asn Val Asn Leu Glu Glu Lys Ile Asp Val
35 40 45

Val Pro Ile Glu Pro His Ala Leu Phe Leu Gly Ile His Gly Gly Lys
50 55 60

Met Cys Leu Ser Cys Val Lys Ser Gly Asp Glu Thr Arg Leu Gln Leu
65 70 75 80

Glu Ala Val Asn Ile Thr Asp Leu Ser Glu Asn Arg Lys Gln Asp Lys
85 90 95

Arg Phe Ala Phe Ile Arg Ser Asp Ser Gly Pro Thr Thr Ser Phe Glu
100 105 110

Ser Ala Ala Cys Pro Gly Trp Phe Leu Cys Thr Ala Met Glu Ala Asp
115 120 125

Gln Pro Val Ser Leu Thr Asn Met Pro Asp Glu Gly Val Met Val Thr
130 135 140

Lys Phe Tyr Phe Gln Glu Asp Glu
145 150

<210> 77
<211> 93
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 77

Leu Gln Cys Phe Cys His Leu Cys Thr Lys Asp Asn Phe Thr Cys Val
1 5 10 15

Thr Asp Gly Leu Cys Phe Val Ser Val Thr Glu Thr Thr Asp Lys Val
20 25 30

Ile His Asn Ser Met Cys Ile Ala Glu Ile Asp Leu Ile Pro Arg Asp
35 40 45

Arg Pro Phe Val Cys Ala Pro Ser Ser Lys Thr Gly Ser Val Thr Thr
50 55 60

Thr Tyr Cys Cys Asn Gln Asp His Cys Asn Lys Ile Glu Leu Pro Thr
65 70 75 80

Thr Val Lys Ser Ser Pro Gly Leu Gly Pro Val Glu Leu
85 90

<210> 78
<211> 136
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 78

Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val Thr
1 5 10 15

```
Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys Asp
            20                  25                  30


Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys
        35                  40                  45


Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val
    50                  55                  60


Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp
65                  70                  75                  80


Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser Pro
                85                  90                  95


Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met
            100                 105                 110


Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu
        115                 120                 125


Glu Tyr Asn Thr Ser Asn Pro Asp
    130                 135


<210>  79
<211>  767
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  79

Gly Pro Glu Pro Gly Ala Leu Cys Glu Leu Ser Pro Val Ser Ala Ser
1               5                   10                  15


His Pro Val Gln Ala Leu Met Glu Ser Phe Thr Val Leu Ser Gly Cys
            20                  25                  30
```

```
Ala Ser Arg Gly Thr Thr Gly Leu Pro Gln Glu Val His Val Leu Asn
        35                  40                  45

Leu Arg Thr Ala Gly Gln Gly Pro Gly Gln Leu Gln Arg Glu Val Thr
    50                  55                  60

Leu His Leu Asn Pro Ile Ser Ser Val His Ile His His Lys Ser Val
65                  70                  75                  80

Val Phe Leu Leu Asn Ser Pro His Pro Leu Val Trp His Leu Lys Thr
                85                  90                  95

Glu Arg Leu Ala Thr Gly Val Ser Arg Leu Phe Leu Val Ser Glu Gly
            100                 105                 110

Ser Val Val Gln Phe Ser Ser Ala Asn Phe Ser Leu Thr Ala Glu Thr
        115                 120                 125

Glu Glu Arg Asn Phe Pro His Gly Asn Glu His Leu Leu Asn Trp Ala
    130                 135                 140

Arg Lys Glu Tyr Gly Ala Val Thr Ser Phe Thr Glu Leu Lys Ile Ala
145                 150                 155                 160

Arg Asn Ile Tyr Ile Lys Val Gly Glu Asp Gln Val Phe Pro Pro Lys
                165                 170                 175

Cys Asn Ile Gly Lys Asn Phe Leu Ser Leu Asn Tyr Leu Ala Glu Tyr
            180                 185                 190

Leu Gln Pro Lys Ala Ala Glu Gly Cys Val Met Ser Ser Gln Pro Gln
        195                 200                 205

Asn Glu Glu Val His Ile Ile Glu Leu Ile Thr Pro Asn Ser Asn Pro
    210                 215                 220
```

```
Tyr Ser Ala Phe Gln Val Asp Ile Thr Ile Asp Ile Arg Pro Ser Gln
225                 230                 235                 240

Glu Asp Leu Glu Val Val Lys Asn Leu Ile Leu Ile Leu Lys Cys Lys
                245                 250                 255

Lys Ser Val Asn Trp Val Ile Lys Ser Phe Asp Val Lys Gly Ser Leu
            260                 265                 270

Lys Ile Ile Ala Pro Asn Ser Ile Gly Phe Gly Lys Glu Ser Glu Arg
        275                 280                 285

Ser Met Thr Met Thr Lys Ser Ile Arg Asp Asp Ile Pro Ser Thr Gln
    290                 295                 300

Gly Asn Leu Val Lys Trp Ala Leu Asp Asn Gly Tyr Ser Pro Ile Thr
305                 310                 315                 320

Ser Tyr Thr Met Ala Pro Val Ala Asn Arg Phe His Leu Arg Leu Glu
