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(54) Title: BISPECIFIC ANTIBODIES TARGETING SIRPα AND PD-L1

(57) Abstract: Provided are antibodies and fragments having binding specificity to the signal regulatory protein alpha (SIRPα) protein and to the programmed death-ligand 1 (PD-L1) protein. The antibodies and fragments can bring peripheral macrophages to PD-L1+ tumor site, thereby enhancing tumor treatment.

WO 2022/127901 A1

BISPECIFIC ANTIBODIES TARGETING SIRPα AND PD-L1

BACKGROUND

[0001] Signal regulatory protein alpha (SIRPα) is a member of the signal-regulatory-protein (SIRP) family, and also belongs to the immunoglobulin superfamily. SIRPα recognizes CD47, an anti-phagocytic signal that distinguishes live cells from dying cells. The extracellular domain of SIRPα binds to CD47 and transmits intracellular signals through its cytoplasmic domain. CD47-binding is mediated through the NH2-terminal V-like domain of SIRPα. The cytoplasmic region contains four ITIMs that become phosphorylated after binding of ligand. The phosphorylation mediates activation of tyrosine kinase SHP2. SIRPα also binds phosphatase SHP1, adaptor protein SCAP2 and FYN-binding protein. Recruitment of SHP phosphatases to the membrane leads to the inhibition of myosin accumulation at the cell surface and results in the inhibition of phagocytosis.

[0002] Cancer cells highly express CD47 that activates SIRPα and inhibits macrophage-mediated destruction. It has been shown that high-affinity variants of SIRPα that antagonized CD47 on cancer cells increased phagocytosis of cancer cells. Anti-SIRPα antibodies have also been shown to help macrophages to reduce cancer growth and metastasis, alone and in synergy with other cancer treatments.

[0003] Programmed death-ligand 1 (PD-L1), also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1), is a 40kDa type 1 transmembrane protein believed to play a major role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease and other disease states such as hepatitis. The binding of PD-L1 to PD-1 or B7.1 transmits an inhibitory signal which reduces the proliferation of CD8+ T cells at the lymph nodes and supplementary to that PD-1 is also able to control the accumulation of foreign antigen specific T cells in the lymph nodes through apoptosis which is further mediated by a lower regulation of the gene Bcl-2.

[0004] Bispecific antibodies targeting both the SIRPα and PD-L1 proteins have been proposed, but development of bispecific antibodies with good stability and activity has been proven to be challenging.

SUMMARY

[0005] The present disclosure, in some embodiments, discloses antibodies targeting both SIRPα and PD-L1 to enhance both T cell function and macrophage phagocytosis for treating cancers. By virtue of the specificity to PD-L1, the antibodies cam bring peripheral M1 macrophages to the tumor site that is positive of PD-L1.The anti-SIRPα specificity can block CD47/SIRPα interaction thereby enhancing the engulfment of tumor cells by macrophages. It can also enable dendritic cells to process and present tumor antigens, leading to priming and boosting of tumor-specific CD8+ effector T cells.

[0006] Dual targeting of these antibodies at both the innate and the adaptive immune checkpoints, therefore, can maximize anti-tumor therapeutic effect and elicit more durable responses. Moreover, these antibodies can have better safety profiles as compared to anti-CD47 antibodies.

[0007] In accordance with one embodiment of the present disclosure, provided is an antibody comprising an anti-signal regulatory protein alpha (SIRPα) unit and an anti-programmed death-ligand 1 (PD-L1) unit, wherein the anti-SIRPα unit comprises an Fab fragment having binding specificity to a human SIRPα protein, and the anti-PD-L1 unit comprises a single-domain antibody (sdAb) having binding specificity to a human PD-L1 protein.

[0008] In some embodiments, the antibody further comprises an Fc fragment. In some embodiments, the sdAb is fused to the heavy chain of the Fab fragment. In some embodiments, the sdAb is fused to the light chain of the Fab fragment. In some embodiments, the sdAb is fused to the C-terminus of the heavy chain. In some embodiments, the sdAb is fused to the N-terminus of the heavy chain.

[0009] Specific sequences for the anti-SIRPα unit and the anti-PD-L1 unit are also disclosed herein.

[0010] Also provided, in some embodiments, are compositions comprising the antibody or fragment thereof and a pharmaceutically acceptable carrier. In some embodiments, the composition further comprises a second antibody having specificity to a tumor antigen. In some embodiments, the second antibody is a tumor-opsonizing antibody.

[0011] Methods and uses for the treatment of diseases and conditions are also provided. In one embodiment, provided is a method of treating cancer in a patient in need thereof, comprising administering to the patient the antibody or fragment thereof of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] FIG. 1 shows cross-binding to SIRPα v1 and v2 by the antibodies.

[0013] FIG. 2 shows binding affinity of the antibody against SIRPα v1.

[0014] FIG. 3 shows competition of SIRPα interaction with CD47 by the antibodies.

[0015] FIG. 4 shows induction of macrophage mediated phagocytosis by the antibodies.

[0016] FIG. 5 shows increase of macrophage mediated phagocytosis of tumor cells by the antibody treatments.

[0017] FIG. 6A-D illustrate four different formats of the bispecific antibodies.

[0018] FIG. 7 shows that the bispecific antibodies had high affinity for PD-L1 expressed on cells.

[0019] FIG. 8 shows that the bispecific antibodies had high affinity for SIRPα expressed on cells.

[0020] FIG. 9 shows that the bispecific antibodies were effective in blocking PD-1 and PD-L1 interactions.

[0021] FIG. 10 shows that the bispecific antibodies were effective in blocking CD47 and SIRPa interactions.

[0022] FIG. 11 shows that the bispecific antibodies had potent activities in inducing phagocytosis.

DETAILED DESCRIPTION

Definitions

[0023] As used herein, an "antibody" or "antigen-binding polypeptide" refers to a polypeptide or a polypeptide complex that specifically recognizes and binds to an antigen. An antibody can be a whole antibody and any antigen binding fragment or a single chain thereof. Thus the term "antibody" includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule having biological activity of binding to the antigen. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein.

[0024] The terms "antibody fragment" or "antigen-binding fragment", as used herein, is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term "antibody fragment" includes aptamers, spiegelmers, and diabodies. The term "antibody fragment" also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex.

[0025] The term antibody encompasses various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon (γ, μ, α, δ, ε) with some subclasses among them (*e.g.*, γ l- γ4). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgG_5, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant disclosure. All immunoglobulin classes are clearly within the scope of the present disclosure, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000 Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a "Y" configuration wherein the light

chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

[0026] Antibodies, antigen-binding polypeptides, variants, or derivatives thereof of the disclosure include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VK or VH domain, fragments produced by a Fab expression library, and anti- idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to LIGHT antibodies disclosed herein). Immunoglobulin or antibody molecules of the disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgGl, IgG2, IgG3, IgG4, IgAl and IgA2) or subclass of immunoglobulin molecule.

[0027] The term "heavy chain-only antibody" or "HCAb" refers to a functional antibody, which comprises heavy chains, but lacks the light chains usually found in 4-chain antibodies. Camelid animals (such as camels, llamas, or alpacas) are known to produce HCAbs.

[0028] The term "single-domain antibody" or "sdAb" refers to a single antigen-binding polypeptide having three complementary determining regions (CDRs). The sdAb alone is capable of binding to the antigen without pairing with a corresponding CDR-containing polypeptide. In some cases, single-domain antibodies are engineered from camelid HCAbs, and their heavy chain variable domains are referred herein as "V_HHs" (Variable domain of the heavy chain of the Heavy chain antibody). Some V_HHs can also be known as nanobodies. Camelid sdAb is one of the smallest known antigen-binding antibody fragments (see, *e.g.*, Hamers-Casterman *et al.*, Nature 363:446-8 (1993); Greenberg *et al.*, Nature 374:168-73 (1995); Hassanzadeh-Ghassabeh *et al.*, Nanomedicine (Lond), 8:1013-26 (2013)). A basic V_HH has the following structure from the N-terminus to the C-terminus: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3.

[0029] By "specifically binds" or "has specificity to," it is generally meant that an antibody binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to "specifically bind" to an epitope when it binds to that

epitope, via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody "A" may be deemed to have a higher specificity for a given epitope than antibody "B," or antibody "A" may be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

[0030] As used herein, the terms "treat" or "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the progression of cancer. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

[0031] By "subject" or "individual" or "animal" or "patient" or "mammal," is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sport, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, and so on.

[0032] As used herein, phrases such as "to a patient in need of treatment" or "a subject in need of treatment" includes subjects, such as mammalian subjects, that would benefit from administration of an antibody or composition of the present disclosure used, *e.g.*, for detection, for a diagnostic procedure and/or for treatment.

Bispecific Antibodies

[0033] PD-L1 is a critical "don't find me" signal to the adaptive immune system. CD47/SIRPα transmits an anti-phagocytic signal, known as the "don't eat me" signal, to the innate immune system. It is contemplated that dual targeting both innate and adaptive

immune checkpoints can help maximize anti-tumor therapeutic effect and elicit more durable responses.

[0034] The present disclosure provides anti-SIRPα antibodies and fragments that have high affinity to both variants v1 and v2. Variant 1 (hSIRPαV1) is the dominant variant among Europeans, Africans, Ad mixed Americans, and South Asians. Variant 2 (hSIRPαV2) is the dominant variant among East Asians. Sequences of hSIRPαV1 and hSIRPαV2 differ within the extracellular Ig-like V-like (IgV) domain. The ability of the instantly disclosed antibodies and fragments to recognize both variants enables them to be effective among the widest patient population.

[0035] The present disclosure also describes single-domain antibodies (sdAb) specifically recognizing PD-L1, as well as heavy chain-only antibody (HCAb). Single-chain antibodies (sdAbs) are different from conventional 4-chain antibodies by having a single monomeric antibody variable domain, such as heavy chain variable domain (V_HH), which can exhibit high affinity to an antigen without the aid of a light chain.

[0036] The anti-SIRPα antibodies and fragments and the anti-PD-L1 sdAbs, in some embodiments, are fused to form a bispecific antibody. Four bispecific antibody formats were tested in this disclosure, which are illustrated in **FIG. 6A-D**. In the first format, the sdAb is fused to the C-terminal end of the heavy chain of the Fab fragment. In the second format, the sdAb is fused to the N-terminal end of the heavy chain of the Fab fragment. In the third format, the sdAb is fused to the C-terminal end of the light chain of the Fab fragment. In the fourth format, the sdAb is fused to the N-terminal end of the light chain of the Fab fragment.

[0037] In some embodiments, the Fab fragment can further include a Fc fragment, as illustrated in the formats of FIG. 6. In a preferred embodiment, the format of FIG. 6A is used, in which an anti-PD-L1 sdAb is fused, through a (G4S)n linker, to the C-terminus of the Fc fragment of the full anti-SIRPα antibody.