                325                 330                 335

Asn Asn Ala Glu Glu Met Gly Asp Glu Glu Val His Thr Ile Pro Pro
            340                 345                 350

Glu Leu Arg Ile Leu Leu Asp Pro Gly Ala Leu Pro Ala Leu Gln Asn
        355                 360                 365

Pro Pro Ile Arg Gly Gly Glu Gly Gln Asn Gly Gly Leu Pro Phe Pro
    370                 375                 380

Phe Pro Asp Ile Ser Arg Arg Val Trp Asn Glu Glu Gly Glu Asp Gly
385                 390                 395                 400

Leu Pro Arg Pro Lys Asp Pro Val Ile Pro Ser Ile Gln Leu Phe Pro
                405                 410                 415

Gly Leu Arg Glu Pro Glu Glu Val Gln Gly Ser Val Asp Ile Ala Leu
            420                 425                 430
```

```
Ser Val Lys Cys Asp Asn Glu Lys Met Ile Val Ala Val Glu Lys Asp
        435                 440                 445

Ser Phe Gln Ala Ser Gly Tyr Ser Gly Met Asp Val Thr Leu Leu Asp
    450                 455                 460

Pro Thr Cys Lys Ala Lys Met Asn Gly Thr His Phe Val Leu Glu Ser
465                 470                 475                 480

Pro Leu Asn Gly Cys Gly Thr Arg Pro Arg Trp Ser Ala Leu Asp Gly
                485                 490                 495

Val Val Tyr Tyr Asn Ser Ile Val Ile Gln Val Pro Ala Leu Gly Asp
            500                 505                 510

Ser Ser Gly Trp Pro Asp Gly Tyr Glu Asp Leu Glu Ser Gly Asp Asn
        515                 520                 525

Gly Phe Pro Gly Asp Met Asp Glu Gly Asp Ala Ser Leu Phe Thr Arg
    530                 535                 540

Pro Glu Ile Val Val Phe Asn Cys Ser Leu Gln Gln Val Arg Asn Pro
545                 550                 555                 560

Ser Ser Phe Gln Glu Gln Pro His Gly Asn Ile Thr Phe Asn Met Glu
                565                 570                 575

Leu Tyr Asn Thr Asp Leu Phe Leu Val Pro Ser Gln Gly Val Phe Ser
            580                 585                 590

Val Pro Glu Asn Gly His Val Tyr Val Glu Val Ser Val Thr Lys Ala
        595                 600                 605

Glu Gln Glu Leu Gly Phe Ala Ile Gln Thr Cys Phe Ile Ser Pro Tyr
    610                 615                 620
```

```
Ser Asn Pro Asp Arg Met Ser His Tyr Thr Ile Ile Glu Asn Ile Cys
625                 630                 635                 640


Pro Lys Asp Glu Ser Val Lys Phe Tyr Ser Pro Lys Arg Val His Phe
                645                 650                 655


Pro Ile Pro Gln Ala Asp Met Asp Lys Lys Arg Phe Ser Phe Val Phe
            660                 665                 670


Lys Pro Val Phe Asn Thr Ser Leu Leu Phe Leu Gln Cys Glu Leu Thr
        675                 680                 685


Leu Cys Thr Lys Met Glu Lys His Pro Gln Lys Leu Pro Lys Cys Val
    690                 695                 700


Pro Pro Asp Glu Ala Cys Thr Ser Leu Asp Ala Ser Ile Ile Trp Ala
705                 710                 715                 720


Met Met Gln Asn Lys Lys Thr Phe Thr Lys Pro Leu Ala Val Ile His
                725                 730                 735


His Glu Ala Glu Ser Lys Glu Lys Gly Pro Ser Met Lys Glu Pro Asn
            740                 745                 750


Pro Ile Ser Pro Pro Ile Phe His Gly Leu Asp Thr Leu Thr Val
        755                 760                 765


<210>  80
<211>  330
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  80

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1               5                   10                  15
```

```
Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220
```

```
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225                 230                 235                 240


Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255


Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270


Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285


Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300


Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320


Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330


<210>  81
<211>  330
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  81

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1               5                   10                  15


Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30


Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45
```

```
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225                 230                 235                 240
```

```
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Ala
                325                 330
```