[0038] In accordance with one embodiment of the present disclosure, provided is an antibody comprising an anti-signal regulatory protein alpha (SIRPα) unit and an anti-programmed death-ligand 1 (PD-L1) unit, wherein the anti-SIRPα unit comprises an Fab fragment having binding specificity to a human SIRPα protein, and the anti-PD-L1 unit comprises a single-domain antibody (sdAb) having binding specificity to a human PD-L1 protein.

[0039] In some embodiments, the antibody further comprises an Fc fragment. In some embodiments, the sdAb is fused to the heavy chain of the Fab fragment. In some embodiments, the sdAb is fused to the light chain of the Fab fragment. In some embodiments, the sdAb is fused to the C-terminus of the heavy chain. In some embodiments, the sdAb is fused to the N-terminus of the heavy chain.

[0040] Examples of anti-PD-L1 sdAbs and anti-SIRPα antibodies are also described herein.

Anti-SIRPα Unit

[0041] In accordance with one embodiment of the present disclosure, therefore, provided are antibodies and antigen-binding fragments thereof that are able to bind to both variants 1 and 2 of SIRPα. Example antibodies include those murine ones listed in **Table 1**, as well as humanized ones of **Tables 2-8**. Also included are those that include the same CDRs as illustrated herein. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as those illustrated here, and those that compete with the instantly disclosed in binding to SIRPα.

[0042] In accordance with one embodiment of the present disclosure, provided is an antibody or fragment thereof that includes the heavy chain and light chain variable domains with the CDR regions disclosed herein, as well as their biological equivalents.

[0043] In one embodiment, the CDRs are those of 248G3F6, as exemplified in **Tables 2B and 2D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16, 21 or 22 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20

or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0044] One embodiment provides an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRPα) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20.

[0045] One embodiment provides an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRPα) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 21, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20.

[0046] One embodiment provides an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRPα) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 22, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20.

[0047] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1 and 23-27, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1 and 23-27.

[0048] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 and 28-29, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:2 and 28-29.

[0049] In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:27 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:29.

[0050] In one embodiment, the CDRs are those of 300A6A6, as exemplified in **Tables 3B and 3D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 31, 36, 37 or 38 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 33 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0051] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRPα) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ

ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 31, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 33, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35.

[0052] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRPα) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 36, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 33, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35.

[0053] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRPα) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 37, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 33, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35.

[0054] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRPα) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 38, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid

sequence of SEQ ID NO: 33, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35.

[0055] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:3 and 39-44, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:3 and 39-44.

[0056] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:4 and 45-46, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:4 and 45-46.

[0057] In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:43 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:45.

[0058] In one embodiment, the CDRs are those of 102A10F2, as exemplified in **Tables 4B and 4D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 48, 53 or 54 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0059] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRPα) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain

variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 48, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52.

[0060] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRPα) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 53, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52.

[0061] In one embodiment, provided is an antibody or fragment thereof having binding specificity to a wild-type human signal regulatory protein alpha (SIRPα) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 54, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52.

[0062] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:5 and 55-60, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:5 and 55-60.

[0063] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:6 and 61-62, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 6 and 61-62.

[0064] In one embodiment, the CDRs are those of 62D2H6, as exemplified in **Tables 5B and 5D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 63 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 64 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 65 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 66 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 67 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 68 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0065] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:7 and 69-72, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:7 and 69-72.

[0066] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:8 and 73-76, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 8 and 73-76.

[0067] In one embodiment, the CDRs are those of 211F8E11, as exemplified in **Tables 6B and 6D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 77 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 78 or a variant thereof having one, two, or three deletions, additions, substitutions or the

combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 79 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 80 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 81 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 82 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0068] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:9 and 83-86, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:9 and 83-86.

[0069] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:10 and 87-90, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 10 and 87-90.

[0070] In one embodiment, the CDRs are those of 217D11E5, as exemplified in **Tables 7B and 7D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 91 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 92 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 93 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 94 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 95 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 96 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0071] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:11 and 97-100, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:11 and 97-100.

[0072] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:12 and 101-102, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12 and 101-102.

[0073] In one embodiment, the CDRs are those of 234B7D5, as exemplified in **Tables 8B and 8D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 103 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 104 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 105 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 106 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 107 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 108 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0074] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:13 and 109-112, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:13 and 109-112.

[0075] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:14 and 113-118, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 14 and 113-118.

[0076] In some embodiments, the anti-SIRPα antibody specifically binds to SIRPα competitively with any one of the anti-SIRPα antibodies described herein. In some embodiments, competitive binding may be determined using an ELISA assay.

[0077] The antibodies that contained these CDR regions, whether mouse, humanized or chimeric, had potent SIRPα binding and inhibitory activities. As shown in **Example 5**, certain residues within the CDR can be modified to retain or improve the property or reduce their potential to have post-translational modifications (PTMs). Such modified CDR can be referred to as affinity matured or de-risked CDRs.

[0078] Non-limiting examples of de-risked CDRs are provided in **Tables 2B, 3B and 4B**. Modified CDRs can include those having one, two or three amino acid addition, deletion and/or substitutions. In some embodiments, the substitutions can be conservative substitutions.

[0079] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in an immunoglobulin polypeptide is preferably replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0080] Non-limiting examples of conservative amino acid substitutions are provided in the table below, where a similarity score of 0 or higher indicates conservative substitution between the two amino acids.

Table A. Amino Acid Similarity Matrix

	C	G	P	S	A	T	D	E	N	Q	H	K	R	V	M	I	L	F	Y	W
W	-8	-7	-6	-2	-6	-5	-7	-7	-4	-5	-3	-3	2	-6	-4	-5	-2	0	0	17
Y	0	-5	-5	-3	-3	-3	-4	-4	-2	-4	0	-4	-5	-2	-2	-1	-1	7	10	
F	-4	-5	-5	-3	-4	-3	-6	-5	-4	-5	-2	-5	-4	-1	0	1	2	9		
L	-6	-4	-3	-3	-2	-2	-4	-3	-3	-2	-2	-3	-3	2	4	2	6			
I	-2	-3	-2	-1	-1	0	-2	-2	-2	-2	-2	-2	-2	4	2	5				
M	-5	-3	-2	-2	-1	-1	-3	-2	0	-1	-2	0	0	2	6					
V	-2	-1	-1	-1	0	0	-2	-2	-2	-2	-2	-2	-2	4						
R	-4	-3	0	0	-2	-1	-1	-1	0	1	2	3	6							
K	-5	-2	-1	0	-1	0	0	0	1	1	0	5								
H	-3	-2	0	-1	-1	-1	1	1	2	3	6									
Q	-5	-1	0	-1	0	-1	2	2	1	4										
N	-4	0	-1	1	0	0	2	1	2											
E	-5	0	-1	0	0	0	3	4												
D	-5	1	-1	0	0	0	4													
T	-2	0	0	1	1	3														
A	-2	1	1	1	2															
S	0	1	1	1																
P	-3	-1	6																	
G	-3	5																		
C	12																			

Table B. Conservative Amino Acid Substitutions

For Amino Acid	**Substitution With**
Alanine	D-Ala, Gly, Aib, β-Ala, L-Cys, D-Cys
Arginine	D-Arg, Lys, D-Lys, Orn D-Orn
Asparagine	D-Asn, Asp, D-Asp, Glu, D-Glu Gln, D-Gln
Aspartic Acid	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr, L-Ser, D-Ser
Glutamine	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	Ala, D-Ala, Pro, D-Pro, Aib, β-Ala
Isoleucine	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	Val, D-Val, Met, D-Met, D-Ile, D-Leu, Ile
Lysine	D-Lys, Arg, D-Arg, Orn, D-Orn
Methionine	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	D-Phe, Tyr, D-Tyr, His, D-His, Trp, D-Trp
Proline	D-Pro
Serine	D-Ser, Thr, D-Thr, allo-Thr, L-Cys, D-Cys
Threonine	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Val, D-Val
Tyrosine	D-Tyr, Phe, D-Phe, His, D-His, Trp, D-Trp
Valine	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

Anti-PD-L1 Unit

[0081] The anti-PD-L1 units described herein can include a single-domain antibody (sdAb). Exemplary sdAbs include, but are not limited to, heavy chain variable domains from heavy-chain only antibodies (*e.g.*, V_HH (Variable domain of the heavy chain of the Heavy chain antibody) in *Camelidae* or V_{NAR} (Variable domain of the shark New Antigen Receptor) in cartilaginous fish), binding molecules naturally devoid of light chains, single domains (such as V_H or V_L) derived from conventional 4-chain antibodies, humanized heavy-chain only antibodies, human single-domain antibodies produced by transgenic mice or rats expressing human heavy chain segments, and engineered domains and single domain scaffolds other than those derived from antibodies. The sdAbs may be derived from any species including, but not limited to mouse, rat, human, camel, llama, lamprey, fish, shark, goat, rabbit, and bovine. Single-domain antibodies contemplated herein also include naturally occurring single-domain antibody molecules from species other than *Camelidae* and sharks.

[0082] In some embodiments, the sdAb is derived from a naturally occurring single-domain antigen binding molecule known as heavy chain antibody devoid of light chains (also referred herein as "heavy chain-only antibodies", or "HCAb"). Such single domain molecules are disclosed in WO 94/04678 and Hamers-Casterman, C. *et al.* (1993) *Nature* 363:446-448, for example. For clarity reasons, the variable domain derived from a heavy chain molecule naturally devoid of light chain is known herein as a V_HH to distinguish it from the conventional VH of four chain immunoglobulins. Such a V_HH molecule can be derived from antibodies raised in *Camelidae* species, for example, camel, llama, vicuna, dromedary, alpaca and guanaco. Other species besides Camelidae may produce heavy chain molecules naturally devoid of light chain, and such V_HHs are within the scope of the present application.

[0083] In some embodiments, the sdAb is derived from a variable region of the immunoglobulin found in cartilaginous fish. For example, the sdAb can be derived from the immunoglobulin isotype known as Novel Antigen Receptor (NAR) found in the serum of shark. Methods of producing single domain molecules derived from a variable region of NAR ("IgNARs") are described in WO 03/014161 and Streltsov (2005) *Protein Sci.* 14:2901-2909.

[0084] In some embodiments, the anti-PD-L1 sdAb includes a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 169-218, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the

amino acid sequence of any one of SEQ ID NOs: 169-318, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 369-418, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the K_d of the binding between the anti-PD-L1 sdAb and PD-L1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-L1 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0085] In some embodiments, the anti-PD-L1 sdAb includes a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 369-418, and the amino acid substitutions are in CDR1 and/or CDR2.

[0086] Thus, in some embodiments, the anti-PD-L1 sdAb includes a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 169-218, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 169-318, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 369-418. In some embodiments, the K_d of the binding between the anti-PD-L1 sdAb and PD-L1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-L1 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0087] In some embodiments, the anti-PD-L1 sdAb includes a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 169-218; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 169-318; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 369-418. In some embodiments, the K_d of the binding between the anti-PD-L1 sdAb and PD-L1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the anti-PD-L1 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0088] The sequences of the CDRs noted herein are provided in Table 24.

[0089] The CDRs can be combined in various pair-wise combinations to generate a number of anti-PD-L1 sdAb.

[0090] The anti-PD-L1 sdAb may comprise one or more "hallmark residues" in one or more of the FR sequences. In some embodiments, the anti-PD-L1 sdAb may comprise a V_HH domain comprising the amino acid sequence of any one of the following: a-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V (such as Y or such as F); a-2) the amino acid residue at position 44 is selected from the group consisting of A, G, E, D, G, Q, R, S, and L (such as G, E, or Q); a-3) the amino acid residue at position 45 is selected from the group consisting of L, R and C (such as L or R); a-4) the amino acid residue at position 103 is selected from the group consisting of G, W, R and S (such as W or R, or such as W); and a-5) the amino acid residue at position 108 is Q; or b-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V (such as Y or such as F); b-2) the amino acid residue at position 44 is selected from the group consisting of E and Q; b-3) the amino acid residue at position 45 is R; b-4) the amino acid residue at position 103 is selected from the group consisting of G, W, R and S (such as W); and b-5) the amino acid residue at position 108 is selected from the group consisting of Q and L (such as Q); wherein the amino acid position is according to Kabat numbering. It should be noted that these "hallmark residues" at amino acid positions 37, 44, 45, 103 and 108 according to Kabat numbering apply to anti-PD-L1 sdAb of natural V_HH sequences, and can be substituted during humanization. For example, Q at amino acid position 108 according to Kabat numbering can be optionally humanized to L. Other humanized substitutions will be clear to those skilled in the art. For example, potentially useful humanizing substitutions can be determined by comparing the FR sequences of a naturally occurring V_HH with the corresponding FR sequences of one or more closely related human V_H, then introducing one or more of such potentially useful humanizing substitutions into said V_HH using methods known in the art (also as described herein). The resulting humanized V_HH sequences can be tested for their PD-L1 binding affinity, for stability, for ease and level of expression, and/or for other desired properties. Possible residue substitutions may also come from an antibody V_H domain wherein the VH/VL interface comprises one or more highly charged amino acid residues. The anti-PD-L1 sdAb described herein can be partially or fully humanized. Preferably, the resulting humanized anti-PD-L1 sdAb binds to PD-L1 with K_d, K_{on}, K_{off} described herein.

[0091] In some embodiments, the anti-PD-L1 sdAb cinludes a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 469-518, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97%, 98%, or 99%) sequence identify to any one of SEQ ID NOs:469-518. In some embodiments, the anti-PD-L1 sdAb includes a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 469-518, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the V_HH domain. In some embodiments, the anti-PD-L1 sdAb includes the V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 469-518 or a variant thereof comprises amino acid substitutions in CDRs, such as the CDR1, and/or the CDR2, and/or the CDR3 of any one of SEQ ID NOs: 469-518. In some embodiments, the anti-PD-L1 sdAb includes the V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 469-518 or a variant thereof comprises CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 469-518, and the amino acid substitutions are in FRs, such as the FR1, and/or the FR2, and/or the FR3, and/or the FR4 of any one of SEQ ID NOs: 469-518.

[0092] In some embodiments, the anti-PD-L1 sdAb specifically binds to PD-L1 competitively with any one of the anti-PD-L1 sdAb described herein. In some embodiments, competitive binding may be determined using an ELISA assay. For example, in some embodiments, there is provided an anti-PD-L1 sdAb that specifically binds to PD-L1 competitively with an anti-PD-L1 sdAb comprising the amino acid sequence of any one of SEQ ID NOs: 469-518. For another example, in some embodiments, there is provided an anti-PD-L1 sdAb that specifically binds to PD-L1 competitively with an anti-PD-L1 sdAb comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 169-218; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 169-318; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 369-418. In some embodiments, the K_d of the binding between the competing anti-PD-L1 sdAb and PD-L1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M). In some embodiments, the competing anti-PD-L1 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0093] In a preferred embodiment, the CDR1 comprises the amino acid sequence of SEQ ID NO:213, the CDR2 comprises the amino acid sequence of SEQ ID NO:313, and the CDR3 comprises the amino acid sequence of SEQ ID NO:413. In some embodiments, the anti-PD-L1 sdAb comprises the amino acid sequence of SEQ ID NO:513.

[0094] Example sequences of the bispecific antibodies are also provided. For instance, the bispecific antibody may include (a) a heavy chain comprising the amino acid sequence of

SEQ ID NO:559 and a light chain comprising the amino acid sequence of SEQ ID NO:560; (b) a heavy chain comprising the amino acid sequence of SEQ ID NO:561 and a light chain comprising the amino acid sequence of SEQ ID NO:562; (c) a heavy chain comprising the amino acid sequence of SEQ ID NO:563 and a light chain comprising the amino acid sequence of SEQ ID NO:560; (d) a heavy chain comprising the amino acid sequence of SEQ ID NO:564 and a light chain comprising the amino acid sequence of SEQ ID NO:562; (e) a heavy chain comprising the amino acid sequence of SEQ ID NO:565 and a light chain comprising the amino acid sequence of SEQ ID NO:566; (f) a heavy chain comprising the amino acid sequence of SEQ ID NO:567 and a light chain comprising the amino acid sequence of SEQ ID NO:568; (g) a heavy chain comprising the amino acid sequence of SEQ ID NO:565 and a light chain comprising the amino acid sequence of SEQ ID NO:569; or (h) a heavy chain comprising the amino acid sequence of SEQ ID NO:567 and a light chain comprising the amino acid sequence of SEQ ID NO:570.

[0095] It will also be understood by one of ordinary skill in the art that antibodies as disclosed herein may be modified such that they vary in amino acid sequence from the naturally occurring binding polypeptide from which they were derived. For example, a polypeptide or amino acid sequence derived from a designated protein may be similar, *e.g.*, have a certain percent identity to the starting sequence, *e.g.*, it may be 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the starting sequence.

[0096] In certain embodiments, the antibody comprises an amino acid sequence or one or more not normally associated with an antibody. Exemplary modifications are described in more detail below. For example, an antibody of the disclosure may comprise a flexible linker sequence, or may be modified to add a functional moiety (*e.g.*, PEG, a drug, a toxin, or a label).

[0097] Antibodies, variants, or derivatives thereof of the disclosure include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to the epitope. For example, but not by way of limitation, the antibodies can be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation,

metabolic synthesis of tunicamycin, etc. Additionally, the antibodies may contain one or more non-classical amino acids.

[0098] In some embodiments, the antibodies may be conjugated to therapeutic agents, prodrugs, peptides, proteins, enzymes, viruses, lipids, biological response modifiers, pharmaceutical agents, or PEG.

[0099] The antibodies may be conjugated or fused to a therapeutic agent, which may include detectable labels such as radioactive labels, an immunomodulator, a hormone, an enzyme, an oligonucleotide, a photoactive therapeutic or diagnostic agent, a cytotoxic agent, which may be a drug or a toxin, an ultrasound enhancing agent, a non-radioactive label, a combination thereof and other such agents known in the art.

[0100] The antibodies can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antigen-binding polypeptide is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0101] The antibodies can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA). Techniques for conjugating various moieties to an antibody are well known, *see, e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. (1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.*, (eds.), Marcel Dekker, Inc., pp. 623- 53 (1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), Academic Press pp. 303-16 (1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* (52:119-58 (1982)).

Polynucleotides Encoding the Antibodies and Methods of Preparing the Antibodies

[0102] The present disclosure also provides isolated polynucleotides or nucleic acid molecules encoding the antibodies, variants or derivatives thereof of the disclosure. The polynucleotides of the present disclosure may encode the entire heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the same polynucleotide molecule or on separate polynucleotide molecules. Additionally, the polynucleotides of the present disclosure may encode portions of the heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the same polynucleotide molecule or on separate polynucleotide molecules.

[0103] Methods of making antibodies are well known in the art and described herein. In certain embodiments, both the variable and constant regions of the antigen-binding polypeptides of the present disclosure are fully human. Fully human antibodies can be made using techniques described in the art and as described herein. For example, fully human antibodies against a specific antigen can be prepared by administering the antigen to a transgenic animal which has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled. Exemplary techniques that can be used to make such antibodies are described in U.S. patents: 6,150,584; 6,458,592; 6,420,140 which are incorporated by reference in their entireties.

Treatment Methods

[0104] As described herein, the antibodies, variants or derivatives of the present disclosure may be used in certain treatment and diagnostic methods.

[0105] The present disclosure is further directed to antibody-based therapies which involve administering the antibodies of the disclosure to a patient such as an animal, a mammal, and a human for treating one or more of the disorders or conditions described herein. Therapeutic compounds of the disclosure include, but are not limited to, antibodies of the disclosure (including variants and derivatives thereof as described herein) and nucleic acids or polynucleotides encoding antibodies of the disclosure (including variants and derivatives thereof as described herein).

[0106] The antibodies of the disclosure can also be used to treat or inhibit cancer. As provided above, SIRPα can be overexpressed in tumor cells, in particular gastric, pancreatic,

esophageal, ovarian, and lung tumors. Inhibition of SIRPα has been shown to be useful for treating the tumors. Some tumors may also overexpress PD-L1 or PD-1, or can be induced to overexpress PD-L1 or PD-1. All of the tumors, it is contemplated, can be effectively treated with the antibodies of the present disclosure.

[0107] Accordingly, in some embodiments, provided are methods for treating a cancer in a patient in need thereof. The method, in one embodiment, entails administering to the patient an effective amount of an antibody of the present disclosure. In some embodiments, at least one of the cancer cells (e.g., stromal cells) in the patient over-express SIRPα, CD47, PD-1 or PD-L1.

[0108] Cellular therapies, such as chimeric antigen receptor (CAR) T-cell therapies, are also provided in the present disclosure. A suitable cell can be used, that is put in contact with an anti-SIRPα antibody of the present disclosure (or alternatively engineered to express an anti-SIRPα antibody of the present disclosure). Upon such contact or engineering, the cell can then be introduced to a cancer patient in need of a treatment. The cancer patient may have a cancer of any of the types as disclosed herein. The cell (e.g., T cell) can be, for instance, a tumor-infiltrating T lymphocyte, a CD4+ T cell, a CD8+ T cell, or the combination thereof, without limitation.

[0109] In some embodiments, the cell was isolated from the cancer patient him- or her-self. In some embodiments, the cell was provided by a donor or from a cell bank. When the cell is isolated from the cancer patient, undesired immune reactions can be minimized.

[0110] Non-limiting examples of cancers include bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, pancreatic cancer, prostate cancer, and thyroid cancer. In some embodiments, the cancer is one or more of gastric, pancreatic, esophageal, ovarian, and lung cancers.

[0111] Additional diseases or conditions associated with increased cell survival, that may be treated, prevented, diagnosed and/or prognosed with the antibodies or variants, or derivatives thereof of the disclosure include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (*e.g.*, acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (*e.g.*, chronic

myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (*e.g.*, Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyo sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma and retinoblastoma.