<210> 82
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 82

```
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1               5                   10                  15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
            20                  25                  30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
        35                  40                  45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
    50                  55                  60
```

```
Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65                  70                  75                  80


Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
                85                  90                  95


Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105


<210>  83
<211>  330
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  83

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1               5                   10                  15


Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30


Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45


Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60


Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80


Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95


Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110
```

```
Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225                 230                 235                 240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320
```

Gln Lys Ser Leu Ser Leu Ser Pro Gly Ala
325 330

<210> 84
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 84

Glu Glu Glu Leu Gln Ile Ile Gln Pro Asp Lys Ser Val Leu Val Ala
1 5 10 15

Ala Gly Glu Thr Ala Thr Leu Arg Cys Thr Ile Thr Ser Leu Phe Pro
20 25 30

Val Gly Pro Ile Gln Trp Phe Arg Gly Ala Gly Pro Gly Arg Val Leu
35 40 45

Ile Tyr Asn Gln Arg Gln Gly Pro Phe Pro Arg Val Thr Thr Val Ser
50 55 60

Asp Thr Thr Lys Arg Asn Asn Met Asp Phe Ser Ile Arg Ile Gly Asn
65 70 75 80

Ile Thr Pro Ala Asp Ala Gly Thr Tyr Tyr Cys Ile Lys Phe Arg Lys
85 90 95

Gly Ser Pro Asp Asp Val Glu Phe Lys Ser Gly Ala Gly Thr Glu Leu
100 105 110

Ser Val Arg Ala Lys Pro Ser
115

<210> 85
<211> 107

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 85

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
            20   25   30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35   40   45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Leu Tyr His Pro Ala
                85   90   95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100   105

<210> 86
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 86

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

```
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
            20                  25                  30


Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110


Leu Val Thr Val Ser Ser
        115


<210>  87
<211>  330
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  87

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1               5                   10                  15


Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30


Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45
```

```
Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Ala Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225                 230                 235                 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255
```

```
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Ala
                325                 330
```

<210> 88
<211> 330
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 88

```
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1               5                   10                  15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80
```

```
Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu
225                 230                 235                 240

Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270
```

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275   280   285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290   295   300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305   310   315   320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Ala
                325   330

<210> 89
<211> 330
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 89

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1   5   10   15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20   25   30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35   40   45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50   55   60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65   70   75   80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85   90   95

```
Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu
225                 230                 235                 240

Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300
```

```
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320


Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330


<210>  90
<211>  213
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  90

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Ala Ala
            20                  25                  30


Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Ser Val Ser Asp Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Thr
                85                  90                  95


Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
            100                 105                 110


Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125
```

```
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205

Asn Arg Gly Glu Cys
    210
```

<210> 91
<211> 587
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 91

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20                  25                  30

Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Ser Leu Glu Trp Met
        35                  40                  45

Gly Asp Ile Asn Pro Asn Asn Ala Glu Thr Leu Tyr Asn His Lys Phe
    50                  55                  60
```

```
Lys Gly Arg Val Thr Val Thr Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Val Lys Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255
```

```
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Ala Gly
        435                 440                 445

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
    450                 455                 460
```

```
Gly Gly Ser Gly Glu Glu Glu Leu Gln Ile Ile Gln Pro Asp Lys Ser
465                 470                 475                 480


Val Leu Val Ala Ala Gly Glu Thr Ala Thr Leu Arg Cys Thr Ile Thr
                485                 490                 495


Ser Leu Phe Pro Val Gly Pro Ile Gln Trp Phe Arg Gly Ala Gly Pro
            500                 505                 510


Gly Arg Val Leu Ile Tyr Asn Gln Arg Gln Gly Pro Phe Pro Arg Val
        515                 520                 525


Thr Thr Val Ser Asp Thr Thr Lys Arg Asn Asn Met Asp Phe Ser Ile
    530                 535                 540


Arg Ile Gly Asn Ile Thr Pro Ala Asp Ala Gly Thr Tyr Tyr Cys Ile
545                 550                 555                 560


Lys Phe Arg Lys Gly Ser Pro Asp Asp Val Glu Phe Lys Ser Gly Ala
                565                 570                 575


Gly Thr Glu Leu Ser Val Arg Ala Lys Pro Ser
            580                 585


<210>  92
<211>  214
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  92

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
            20                  25                  30
```

```
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Leu Tyr His Pro Ala
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys
    210
```

<210> 93
<211> 588
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 93

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
            20                  25                  30


Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110


Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125


Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
    130                 135                 140


Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160
```

```
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
        195                 200                 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
    210                 215                 220

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
225                 230                 235                 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                245                 250                 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            260                 265                 270

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        275                 280                 285

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    290                 295                 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305                 310                 315                 320

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
                325                 330                 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            340                 345                 350

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
        355                 360                 365
```

```
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    370                 375                 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385                 390                 395                 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                405                 410                 415

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            420                 425                 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Ala
        435                 440                 445

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    450                 455                 460

Gly Gly Gly Ser Gly Glu Glu Glu Leu Gln Ile Ile Gln Pro Asp Lys
465                 470                 475                 480

Ser Val Leu Val Ala Ala Gly Glu Thr Ala Thr Leu Arg Cys Thr Ile
                485                 490                 495

Thr Ser Leu Phe Pro Val Gly Pro Ile Gln Trp Phe Arg Gly Ala Gly
            500                 505                 510

Pro Gly Arg Val Leu Ile Tyr Asn Gln Arg Gln Gly Pro Phe Pro Arg
        515                 520                 525

Val Thr Thr Val Ser Asp Thr Thr Lys Arg Asn Asn Met Asp Phe Ser
    530                 535                 540

Ile Arg Ile Gly Asn Ile Thr Pro Ala Asp Ala Gly Thr Tyr Tyr Cys
545                 550                 555                 560
```

```
Ile Lys Phe Arg Lys Gly Ser Pro Asp Asp Val Glu Phe Lys Ser Gly
                565                 570                 575


Ala Gly Thr Glu Leu Ser Val Arg Ala Lys Pro Ser
            580                 585


<210>  94
<211>  587
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  94

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20                  25                  30


Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Ser Leu Glu Trp Met
        35                  40                  45


Gly Asp Ile Asn Pro Asn Asn Ala Glu Thr Leu Tyr Asn His Lys Phe
    50                  55                  60


Lys Gly Arg Val Thr Val Thr Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Val Lys Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125
```

```
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile
                325                 330                 335
```

```
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Ala Gly
        435                 440                 445

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
    450                 455                 460

Gly Gly Ser Gly Glu Glu Glu Leu Gln Ile Ile Gln Pro Asp Lys Ser
465                 470                 475                 480

Val Leu Val Ala Ala Gly Glu Thr Ala Thr Leu Arg Cys Thr Ile Thr
                485                 490                 495

Ser Leu Phe Pro Val Gly Pro Ile Gln Trp Phe Arg Gly Ala Gly Pro
            500                 505                 510

Gly Arg Val Leu Ile Tyr Asn Gln Arg Gln Gly Pro Phe Pro Arg Val
        515                 520                 525
```

```
Thr Thr Val Ser Asp Thr Thr Lys Arg Asn Asn Met Asp Phe Ser Ile
    530                 535                 540

Arg Ile Gly Asn Ile Thr Pro Ala Asp Ala Gly Thr Tyr Tyr Cys Ile
545                 550                 555                 560

Lys Phe Arg Lys Gly Ser Pro Asp Asp Val Glu Phe Lys Ser Gly Ala
                565                 570                 575

Gly Thr Glu Leu Ser Val Arg Ala Lys Pro Ser
            580                 585
```