[0112] A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular antibodies, variant or derivative thereof used, the patient's age, body weight, general health, sex, and diet, and the time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated. Judgment of such factors by medical caregivers is within the ordinary skill in the art. The amount will also depend on the individual patient to be treated, the route of administration, the type of formulation, the characteristics of the compound used, the severity of the disease, and the desired effect. The amount used can be determined by pharmacological and pharmacokinetic principles well known in the art.

[0113] Methods of administration of the antibodies, variants or include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The antigen-binding polypeptides or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Thus, pharmaceutical compositions containing the antigen-binding polypeptides of the disclosure may be

administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray.

[0114] The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intra-articular injection and infusion.

[0115] Administration can be systemic or local. In addition, it may be desirable to introduce the antibodies of the disclosure into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0116] It may be desirable to administer the antigen-binding polypeptides or compositions of the disclosure locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction, with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the disclosure, care must be taken to use materials to which the protein does not absorb.

[0117] The amount of the antibodies of the disclosure which will be effective in the treatment, inhibition and prevention of an inflammatory, immune or malignant disease, disorder or condition can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease, disorder or condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0118] As a general proposition, the dosage administered to a patient of the antigen-binding polypeptides of the present disclosure is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight, between 0.1 mg/kg and 20 mg/kg of the patient's body weight, or 1 mg/kg to 10

mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the disclosure may be reduced by enhancing uptake and tissue penetration (*e.g.*, into the brain) of the antibodies by modifications such as, for example, lipidation.

[0119] In an additional embodiment, the compositions of the disclosure are administered in combination with cytokines. Cytokines that may be administered with the compositions of the disclosure include, but are not limited to, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, anti-CD40, CD40L, and TNF-α.

[0120] In additional embodiments, the compositions of the disclosure are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Compositions

[0121] The present disclosure also provides pharmaceutical compositions. Such compositions comprise an effective amount of an antibody, and an acceptable carrier. In some embodiments, the composition further includes a second anticancer agent (e.g., an immune checkpoint inhibitor).

[0122] In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. Further, a "pharmaceutically acceptable carrier" will generally be a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

[0123] The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose,

sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents such as acetates, citrates or phosphates. Antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; and agents for the adjustment of tonicity such as sodium chloride or dextrose are also envisioned. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences by E. W. Martin, incorporated herein by reference. Such compositions will contain a therapeutically effective amount of the antigen-binding polypeptide, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0124] In an embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0125] The compounds of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

EXAMPLES

Example 1: Generation of murine monoclonal antibodies against human SIRPα

[0126] The human SIRPa protein was used to immunize different strains of mice and hybridomas were generated accordingly. Eight fusions were made to generate sufficient number of hybridoma clones. SIRPa v1/v2 positive binders were selected and subcloned. Subsequently, *in vitro* binding and functional screening were carried out with about 30 purified antibodies and lead antibodies with highest binding affinity and strongest functional potency were identified. The lead antibodies were humanized.

[0127] The VH/VL sequences of the lead murine antibodies are provided in the table below.

Table 1. VH/VL sequence of the lead murine antibodies

Name	Sequence (CDRs are underlined)	SEQ ID NO:
248G3F6 VH	EVQLQQSGAELVKPGASVKLSCTASGFNFE**DTYMH**WVKQRPDQGLEWIG**R IDPADGDTKYNPKFQD**KATITVDTSSNTAYLQLSSLTSEDTAVYYCVR**GN YVN**WGQGTTLTVSS	1
248G3F6 VL	QIVLIQSPAIMSASPGERVTLTC**RASSSVSSSYLY**WYQQKPGSSPKLWIY **STSNLAS**GVPARFSGSGSGTSYSLTISSMEAEDAASYFC**HQWYSYPRT**FG GGTKLEIK	2
300A6A6 VH	QVQLQQSGTELVKPGSSVKISCKASGYTFT**SNYIH**WIRQQPGNGLEWIG**W IYPGDGDTNYNQKFNG**KATLTADKSSSTAYMQLSSLTSEDYAVYFCAI**NY GGIWFAY**WGQGTLVTVSS	3
300A6A6 VL	DIQMTQSPSSMSASLGDRVTITC**QASQDIGNKLI**WFQQKPGKSPRLMIH**Y VTNLPG**GVPLRFSGSRSGSDYSLTISSLESEDMADYYC**LQYKQNPLT**FGS GTKLEIK	4
102A10F2 VH	QVTLKESGPGILQPSQTLSLTCSFSGFSLN**TYDIGMG**WIRQPSGKGLEWL A**HIWWNDREYYNSALQS**RVTISKDTSNTQVFLKIASVDTADTATYYCVR**I DYFGSGQAWFTY**WGQGTLVTVSA	5
102A10F2 VL	EIVLTQSPPTMAASPGEKITITC**SSSSTISSTYLH**WYQQKPGFSPKLLIS **GTSNLAS**GVPPRFSGSGSGTSYSLTIGTLEAEDVATYYC**QQGSRIPFT**FG SGTKLEIK	6
62D2H6 VH	EVQLQQSGAELVKPGASVKLSCTASGFNIK**DYYMH**WVKQRTEQGLEWIG**R IDPEDGETKYAPKFQG**KATITADTSSNTAYLQLSSLTSEDTAVYYCSR**SW AY**WGQGTTLTVSS	7
62D2H6 VL	QIVLTQSPAIMSASPGEKVTLTC**SASSSVSSSYLY**WYQQKPGSSPKLWIY **STSNLAS**GVPARFSGSGSGTSYSLTISSMEAEDAASYFC**HQWSSYPRT**FG GGTKLEIK	8

211F8E11 VH	EVQLQQSGAELVKPGASVKLSCTASGFNIK**DTYMH**WVKQRPEQGLEWVG**R IDPANVNTIYDPKFQG**KATITADTSSNTAYLQLSSLTSEDTAVYYCAR**VG AYDGYDFDY**WGQGTTLTVSS	9
211F8E11 VL	DIVLTQSPASLAVSLGQRATISC**RASESVDNYGNSFMH**WYQQKPGQPPKL LIY**RASNLES**GIPARFSGSGSRTDFTLTINPVEADDVATYYC**QQNNEDPL T**FGAGTKLELK	10
217D11E5 VH	EVQLQQSGPELVKPGASVKMSCKASGYTFT**SYVMH**WVKQKPGQGLEWIG**Y INPYNDGTKYNEKFKG**KATLTSDKSSSTAYMELSSLTSEDSAVYYCAR**SY YDYDGSFDY**WGQGTTLTVSS	11
217D11E5 VL	DIVMTQSHKFMSTSVGDRVSITC**KASQDVTTAVA**WYQQKSGQSPKLLIY**S ASYRYT**GDPDRFTGSGSGTDFTFTISSVQAEDLAVYYC**QQHYSTPWT**FGG GTKLEIK	12
234B7D5 VH	EVQLQQSGAELVKPGASVKLSCTASGFNFE**DTYIH**WVKQRPDQGLEWIG**R IDPADGDTKHNPKFHD**KATVTVDTSSNTAYLELSSLTSEDTAVYYCVR**GN YVN**WGQGTTLTVSS	13
234B7D5 VL	QIVLIQSPAIMSASPGERVTLTC**RASSSVTSSYLY**WYQQKPGSSPKLWIY **SASNLAS**GVPARFSGSGSGTSYSLTISSVEAEDAASYFC**HQWYSYPRT**FG GGTKLEIK	14

Example 2. Cross-binding of SIRPa v1 and v2

[0128] This example measured the dose response of ELISA binding of mouse anti-SIRPα mAb to recombinant human SIRPα variant 1 and variant 2 protein (0.5 μg/ml@100 μl). Recombinant human SIRPα v1 or v2 protein (Biointron) was coated at 0.5 μg/ml in PBS onto microtiter plates for 2 h at RT. After coating of the antigen, the wells were blocked with PBS/0.05% Tween (PBST) with 1% BSA for 1 h at RT.

[0129] After washing of the wells with PBST, different concentrations of anti-SIRPα antibodies were added to the well and incubated for 1 at RT. For detection of the binding antibodies, the HRP conjugated secondary antibodies against mouse Fc (Jackson Immuno Research) were added followed by the addition of fluorogenic substrates (Roche). Between all incubation steps, the wells of the plate were washed with PBST three times. Fluorescence was measured in a TECAN Spectrafluor plate reader.

[0130] The results are shown in **FIG. 1**. Both tested antibodies, 248G3F6 and 300A6A6, exhibited nanogram level affinity to both variants 1 and 2.

[0131] The binding kinetics assay of antibody to variant 1 was performed using Biacore 8K system through human antibody capture approach. The anti-mouse Fc lgG were immobilized on CM5 sensor chip according to the manufactory's instruction. The test antibody was injected and captured by the immobilized anti-human Fc lgG. Serial concentrations of antigen was individually injected, and the binding profile was recorded for each concentration antigen analyte, respectively.

[0132] The assay system was regenerated by injection of 10 mM Glycine-HCl pH 1.5 for 30 seconds. The running buffer was HBS-EP+ (10mM HEPES, pH 7.4, 150mM NaCl, 3mM EDTA and 0.05% P20). The assay temperature was 25 °C, and the association and dissociation time were 180 and 600 seconds, respectively. The Biacore data were fitted using Biacore K8 evaluation software 1.0 according to 1:1 binding model to calculate the association (ka) and dissociation (kd) rate constants as well as the equilibrium constant (KD).

[0133] The results are shown in **FIG. 2**, and summarized in the table below. Both tested antibodies exhibited excellent binding affinity.

Sample	ka (1/Ms)	kd (1/s)	KD (M)
248G3F6	1.87E+05	3.74E-04	2.00E-09
300A6A6	1.31E+05	1.48E-04	1.13E-09

Example 3. Competition with CD47

[0134] This example tested the ability of the anti-SIRPα antibodies to compete with CD47 in binding to SIRPα.

[0135] Recombinant CD47-Fc fusion protein (Acrobiosystems) was coated at 1 μg/ml in PBS onto microtiter plates for 16 hours at 4 °C. After blocking for 1 h with 1% BSA in PBST at RT, 1 μg/mL of SIRPα-His protein was added either in the absence or presence of different concentrations of anti-SIRPα antibodies at RT for 1 h. Plates were subsequently washed three times and incubated with an HRP-conjugated anti-His secondary antibody for 1 h at RT. After washing, the TMB solution was added to each well for 30 min and the reaction was stopped with 2M H_2SO_4, and OD was measured at 490 nm.

[0136] As shown in **FIG. 3**, both 248G3F6 and 300A6A6 potently and dose-dependently inhibited the binding of CD47 to SIRPα.

Example 4. Induction of macrophage mediated phagocytosis

[0137] This example tested the ability of the anti-SIRPα antibodies to induce macrophage mediated phagocytosis.

[0138] PBMCs were isolated from human blood, and the monocytes were differentiated into macrophages for 6 days. The monocyte derived macrophages (MDMs) were scraped and re-plated in 24-well dishes and allowed to adhere for 24 hours. The human tumor cell line Raji

which endogenously expressed CD47 were transfected with human PD-L1 to overexpress human PD-L1 on the surface. This PD-L1 overexpressed Raji cells were chosen as target cells and labeled with 1 μM CFSE for 10 minutes, then added to MDMs at a ratio of 5:1 tumor cells per phagocyte.

[0139] Anti-SIRPalpha antibodies and anti-PD-L1 antibody were added in the culture system. After incubation for 3 hours, non-phagocytosed target cells were washed away with PBS and the remaining phagocytes were scraped off, stained with macrophage marker CD14 antibody, and analyzed by flow cytometry. Phagocytosis was measured by gating on $CD14^+$ cells and then assessing the percent of $CFSE^+$ cells.

[0140] The results of phagocytosis of PD-L1 expressing tumor cells by combo-treatment of anti-SIRPα antibody with anti-PD-L1 antibody are shown in **FIG. 4**. The combination of anti-PD-L1 antibody with either of the anti-SIRPα antibodies exhibited the highest phagocytosis (the two columns on the right).

Example 5. Humanization of the mouse mAbs

[0141] The murine antibody variable region genes were employed to create humanized mAbs. In the first step of this process, the amino acid sequences of the VH and VL of mAb were compared against the available database of human Ig gene sequences to find the overall best-matching human germline Ig gene sequences.

[0142] The amino acid sequences of the humanized antibody are provided below.

Humanized sequences

A. 248G3F6

Table 2A. Humanization of 248G3F6 – VH

Name	Sequence	SEQ ID NO:
248G3F6 VH	EVQLQQSGAELVKPGASVKLSCTASGFNFE**DTYMH**WVKQRPDQGLEWIG**R IDPADGDTKYNPKFQD**KATITVDTSSNTAYLQLSSLTSEDTAVYYCVR**GN YVN**WGQGTTLTVSS	1
V1 (CDR grafting)	QVQLVQSGAEVKKPGASVKVSCKASGFNFE**DTYMH**WVRQAPGQGLEWMG**R IDPADGDTKYNPKFQD**RVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR**GN YVN**WGQGTTVTVSS	23
V2 (with back mutations)	QVQLVQSGAEVKKPGASVKVSCKASGFNFEDTYMHWVRQAPGQGLEWMGR IDPADGDTKYNPKFQDRVTMT**V**DTSI**N**T**A**YMELSSLRSEDTAVYYC**V**RGN YVNWGQGTTVTVSS	24

V3 (with back mutations)	QVQLVQSGAEVKKPGASVKVSCKASGFNFEDTYMHWVRQAPGQGLEWMGR IDPADGDTKYNPKFQDRVT**I**T**V**DTSI**N**T**A**YMELSSLRSEDTAVYYC**V**RGN YVNWGQGTTVTVSS	25
V4 (with back mutations)	QVQLVQSGAEVKKPGASVKVSCKASGFNFEDTYMHWVRQAPGQGLEWMGR IDPA**E**GDTKYNPKFQDRVT**I**T**V**DTSI**N**T**A**YMELSSLRSEDTAVYYC**V**RGN YVNWGQGTTVTVSS	26
V5 (with back mutations)	QVQLVQSGAEVKKPGASVKVSCKASGFNFEDTYMHWVRQAPGQGLEWMGR IDPAD**A**DTKYNPKFQDRVT**I**T**V**DTSI**N**T**A**YMELSSLRSEDTAVYYC**V**RGN YVNWGQGTTVTVSS	27

Table 2B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	DTYMH	15
CDR-H2	RIDPADGDTKYNPKFQD	16
CDR-H3	GNYVN	17
CDR-H2 (v4)	RIDPA**E**GDTKYNPKFQD	21
CDR-H2 (v5)	RIDPAD**A**DTKYNPKFQD	22

Table 2C. Humanization of 248G3F6 – VL

Name	Sequence	SEQ ID NO:
248G3F6 VL	QIVLIQSPAIMSASPGERVTLTC**RASSSVSSSYLY**WYQQKPGSSPKLWIY **STSNLAS**GVPARFSGSGSGTSYSLTISSMEAEDAASYFC**HQWYSYPRT**FG GGTKLEIK	2
V1 (CDR grafting)	EIVLTQSPGTLSLSPGERATLSC**RASSSVSSSYLY**WYQQKPGQAPRLLIY **STSNLAS**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**HQWYSYPRT**FG GGTKVEIK	28
V2 (with back mutations)	EIVLTQSPGTLSLSPGERATLSCRASSSVSSSYLYWYQQKPGQAPRLLIY STSNLASGIPDRFSGSGSGTD**Y**TLTISRLEPED**A**AVY**F**CHQWYSYPRTFG GGTKVEIK	29

Table 2D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	RASSSVSSSYLY	18
CDR-L2	STSNLAS	19
CDR-L3	HQWYSYPRT	20

Table 2E. Humanized antibodies

	VL	VL v1	VL v2
VH	HSP210-02-Chi		
VH v1		HSP210-02-hz11	HSP210-02-hz12
VH v2		HSP210-02-hz21	HSP210-02-hz22
VH v3		HSP210-02-hz31	HSP210-02-hz32
VH v4		HSP210-02-hz41	HSP210-02-hz42
VH v5		HSP210-02-hz51	**HSP210-02-hz52**

B. 300A6A6

Table 3A. Humanization of 300A6A6 – VH

Name	Sequence	SEQ ID NO:
300A6A6 VH	QVQLQQSGTELVKPGSSVKISCKASGYTFT**SNYIH**WIRQQPGNGLEWIG**W IYPGDGDTNYNQKFNG**KATLTADKSSSTAYMQLSSLTSEDYAVYFCAI**NY GGIWFAY**WGQGTLVTVSS	3
V1 (CDR grafting)	QVQLVQSGAEVKKPGSSVKVSCKASGYTFT**SNYIH**WVRQAPGQGLEWMG**W IYPGDGDTNYNQKFNG**RVTITADKSTSTAYMELSSLRSEDTAVYYCAR**NY GGIWFAY**WGQGTLVTVSS	39
V2 (with back mutations)	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPGDGDTNYNQKFNGRVTITADKSTSTAYMELSSLRSEDTAVYYCA**I**NY GGIWFAYWGQGTLVTVSS	40
V3 (with back mutations)	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPGDGDTNYNQKFNGRVT**L**TADKSTSTAYMELSSLRSEDTAVYYCA**I**NY GGIWFAYWGQGTLVTVSS	41
V4 (with back mutations)	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPG**E**GDTNYNQKFNGRVT**L**TADKSTSTAYMELSSLRSEDTAVYYCA**I**NY GGIWFAYWGQGTLVTVSS	42
V5 (with back mutations)	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPGD**A**DTNYNQKFNGRVT**L**TADKSTSTAYMELSSLRSEDTAVYYCA**I**NY GGIWFAYWGQGTLVTVSS	43
V6 (with back mutations)	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPGDGDTNYNQKF**Q**GRVT**L**TADKSTSTAYMELSSLRSEDTAVYYCA**I**NY GGIWFAYWGQGTLVTVSS	44

Table 3B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	SNYIH	30
CDR-H2	WIYPGDGDTNYNQKFNG	31
CDR-H3	NYGGIWFAY	32
CDR-H2 (v4)	WIYPG**E**GDTNYNQKFNG	36
CDR-H2 (v5)	WIYPGD**A**DTNYNQKFNG	37
CDR-H2 (v6)	WIYPGDGDTNYNQKF**Q**G	38

Table 3C. Humanization of 300A6A6 – VL

Name	Sequence	SEQ ID NO:
300A6A6 VL	DIQMTQSPSSMSASLGDRVTITC**QASQDIGNKLI**WFQQKPGKSPRLMIH**Y VTNLPG**GVPLRFSGSRSGSDYSLTISSLESEDMADYYC**LQYKQNPLT**FGS GTKLEIK	4
V1 (CDR grafting)	DIQMTQSPSSLSASVGDRVTITC**QASQDIGNKLI**WYQQKPGKAPKLLIY**Y VTNLPG**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**LQYKQNPLT**FGQ GTKLEIK	45
V2 (with back mutations)	DIQMTQSPSSLSASVGDRVTITCQASQDIGNKLIW**F**QQKPGKAPKLLI**H**Y VTNLPGGVPSRFSGS**R**SG**S**D**Y**TLTISSLQPEDFATYYCLQYKQNPLTFGQ GTKLEIK	46

Table 3D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	QASQDIGNKLI	33

CDR-L2	YVTNLPG	34
CDR-L3	LQYKQNPLT	35

Table 3E. Humanized antibodies

	VL	VL v1	VL v2
VH	HSP210-03-Chi		
VH v1		HSP210-03-hz11	HSP210-03-hz12
VH v2		HSP210-03-hz21	HSP210-03-hz22
VH v3		HSP210-03-hz31	HSP210-03-hz32
VH v4		HSP210-03-hz41	HSP210-03-hz42
VH v5		**HSP210-03-hz51**	HSP210-03-hz52
VH v6		HSP210-03-hz61	HSP210-03-hz62

C. 102A10F2

Table 4A. Humanization of 102A10F2 – VH

Name	Sequence	SEQ ID NO:
102A10F2 VH	QVTLKESGPGILQPSQTLSLTCSFSGFSLN**TYDIGMG**WIRQPSGKGLEWLA**H** **IWWNDREYYNSALQS**RVTISKDTSNTQVFLKIASVDTADTATYYCVR**IDYFG** **SGQAWFTY**WGQGTLVTVSA	5
V1 (CDR grafting)	QLQLQESGPGLVKPSETLSLTCTVSGFSLN**TYDIGMG**WIRQPPGKGLEWIG**H** **IWWNDREYYNSALQS**RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR**IDYFG** **SGQAWFTY**WGQGTLVTVSS	55
V2 (with back mutations)	Q**V**QLQESGPGLVKPSETLSLTCT**F**SGFSLNTYDIGMGWIRQPPGKGLEWIGH IWWNDREYYNSALQSRVTIS**K**DTSK**T**Q**V**SLKLSSVTAADTAVYYC**V**RIDYFG SGQAWFTYWGQGTLVTVSS	56
V3 (with back mutations)	Q**V**QLQESGPGLVKPSETLSLTCT**F**SGFSLNTYDIGMGWIRQPPGKGLEWI**A**H IWWNDREYYNSALQSRVTIS**K**DTSK**T**Q**V**SLKLSSVTAADTAVYYC**V**RIDYFG SGQAWFTYWGQGTLVTVSS	57
V4 (with back mutations)	Q**V**QLQESGPGLVKPSETLSLTCT**F**SGFSL**S**TYDIGMGWIRQPPGKGLEWI**A**H IWWNDREYYNSALQSRVTIS**K**DTSK**T**Q**V**SLKLSSVTAADTAVYYC**V**RIDYFG SGQAWFTYWGQGTLVTVSS	58
V5 (with back mutations)	Q**V**QLQESGPGLVKPSETLSLTCT**F**SGFSLNTYDIGMGWIRQPPGKGLEWI**A**H IWWNDREYY**S**SALQSRVTIS**K**DTSK**T**Q**V**SLKLSSVTAADTAVYYC**V**RIDYFG SGQAWFTYWGQGTLVTVSS	59
V6 (with back mutations)	Q**V**QLQESGPGLVKPSETLSLTCT**F**SGFSLNTYDIGMGWIRQPPGKGLEWI**A**H IWWNDREYYN**P**ALQSRVTIS**K**DTSK**T**Q**V**SLKLSSVTAADTAVYYC**V**RIDYFG SGQAWFTYWGQGTLVTVSS	60