<210> 95
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 95

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20                  25                  30

Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Ser Leu Glu Trp Met
        35                  40                  45

Gly Asp Ile Asn Pro Asn Asn Ala Glu Thr Leu Tyr Asn His Lys Phe
    50                  55                  60

Lys Gly Arg Val Thr Val Thr Val Asp Lys Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
```

```
Val Lys Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300
```

```
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320


Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr Ile
                325                 330                 335


Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro
            340                 345                 350


Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala
        355                 360                 365


Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380


Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400


Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415


Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430


His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445


<210>  96
<211>  588
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  96

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15
```

```
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
            20                  25                  30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
    130                 135                 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
        195                 200                 205
```

```
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
    210                 215                 220

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser
225                 230                 235                 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                245                 250                 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            260                 265                 270

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        275                 280                 285

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    290                 295                 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305                 310                 315                 320

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr
                325                 330                 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            340                 345                 350

Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys
        355                 360                 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    370                 375                 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385                 390                 395                 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                405                 410                 415
```

```
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            420                 425                 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Ala
        435                 440                 445

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    450                 455                 460

Gly Gly Gly Ser Gly Glu Glu Glu Leu Gln Ile Ile Gln Pro Asp Lys
465                 470                 475                 480

Ser Val Leu Val Ala Ala Gly Glu Thr Ala Thr Leu Arg Cys Thr Ile
                485                 490                 495

Thr Ser Leu Phe Pro Val Gly Pro Ile Gln Trp Phe Arg Gly Ala Gly
            500                 505                 510

Pro Gly Arg Val Leu Ile Tyr Asn Gln Arg Gln Gly Pro Phe Pro Arg
        515                 520                 525

Val Thr Thr Val Ser Asp Thr Thr Lys Arg Asn Asn Met Asp Phe Ser
    530                 535                 540

Ile Arg Ile Gly Asn Ile Thr Pro Ala Asp Ala Gly Thr Tyr Tyr Cys
545                 550                 555                 560

Ile Lys Phe Arg Lys Gly Ser Pro Asp Asp Val Glu Phe Lys Ser Gly
                565                 570                 575