Table 4B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	TYDIGMG	47
CDR-H2	HIWWNDREYYNSALQS	48
CDR-H3	IDYFGSGQAWFTY	49
CDR-H2 (v5)	HIWWNDREYY**S**SALQS	53
CDR-H2 (v6)	HIWWNDREYYN**P**ALQS	54

Table 4C. Humanization of 102A10F2 – VL

Name	Sequence	SEQ ID NO:
102A10F2 VL	EIVLTQSPPTMAASPGEKITITC**SSSSTISSTYLH**WYQQKPGFSPKLLIS **GTSNLAS**GVPPRFSGSGSGTSYSLTIGTLEAEDVATYYC**QQGSRIPFT**FG SGTKLEIK	6
V1 (CDR grafting)	EIVLTQSPGTLSLSPGERATLSC**SSSSTISSTYLH**WYQQKPGQAPRLLIY **GTSNLAS**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQGSRIPFT**FG QGTKLEIK	61
V2 (with back mutations)	EIVLTQSPGTLSLSPGERATLSCSSSSTISSTYLHWYQQKPGQAPRLLI**S** GTSNLASGIPDRFSGSGSGTD**Y**TLTISRLEPED**V**AVYYCQQGSRIPFTFG QGTKLEIK	62

Table 4D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	SSSSTISSTYLH	50
CDR-L2	GTSNLAS	51
CDR-L3	QQGSRIPFT	52

Table 3E. Humanized antibodies

	VL	VL v1	VL v2
VH	HSP210-01-Chi		
VH v1		HSP210-01-hz11	HSP210-01-hz12
VH v2		HSP210-01-hz21	HSP210-01-hz22
VH v3		HSP210-01-hz31	HSP210-01-hz32
VH v4		HSP210-01-hz41	HSP210-01-hz42
VH v5		HSP210-01-hz51	HSP210-01-hz52
VH v6		HSP210-01-hz61	HSP210-01-hz62

D. 62D2H6

Table 5A. Humanization of 62D2H6 – VH

Name	Sequence	SEQ ID NO:
62D2H6 VH	EVQLQQSGAELVKPGASVKLSCTASGFNIK**DYYMH**WVKQRTEQGLEWIG**R** **IDPEDGETKYAPKFQG**KATITADTSSNTAYLQLSSLTSEDTAVYYCSR**SW** **AY**WGQGTTLTVSS	7
V1 (CDR grafting)	EVQLVQSGAEVKKPGATVKISCKVSGFNIK**DYYMH**WVQQAPGKGLEWMG**R** **IDPEDGETKYAPKFQG**RVTITADTSTDTAYMELSSLRSEDTAVYYCAT**SW** **AY**WGQGTTVTVSS	69
V2 (with back mutations)	EVQLVQSGAEVKKPGATVKISCK**A**SGFNIKDYYMHWVQQAPGKGLEWMGR IDPEDGETKYAPKFQGRVTITADTST**N**TAYMELSSLRSEDTAVYYC**SR**SW AYWGQGTTVTVSS	70
V3 (chimeric 2)	**Q**VQLVQSGAEVKKPGA**S**VK**V**SCK**A**SGFNIKDYYMHWV**R**QAPG**Q**GLEWMGR IDPEDGETKYAPKFQGRVT**M**T**R**DTST**S**T**V**YMELSSLRSEDTAVYYCA**R**SW AYWGQGTTVTVSS	71
V4 (with back mutations)	**Q**VQLVQSGAEVKKPGA**S**VK**V**SCK**A**SGFNIKDYYMHWV**R**QAPG**Q**GLEWMGR IDPEDGETKYAPKFQGRVT**M**TADTST**N**TAYMELSSLRSEDTAVYYC**SR**SW AYWGQGTTVTVSS	72

Table 5B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	DYYMH	63
CDR-H2	RIDPEDGETKYAPKFQG	64
CDR-H3	SWAY	65

Table 5C. Humanization of 62D2H6 – VL

Name	Sequence	SEQ ID NO:
62D2H6 VL	QIVLTQSPAIMSASPGEKVTLTC**SASSSVSSSYLY**WYQQKPGSSPKLWIY**ST SNLAS**GVPARFSGSGSGTSYSLTISSMEAEDAASYFC**HQWSSYPRT**FGGGTK LEIK	8
V1 (CDR grafting)	EIVLTQSPATLSLSPGERATLSC**SASSSVSSSYLY**WYQQKPGQAPRLLIY**ST SNLAS**GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC**HQWSSYPRT**FGGGTK VEIK	73
V2 (with back mutations)	EIVLTQSPATLSLSPGERATLSCSASSSVSSSYLYWYQQKPGQAPRLLIYST SNLASGIPARFSGSGSGTD**Y**TLTISSLEPED**A**AVY**F**CHQWSSYPRTFGGGTK VEIK	74
V3 (chimeric 2)	EIV**M**TQSP**P**TLSLSPGER**V**TLSCSASSSVSSSYLYWYQQKPGQAPRLLIYST SNLASGIPARFSGSGSGTDFTLTISSL**Q**PEDFAVYYCHQWSSYPRTFGGGTK VEIK	75
V4 (with back mutations)	EIV**M**TQSP**P**TLSLSPGER**V**TLSCSASSSVSSSYLYWYQQKPGQAPRLLIYST SNLASGIPARFSGSGSGTD**Y**TLTISSL**Q**PED**A**AVY**F**CHQWSSYPRTFGGGTK VEIK	76

Table 5D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	SASSSVSSSYLY	66
CDR-L2	STSNLAS	67
CDR-L3	HQWSSYPRT	68

Table 5E. Humanized antibodies

	VL	VL v1	VL v2	VL v3	VL v4
VH	Chimeric				
VH v1		hz11			
VH v2			hz22		hz24
VH v3				hz33	
VH v4			hz42		hz44

E. 211F8E11

Table 6A. Humanization of 211F8E11 – VH

Name	Sequence	SEQ ID NO:
211F8E11 VH	EVQLQQSGAELVKPGASVKLSCTASGFNIK**DTYMH**WVKQRPEQGLEWVG**R IDPANVNTIYDPKFQG**KATITADTSSNTAYLQLSSLTSEDTAVYYCAR**VG AYDGYDFDY**WGQGTTLTVSS	9

V1 (CDR grafting)	EVQLVQSGAEVKKPGATVKISCKVSGFNIK**DTYMH**WVQQAPGKGLEWMG**R IDPANVNTIYDPKFQG**RVTITADTSIDTAYMELSSLRSEDTAVYYCAT**VG AYDGYDFDY**WGQGTTVTVSS	83
V2 (with back mutations)	EVQLVQSGAEVKKPGATVKISCKASGFNIKDTYMHWVQQAPGKGLEWMGR IDPANVNTIYDPKFQGRVTITADTSTNTAYMELSSLRSEDTAVYYCARVG AYDGYDFDYWGQGTTVTVSS	84
V3 (chimeric 2)	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDTYMHWVRQAPGQGLEWMGR IDPANVNTIYDPKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARVG AYDGYDFDYWGQGTTVTVSS	85
V4 (with back mutations)	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDTYMHWVRQAPGQGLEWMGR IDPANVNTIYDPKFQGRVTMTADTSTNTAYMELSSLRSEDTAVYYCARVG AYDGYDFDYWGQGTTVTVSS	86

Table 6B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	DTYMH	77
CDR-H2	RIDPANVNTIYDPKFQG	78
CDR-H3	VGAYDGYDFDY	79

Table 6C. Humanization of 211F8E11 – VL

Name	Sequence	SEQ ID NO:
211F8E11 VL	DIVLTQSPASLAVSLGQRATISC**RASESVDNYGNSFMH**WYQQKPGQPPKLLI Y**RASNLES**GIPARFSGSGSRTDFTLTINPVEADDVATYYC**QQNNEDPLT**FGA GTKLELK	10
V1 (CDR grafting)	DIVMTQSPDSLAVSLGERATINC**RASESVDNYGNSFMH**WYQQKPGQPPKLLI Y**RASNLES**GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC**QQNNEDPLT**FGQ GTKLEIK	87
V2 (with back mutations)	DIVLTQSPDSLAVSLGERATINCRASESVDNYGNSFMHWYQQKPGQPPKLLI YRASNLESGVPDRFSGSGSRTDFTLTISSLQAEDVAVYYCQQNNEDPLTFGQ GTKLEIK	88
V3 (chimeric 2)	DIQMTQSPSSLSASVGDRVTITCRASESVDNYGNSFMHWYQQKPGKVPKLLI YRASNLESGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQQNNEDPLTFGQ GTKLEIK	89
V4 (with back mutations)	DIQLTQSPSSLSASVGDRVTITCRASESVDNYGNSFMHWYQQKPGKVPKLLI YRASNLESGVPSRFSGSGSRTDFTLTISSLQPEDVATYYCQQNNEDPLTFGQ GTKLEIK	90

Table 6D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	RASESVDNYGNSFMH	80
CDR-L2	RASNLES	81
CDR-L3	QQNNEDPLT	82

Table 6E. Humanized antibodies

	VL	VL v1	VL v2	VL v3	VL v4
VH	Chimeric				
VH v1		hz11			
VH v2			hz22		hz24

VH v3				hz33	
VH v4			hz42		hz44

F. 217D11E5

Table 7A. Humanization of 217D11E5 – VH

Name	Sequence	SEQ ID NO:
217D11E5 VH	EVQLQQSGPELVKPGASVKMSCKASGYTFT**SYVMH**WVKQKPGQGLEWIG**Y INPYNDGTKYNEKFKG**KATLTSDKSSSTAYMELSSLTSEDSAVYYCAR**SY YDYDGSFDY**WGQGTTLTVSS	11
V1 (CDR grafting)	QVQLVQSGAEVKKPGASVKVSCKASGYTFT**SYVMH**WVRQAPGQRLEWMG**Y INPYNDGTKYNEKFKG**RVTITRDTSASTAYMELSSLRSEDTAVYYCAR**SY YDYDGSFDY**WGQGTTVTVSS	97
V2 (with back mutations)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVMHWVRQAPGQRLEWMGY INPYNDGTKYNEKFKGRVTITSDKSASTAYMELSSLRSEDTAVYYCARSY YDYDGSFDYWGQGTTVTVSS	98
V3 (chimeric 2)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVMHWVRQAPGQGLEWMGY INPYNDGTKYNEKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARSY YDYDGSFDYWGQGTTVTVSS	99
V4 (with back mutations)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVMHWVRQAPGQGLEWMGY INPYNDGTKYNEKFKGRVTMTSDKSTSTAYMELSSLRSEDTAVYYCARSY YDYDGSFDYWGQGTTVTVSS	100

Table 7B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	SYVMH	91
CDR-H2	YINPYNDGTKYNEKFKG	92
CDR-H3	SYYDYDGSFDY	93

Table 7C. Humanization of 217D11E5 – VL

Name	Sequence	SEQ ID NO:
217D11E5 VL	DIVMTQSHKFMSTSVGDRVSITC**KASQDVTTAVA**WYQQKSGQSPKLLIY**SAS YRYT**GDPDRFTGSGSGTDFTFTISSVQAEDLAVYYC**QQHYSTPWT**FGGGTKL EIK	12
V1 (CDR grafting)	DIQMTQSPSSLSASVGDRVTITC**KASQDVTTAVA**WYQQKPGKAPKLLIY**SAS YRYT**GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC**QQHYSTPWT**FGGGTKV EIK	101
V2 (chimeric 2)	DIQMTQSPSSLSASVGDRVTITCKASQDVTTAVAWYQQKPGKVPKLLIYSAS YRYTGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQQHYSTPWTFGGGTKV EIK	102

Table 7D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	KASQDVTTAVA	94
CDR-L2	SASYRYT	95
CDR-L3	QQHYSTPWT	96

Table 7E. Humanized antibodies

	VL	VL v1	VL v2
VH	Chimeric		
VH v1		hz11	
VH v2		hz21	hz22
VH v3			
VH v4		hz41	hz42

G. 234B7D5

Table 8A. Humanization of 234B7D5 – VH

Name	Sequence	SEQ ID NO:
234B7D5 VH	EVQLQQSGAELVKPGASVKLSCTASGFNFE**DTYIH**WVKQRPDQGLEWIG**RID PADGDTKHNPKFHD**KATVTVDTSSNTAYLELSSLTSEDTAVYYCVR**GNYVN**W GQGTTLTVSS	13
V1 (CDR grafting)	EVQLVQSGAEVKKPGATVKISCKVSGFNFE**DTYIH**WVQQAPGKGLEWMG**RID PADGDTKHNPKFHD**RVTITADTSTDTAYMELSSLRSEDTAVYYCAT**GNYVN**W GQGTTVTVSS	109
V2 (with back mutations)	EVQLVQSGAEVKKPGATVKISCKASGFNFEDTYIHWVQQAPGKGLEWMGRID PADGDTKHNPKFHDRVTITVDTSTNTAYMELSSLRSEDTAVYYCVRGNYVNW GQGTTVTVSS	110
V3 (chimeric 2)	QVQLVQSGAEVKKPGASVKVSCKASGFNFEDTYIHWVRQAPGQGLEWMGRID PADGDTKHNPKFHDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGNYVNW GQGTTVTVSS	111
V4 (with back mutations)	QVQLVQSGAEVKKPGASVKVSCKASGFNFEDTYIHWVRQAPGQGLEWMGRID PADGDTKHNPKFHDRVTMTVDTSTNTAYMELSSLRSEDTAVYYCVRGNYVNW GQGTTVTVSS	112

Table 8B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	DTYIH	103
CDR-H2	RIDPADGDTKHNPKFHD	104
CDR-H3	GNYVN	105

Table 7C. Humanization of 234B7D5 – VL

Name	Sequence	SEQ ID NO:
234B7D5 VL	QIVLIQSPAIMSASPGERVTLTC**RASSSVTSSYLY**WYQQKPGSSPKLWIY**SA SNLAS**GVPARFSGSGSGTSYSLTISSVEAEDAASYFC**HQWYSYPRT**FGGGTK LEIK	14
V1 (CDR grafting)	EIVLTQSPATLSLSPGERATLSC**RASSSVTSSYLY**WYQQKPGQAPRLLIY**SA SNLAS**GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC**HQWYSYPRT**FGGGTK VEIK	113
V2 (with back mutations)	EIVLTQSPATLSLSPGERATLSCRASSSVTSSYLYWYQQKPGQAPRLLIYSA SNLASGIPARFSGSGSGTDYTLTISSLEPEDAAVYFCHQWYSYPRTFGGGTK VEIK	114

V3 (chimeric 2)	EIVLTQSPGTLSLSPGERATLSCRASSSVTSSYLYWYQQKPGQAPRLLIYSA SNLASGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQWYSYPRTFGGGTK VEIK	115
V4 (with back mutations)	EIVLTQSPGTLSLSPGERATLSCRASSSVTSSYLYWYQQKPGQAPRLLIYSA SNLASGIPDRFSGSGSGTDYTLTISRLEPEDAAVYFCHQWYSYPRTFGGGTK VEIK	116
V5 (chimeric 3)	EIVMTQSPPTLSLSPGERVTLSCRASSSVTSSYLYWYQQKPGQAPRLLIYSA SNLASGIPARFSGSGSGTDFTLTISSLQPEDFAVYYCHQWYSYPRTFGGGTK VEIK	117
V6 (with back mutations)	EIVMTQSPPTLSLSPGERVTLSCRASSSVTSSYLYWYQQKPGQAPRLLIYSA SNLASGIPARFSGSGSGTDYTLTISSLQPEDAAVYFCHQWYSYPRTFGGGTK VEIK	118

Table 8D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	RASSSVTSSYLY	106
CDR-L2	SASNLAS	107
CDR-L3	HQWYSYPRT	108

Table 8E. Humanized antibodies

	VL	VL v1	VL v2	VL v3	VL v4	VL v5	VL v6
VH	Chimeric						
VH v1		hz11					
VH v2			hz22		hz24		hz26
VH v3				hz33			
VH v4			hz42		hz44		hz46

Example 6. Testing of Humanized Antibodies

[0143] This example tested some of the humanized antibodies for the ability to block interactions between SIRPα and CD47.

[0144] Recombinant CD47-Fc fusion protein (Acrobiosystems) was coated at 1 μg/ml in PBS onto microtiter plates for 16 hours at 4 °C. After blocking for 1 h with 1% BSA in PBST at RT, 1 μg/mL of SIRPα-His protein was added either in the absence or presence of different concentrations of the anti-SIRPα antibodies at RT for 1 h. Plates were subsequently washed three times and incubated with an HRP-conjugated anti-His secondary antibody for 1 h at RT. After washing, the TMB solution was added to each well for 30 min and the reaction was stopped with 2M H_2SO_4, and OD was measured at 490 nm.

[0145] All of the antibodies listed in **Tables 2E** (248G3F6), **3E** (300A6A6), and **4E** (102A10F2) were tested and exhibited high IC50 (**Table 9**).

Table 9. Activities of humanized antibodies to block SIRPα interaction with CD47

	Antibody	IC_{50} (nM)
248G3F6	02-chi	0.14
	02-hz22	0.092
	02-hz32	0.11
	02-hz42	0.12
	02-hz52	0.11
300A6A6	03-chi	0.14
	03-hz22	0.16
	03-hz32	0.145
	03-hz42	0.13
	03-hz52	0.13
102A10F2	01-hz22	0.21
	01-hz32	0.16
	01-hz52	0.23
	01-hz61	0.21
	01-hz62	0.16

Example 7. Increase of macrophage mediated phagocytosis of tumor cells

[0146] This example tested some of the humanized antibodies for their ability to increase macrophage mediated phagocytosis of tumor cells.

[0147] PBMCs were isolated from human blood, and monocytes were differentiated into macrophages using a standard protocol. The monocyte derived macrophages (MDMs) were scraped and re-plated in 24-well dishes and allowed to adhere for 24 hrs. The human tumor cell line Raji that endogenously expressed CD47 were selected as target cells and labeled with 1uM CFSE for 10 mins, then added to MDMs at a ratio of 5:1 tumor cells per phagocyte and different concentrations of anti-SIRPα antibodies was added at the indicated concentrations. After 3hr incubation, non-phagocytosed target cells were washed away with PBS and the remaining phagocytes were scraped off, stained with CD14 antibody, and analyzed by flow cytometry. Phagocytosis was measured by gating on $CD14^+$ cells and then assessing the percentage of $CFSE^+$ cells.

[0148] The results are presented in **FIG. 5**. Out of the tested antibodies, 02-hz52 (248G3F6) and 03-hz51 (300A6A6) exhibited the highest activities, and all others showed excellent activities as well.

Example 8. Binding affinity to SIRPα v1 and v2

[0149] Humanized antibodies 02-hz52 (248G3F6) and 03-hz51 (300A6A6) were tested for their binding affinities to SIRPα v1 and v2 in this example.

[0150] The binding kinetics assay of antibody to antigen was performed using Biacore 8K system through human antibody capture approach. The anti-mouse Fc lgG were immobilized on CM5 sensor chip according to the manufactory's instruction. The test antibody was injected and captured by the immobilized anti-human Fc lgG. And then serial concentrations of human SIRPα v1 or SIRPα v2 protein were individually injected, and the binding profile was recorded for each concentration antigen analyte, respectively. The assay system was regenerated by injection of 10 mM Glycine-HCl pH 1.5 for 30 seconds. The running buffer was HBS-EP+ (10mM HEPES, pH 7.4, 150mM NaCl, 3mM EDTA and 0.05% P20). The assay temperature was 25 °C, and the association and dissociation time were 180 and 600 seconds, respectively. The Biacore data were fitted using Biacore K8 evaluation software 1.0 according to 1:1 binding model to calculate the association (ka) and dissociation (kd) rate constants as well as the equilibrium constant (KD).

[0151] The testing results are shown in **Table 10A-B**.