Ala Gly Thr Glu Leu Ser Val Arg Ala Lys Pro Ser
            580                 585
```

<210> 97
<211> 448
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 97

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
            20                  25                  30


Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110


Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125


Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
    130                 135                 140


Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160


Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175
```

```
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
        195                 200                 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
    210                 215                 220

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser
225                 230                 235                 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                245                 250                 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            260                 265                 270

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        275                 280                 285

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    290                 295                 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305                 310                 315                 320

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr
                325                 330                 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu
            340                 345                 350

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys
        355                 360                 365

Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    370                 375                 380
```

```
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385                 390                 395                 400


Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser
                405                 410                 415


Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            420                 425                 430


Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445


<210>  98
<211>  119
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  98

Glu Glu Glu Leu Gln Val Ile Gln Pro Asp Lys Ser Val Leu Val Ala
1               5                   10                  15


Ala Gly Glu Thr Ala Thr Leu Arg Cys Thr Val Thr Ser Leu Ile Pro
            20                  25                  30


Val Gly Pro Ile Gln Trp Phe Arg Gly Ala Gly Pro Gly Arg Glu Leu
        35                  40                  45


Ile Tyr Asn Gln Lys Glu Gly His Phe Pro Arg Val Thr Thr Val Ser
    50                  55                  60


Asp Ser Thr Lys Arg Asn Asn Met Asp Phe Ser Ile Arg Ile Gly Asn
65                  70                  75                  80


Ile Thr Pro Ala Asp Ala Gly Thr Tyr Tyr Cys Val Lys Phe Arg Lys
                85                  90                  95
```

```
Gly Ser Pro Asp Asp Val Glu Phe Lys Ser Gly Ala Gly Thr Glu Leu
            100                 105                 110


Ser Val Arg Ala Lys Pro Ser
        115


<210>  99
<211>  150
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  99

Leu Gln Pro Gly Ala Glu Val Pro Val Val Trp Ala Gln Glu Gly Ala
1               5                   10                  15


Pro Ala Gln Leu Pro Cys Ser Pro Thr Ile Pro Leu Gln Asp Leu Ser
            20                  25                  30


Leu Leu Arg Arg Ala Gly Val Thr Trp Gln His Gln Pro Asp Ser Gly
        35                  40                  45


Pro Pro Ala Ala Ala Pro Gly His Pro Leu Ala Pro Gly Pro His Pro
    50                  55                  60


Ala Ala Pro Ser Ser Trp Gly Pro Arg Pro Arg Arg Tyr Thr Val Leu
65                  70                  75                  80


Ser Val Gly Pro Gly Gly Leu Arg Ser Gly Arg Leu Pro Leu Gln Pro
                85                  90                  95


Arg Val Gln Leu Asp Glu Arg Gly Arg Gln Arg Gly Asp Phe Ser Leu
            100                 105                 110


Trp Leu Arg Pro Ala Arg Arg Ala Asp Ala Gly Glu Tyr Arg Ala Ala
        115                 120                 125
```

```
Val His Leu Arg Asp Arg Ala Leu Ser Cys Arg Leu Arg Leu Arg Leu
    130                 135                 140


Gly Gln Ala Ser Met Thr
145                 150


<210>  100
<211>  260
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  100

Leu Gln Pro Gly Ala Glu Val Pro Val Val Trp Ala Gln Glu Gly Ala
1               5                   10                  15


Pro Ala Gln Leu Pro Cys Ser Pro Thr Ile Pro Leu Gln Asp Leu Ser
            20                  25                  30


Leu Leu Arg Arg Ala Gly Val Thr Trp Gln His Gln Pro Asp Ser Gly
        35                  40                  45


Pro Pro Ala Ala Ala Pro Gly His Pro Leu Ala Pro Gly Pro His Pro
    50                  55                  60


Ala Ala Pro Ser Ser Trp Gly Pro Arg Pro Arg Arg Tyr Thr Val Leu
65                  70                  75                  80


Ser Val Gly Pro Gly Gly Leu Arg Ser Gly Arg Leu Pro Leu Gln Pro
                85                  90                  95


Arg Val Gln Leu Asp Glu Arg Gly Arg Gln Arg Gly Asp Phe Ser Leu
            100                 105                 110


Trp Leu Arg Pro Ala Arg Arg Ala Asp Ala Gly Glu Tyr Arg Ala Ala
        115                 120                 125
```

```
Val His Leu Arg Asp Arg Ala Leu Ser Cys Arg Leu Arg Leu Arg Leu
    130                 135                 140

Gly Gln Ala Ser Met Thr Ala Ser Pro Pro Gly Ser Leu Arg Ala Ser
145                 150                 155                 160

Asp Trp Val Ile Leu Asn Cys Ser Phe Ser Arg Pro Asp Arg Pro Ala
                165                 170                 175

Ser Val His Trp Phe Arg Asn Arg Gly Gln Gly Arg Val Pro Val Arg
            180                 185                 190

Glu Ser Pro His His His Leu Ala Glu Ser Phe Leu Phe Leu Pro Gln
        195                 200                 205

Val Ser Pro Met Asp Ser Gly Pro Trp Gly Cys Ile Leu Thr Tyr Arg
    210                 215                 220

Asp Gly Phe Asn Val Ser Ile Met Tyr Asn Leu Thr Val Leu Gly Leu
225                 230                 235                 240

Glu Pro Pro Thr Pro Leu Thr Val Tyr Ala Gly Ala Gly Ser Arg Val
                245                 250                 255

Gly Leu Pro Cys
            260
```

<210> 101
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 101

```
Gly Pro Pro Ala Ala Ala Pro Gly His Pro Leu Ala Pro Gly Pro His
1               5                   10                  15
```

Pro Ala Ala Pro Ser Ser Trp Gly Pro Arg Pro Arg Arg
            20   25

<210> 102
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 102

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1   5   10   15

Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser
            20   25   30

Gly Met Gly Val Gly Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu
        35   40   45

Trp Leu Ala His Ile Trp Trp Asp Gly Asp Glu Ser Tyr Asn Pro Ser
    50   55   60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65   70   75   80

Ser Leu Lys Ile Thr Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Phe
                85   90   95

Cys Ala Arg Asn Arg Tyr Asp Pro Pro Trp Phe Val Asp Trp Gly Gln
            100   105   110

Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> 103
<211> 107

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 103

Asp Ile Gln Met Thr Gln Ser Thr Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
            20   25   30

Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Val Lys Leu Leu Ile
        35   40   45

Tyr Tyr Thr Ser Lys Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Gln
65   70   75   80

Glu Asp Phe Ala Thr Tyr Phe Cys Leu Gln Gly Lys Met Leu Pro Trp
                85   90   95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100   105

<210> 104
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 104

Thr Ser Gly Met Gly Val Gly
1   5

<210> 105
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 105

His Ile Trp Trp Asp Gly Asp Glu Ser Tyr Asn Pro Ser Leu Lys Ser
1   5   10   15

<210> 106
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 106

Asn Arg Tyr Asp Pro Pro Trp Phe Val Asp
1   5   10

<210> 107
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 107

Arg Ala Ser Gln Asp Ile Ser Asn Tyr Leu Ser
1   5   10

<210> 108
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 108

Tyr Thr Ser Lys Leu His Ser
1 5

<210> 109
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 109

Leu Gln Gly Lys Met Leu Pro Trp Thr
1 5

<210> 110
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 110

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Val Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Ile Ile Trp Tyr Asp Gly Asp Asn Gln Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

```
Leu Gln Met Asn Gly Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Asp Leu Arg Thr Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110


Leu Val Thr Val Ser Ser
        115


<210>  111
<211>  107
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<400>  111

Glu Ile Val Leu Thr Gln Ser Pro Asp Phe Gln Ser Val Thr Pro Lys
1               5                   10                  15


Glu Lys Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Ser Ser
            20                  25                  30


Leu His Trp Tyr Gln Gln Lys Pro Asp Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45


Lys Tyr Ala Ser Gln Ser Phe Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Glu Ala
65                  70                  75                  80


Glu Asp Ala Ala Ala Tyr Tyr Cys His Gln Ser Ser Ser Leu Pro Phe
                85                  90                  95


Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
            100                 105
```

<210> 112
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 112

Val Tyr Gly Met Asn
1   5

<210> 113
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 113

Ile Ile Trp Tyr Asp Gly Asp Asn Gln Tyr Tyr Ala Asp Ser Val Lys
1   5   10   15

Gly

<210> 114
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 114

Asp Leu Arg Thr Gly Pro Phe Asp Tyr
1   5

<210> 115
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 115

Arg Ala Ser Gln Ser Ile Gly Ser Ser Leu His
1   5   10

<210> 116
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 116

Tyr Ala Ser Gln Ser Phe Ser
1   5

<210> 117
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 117

His Gln Ser Ser Ser Leu Pro Phe Thr
1   5

<210> 118
<211> 713
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 118

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1   5   10   15

```
Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser
            20                  25                  30

Gly Met Gly Val Gly Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu
        35                  40                  45

Trp Leu Ala His Ile Trp Trp Asp Gly Asp Glu Ser Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80

Ser Leu Lys Ile Thr Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Phe
                85                  90                  95

Cys Ala Arg Asn Arg Tyr Asp Pro Pro Trp Phe Val Asp Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205
```

```
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415
```

```
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
    450                 455                 460

Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu
465                 470                 475                 480

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
                485                 490                 495

Tyr Val Phe Thr Asp Tyr Tyr Met Asn Trp Val Arg Gln Ala Pro Gly
            500                 505                 510

Gln Ser Leu Glu Trp Met Gly Asp Ile Asn Pro Asn Asn Ala Glu Thr
        515                 520                 525

Leu Tyr Asn His Lys Phe Lys Gly Arg Val Thr Val Thr Val Asp Lys
    530                 535                 540

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
545                 550                 555                 560

Thr Ala Val Tyr Tyr Cys Val Lys Trp Gly Asp Gly Pro Phe Ala Tyr
                565                 570                 575

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser
            580                 585                 590

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp
        595                 600                 605
```

```
Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp
    610                 615                 620

Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Ala Ala Val
625                 630                 635                 640

Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
                645                 650                 655

Ser Val Ser Asp Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly Ser
            660                 665                 670

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
        675                 680                 685

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Thr Phe
    690                 695                 700

Gly Gln Gly Thr Lys Leu Thr Val Leu
705                 710
```

<210> 119
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 119

```
Asp Ile Gln Met Thr Gln Ser Thr Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Val Lys Leu Leu Ile
        35                  40                  45
```

```
Tyr Tyr Thr Ser Lys Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Gln
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Phe Cys Leu Gln Gly Lys Met Leu Pro Trp
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys
    210
```

<210> 120
<211> 711
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic

<400> 120

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Val Tyr
            20                  25                  30


Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ala Ile Ile Trp Tyr Asp Gly Asp Asn Gln Tyr Tyr Ala Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80


Leu Gln Met Asn Gly Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Asp Leu Arg Thr Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110


Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125


Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
    130                 135                 140


Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160


Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175


Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190
```

```
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
        195                 200                 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
    210                 215                 220

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser
225                 230                 235                 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                245                 250                 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            260                 265                 270

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        275                 280                 285

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    290                 295                 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305                 310                 315                 320

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Ser Ile Glu Lys Thr
                325                 330                 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            340                 345                 350

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
        355                 360                 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    370                 375                 380
```

```
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385                 390                 395                 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                405                 410                 415

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            420                 425                 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Ala
        435                 440                 445

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    450                 455                 460

Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys
465                 470                 475                 480

Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Val
                485                 490                 495

Phe Thr Asp Tyr Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Cys
            500                 505                 510

Leu Glu Trp Met Gly Asp Ile Asn Pro Asn Asn Ala Glu Thr Leu Tyr
        515                 520                 525

Asn His Lys Phe Lys Gly Arg Val Thr Val Thr Val Asp Lys Ser Ile
    530                 535                 540

Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala
545                 550                 555                 560

Val Tyr Tyr Cys Val Lys Trp Gly Asp Gly Pro Phe Ala Tyr Trp Gly
                565                 570                 575

Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
            580                 585                 590
```

```
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln
        595                 600                 605


Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
    610                 615                 620


Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Ala Ala Val Ala Trp
625                 630                 635                 640


Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ser Val
                645                 650                 655


Ser Asp Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser
            660                 665                 670


Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile
        675                 680                 685


Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Asn Tyr Pro Thr Phe Gly Cys
    690                 695                 700


Gly Thr Lys Leu Thr Val Leu
705                 710
```

<210> 121
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 121

```
Glu Ile Val Leu Thr Gln Ser Pro Asp Phe Gln Ser Val Thr Pro Lys
1               5                   10                  15


Glu Lys Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Ser Ser
            20                  25                  30
```

```
Leu His Trp Tyr Gln Gln Lys Pro Asp Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45

Lys Tyr Ala Ser Gln Ser Phe Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Glu Ala
65                  70                  75                  80

Glu Asp Ala Ala Ala Tyr Tyr Cys His Gln Ser Ser Ser Leu Pro Phe
                85                  90                  95

Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys
    210
```

<210> 122
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic

<400> 122

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser
20