Table 10A. Binding affinity against SIRPα v1

Sample	ka (1/Ms)	kd (1/s)	KD (M)
02-hz52	7.04E+05	2.98E-04	4.23E-10
03-hz51	8.05E+05	8.04E-04	9.99E-10

Table 10B. Binding affinity against SIRPα v2

Sample	ka (1/Ms)	kd (1/s)	KD (M)
02-hz52	3.33E+05	2.47E-03	7.40E-09
03-hz51	3.31E+05	1.00E-03	3.03E-09

Example 9: Generation of anti-PD-L1 Single Domain Antibodies (sdAbs)

Immunization

[0152] Two llamas were immunized with recombinant PD-L1 ECD protein under all current animal welfare regulations. For immunization, the antigen was formulated as an emulsion with CFA (primary immunization) or IFA (boost immunization). The antigen was administered by double-spot injections intramuscularly at the neck. Each animal received two injections of the emulsion, containing 100 μg of PD-L1 ECD and 4 subsequent injections

containing 50 μg of antigen at weekly intervals. At different time points during immunization, 10 ml blood samples were collected from the animal and sera were prepared. The induction of an antigen specific humoral immune response was verified using the serum samples in an ELISA-based experiment with immobilized PD-L1 ECD protein. Five days after the last immunization, a blood sample of 300 ml was collected. Peripheral blood lymphocytes (PBLs), as the genetic source of the llama heavy chain immunoglobulins (HCAbs), were isolated from the 300 ml blood sample using a Ficoll-Paque gradient (Amersham Biosciences), yielding 1×10^9 PBLs. The maximal diversity of antibodies is expected to be equal to the number of sampled B-lymphocytes, which is about 10% of the number of PBLs (1×10^8). The fraction of heavy-chain antibodies in llama is up to 20% of the number of B-lymphocytes. Therefore, the maximal diversity of HCAbs in the 300 ml blood sample is calculated as 2×10^7 different molecules.

Library construction

[0153] RNA extracted from PBLs and lymph node was used as starting material for RT-PCR to amplify sdAb encoding gene fragments. These fragments were cloned into an in-house phagemid vector. In frame with the sdAb coding sequence, the vector coded for a C-terminal (His)6 tag. The library size is more than 1×10^9. The library phage was prepared according to a standard protocol and stored after filter sterilization at 4˚C for further use.

Selections and high-throughput screening

[0154] Selections were carried out with the above libraries using solid panning as well as cell-based panning. Only a single round of selection was performed for both conditions. Each selection output was analyzed for enrichment factor (# phage present in eluate relative to control), diversity and percentage of PD-L1 positive clones (ELISA). Based on these parameters the best selections were chosen for further analysis. To this end, the output from each selection was recloned as a pool into a soluble expression vector for high-throughput screening. In frame with the sdAb coding sequence, the vector coded for a C-terminal (His)6 tag. Colonies were picked and grown in 96 deep well plates (1 ml volume) and induced by adding IPTG and 0.1% Triton for sdAb expression in the supernatant.

[0155] The supernatant was analyzed for their ability to bind to PD-L1 ECD protein (by ELISA) and PD-L1 stable cell line (by FACS). The positive binders were sequenced and the unique clones were selected for further characterization.

[0156] The unique clones were grown in 2XYT medium and induced by IPTG for sdAb expression in the supernatant. The supernatant of unique binders were analyzed for their ability to inhibit PD-L1-PD-1 interaction. To this end, the supernatant was incubated with PD-L1 ECD protein, then the complex was added to PD-1 stable cell line for binding evaluation. sdAbs with negative signal on PD-1 cell line are considered as PD-L1 inhibitors.

[0157] All potential inhibitors were selected for off-rate analysis by surface plasmon resonance (SPR) on a BIAcore T200 instrument. The dissociation phase was used to calculate the k_{off} values for each individual sdAb.

sdAb production

[0158] The His6-tagged sdAbs were purified from periplasmic extracts by ÄKTA. The NTA resin was processed according to the manufacturer's instructions. Periplasmic extracts prepared were incubated with the resin for 30 min at RT on a rotator. The resin was washed with PBS and transferred to a column. The packed resin was washed with 15 mM Imidazole. sdAbs were eluted from the column using 150 mM Imidazole. The eluted fractions were analyzed by spotting on Hybond Membrane and visualized with Ponceau. Fractions containing protein were pooled and dialyzed against PBS. Dialyzed protein was collected, filter sterilized, concentration determined and stored at -20°C.

[0159] To determine the purity, protein samples were analyzed on a 12% SDS-PAGE gel. 10 µl Laemmli sample buffer was added to 10 µl (2 µg) purified protein, then the sample was heated for 10 minutes at 95°C, cooled and loaded onto a 12% SDS-PAGE gel. The gel was processed according to general procedures and stained with Coomassie Brilliant Blue (CBB).

HCAb production

[0160] Heavy chain-only antibody (HCAb) constructs were generated by fusing sdAbs with human Fc region. The maxiprep of the HCAb constructs were prepared for CHO-K1 cell transient expression and purification. The expressed HCAbs were purified by chromatography through a column containing Protein A agarose resin followed by a size exclusion column.

[0161] To determine the purity, protein samples were analyzed on a 12% SDS-PAGE gel. 10 µl Laemmli sample buffer was added to 10 µl (2 µg) purified protein, then the sample was

heated for 10 minutes at 95°C, cooled and loaded onto a 12% SDS-PAGE gel. The gel was processed according to general procedures and stained with Coomassie Brilliant Blue (CBB). The purity of purified HCAbs are >85%. The data were summarized in Table 11.

Table 11. Summary of HCAb purification

Sample	AS06617	AS06618	AS06628	AS06682	AS06686
Conc.(mg/ml)	1.50	1.70	1.58	1.55	1.48
Amount(mg)	10.48	20.42	16.60	16.26	14.83
Purity	>85%	>85%	>85%	>85%	>85%
Endotoxin level(EU/μg)	<0.01	<0.01	<0.01	<0.01	<0.01
Sample	AS06703	AS06730	AS06750	AS06775	AS06778
Conc.(mg/ml)	1.35	1.81	1.81	1.63	1.41
Amount(mg)	13.45	21.77	21.70	19.51	12.71
Purity	>85%	>85%	>85%	>85%	>85%
Endotoxin level(EU/μg)	<0.01	<0.01	<0.01	<0.01	<0.01
Sample	AS06791	AS11947	AS11948	AS12003	
Conc.(mg/ml)	1.82	1.80	1.58	2.02	
Amount(mg)	21.82	19.81	17.38	24.25	
Purity	>85%	>85%	>85%	>85%	
Endotoxin level(EU/μg)	<0.01	0.01~0.1	<0.01	<0.01	

sdAb affinity determination and HCAb affinity determination

[0162] Affinity constant (K_d) of each sdAb and HCAb was determined by surface plasmon resonance (SPR) on a BIAcore T200 instrument. Briefly, PD-L1 His was amine-coupled to a CM5 sensor chip at a density of no higher than 100 RU. Anti-PD-L1 sdAbs or anti-PD-L1 HCAbs were injected at 5 different concentrations between 0.33 and 27 nM. Flow rate was 30 μl/min in all experiments. Association and dissociation phases were 5 and 10 min, respectively. The chip was regenerated using Glycine/HCl pH 1.5. Binding curves at different concentrations of sdAbs and HCAbs were used to calculate the kinetic parameters k_{on}, k_{off} and K_D. The kinetics data were summarized in Table 12 and Table 13.

Table 12. affinity determination of sdAbs against PD-L1

Ligand	Analyte	k_a (1/Ms)	k_d (1/s)	K_D (M)
PD-L1/His	AS06617 sdAb	1.86E+06	4.64E-04	2.49E-10
	AS06618 sdAb	1.44E+06	2.45E-04	1.70E-10
	AS06628 sdAb	4.10E+06	2.15E-04	5.26E-11
	AS06682 sdAb	1.68E+06	3.42E-04	2.03E-10
	AS06686 sdAb	2.79E+06	9.65E-04	3.46E-10
	AS06703 sdAb	1.80E+06	6.96E-05	3.87E-11
	AS06730 sdAb	7.11E+06	1.39E-04	1.95E-11

	AS06750 sdAb	2.05E+06	2.23E-04	1.09E-10
	AS06775 sdAb	1.58E+06	2.47E-04	1.56E-10
	AS06778 sdAb	1.90E+06	4.42E-05	2.33E-11
	AS06791 sdAb	1.56E+06	2.39E-04	1.53E-10
	AS11947 sdAb	1.92E+06	1.18E-03	6.17E-10
	AS11948 sdAb	2.37E+06	3.94E-04	1.67E-10
	AS12003 sdAb	2.76E+06	1.55E-03	5.60E-10

Table 13. affinity determination of HCAbs against PD-L1

Ligand	Analyte	k_a (1/Ms)	k_d (1/s)	K_D (M)
PD-L1/His	AS06617 HCAb	5.20E+05	5.51E-05	1.06E-10
	AS06618 HCAb	4.21E+05	1.41E-05	3.35E-11
	AS06628 HCAb	1.27E+06	1.98E-05	1.55E-11
	AS06730 HCAb	1.52E+06	2.44E-05	1.61E-11
	AS06682 HCAb	5.99E+05	3.34E-05	5.57E-11
	AS06686 HCAb	9.79E+05	1.53E-04	1.56E-10
	AS06703 HCAb	5.55E+05	<1.00E-05*	<1.80E-11*
	AS06750 HCAb	7.19E+05	2.81E-05	3.92E-11
	AS06775 HCAb	4.76E+05	3.29E-05	6.90E-11
	AS06778 HCAb	5.53E+05	<1.00E-05*	<1.81E-11*
	AS06791 HCAb	7.81E+05	5.33E-05	6.83E-11
	AS11947 HCAb	7.30E+05	2.26E-04	3.10E-10
	AS11948 HCAb	7.61E+05	8.25E-05	1.08E-10
	AS12003 HCAb	5.69E+05	2.47E-04	4.34E-10

*kd is outside the limits that can be measured by Biacore T200

Target binding assays

[0163] The ability of the purified antigen binding proteins to bind PD-L1 was determined using Surface Plasmon Resonance method (e.g., BIACORE®), an enzyme-linked immunosorbent assay, a Fluorescence-Assisted Cell Sorting method (FACS), or a combination thereof. The analyses can be performed on PD-L1 transfected cells.

[0164] CHO-K1 cells expressing human PD-L1 were dissociated from adherent culture flasks and mixed with varying concentrations of antibodies and a constant concentration of anti-PD-L1 sdAbs or HCAbs (in a 96-well plate). Tecentriq® was used as an anti-PD-L1 antibody positive control. The antibody and cell incubation was equilibrated for 30 minutes at room temperature, washed three times with FACS buffer (PBS containing 1% BSA). FITC conjugated anti-human IgG secondary antibody was then added and incubated for 15 minutes at room temperature. Cells were washed again with FACS buffer and analyzed by flow

cytometry. Data were analyzed with Prism (GraphPad Software, San Diego, CA) using non-linear regression, and EC_{50} values were calculated.

Inhibition of ligand binding by FACS analysis

[0165] Blockade of ligand binding was studied using flow cytometry. For anti-PD-L1 HCAbs evaluation, CHO-K1 cells expressing human PD-L1 were dissociated from adherent culture flasks and mixed with varying concentrations of antibodies and a constant concentration of biotin-labeled hPD-1/Fc protein (both in a 96-well plate). Tecentriq® was used as an anti-PD-L1 antibody positive control. The mixture was equilibrated for 30 minutes at room temperature, washed three times with FACS buffer (PBS containing 1% BSA). PE/Cy5 Streptavidin secondary antibody was then added and incubated for 15 minutes at room temperature. Cells were washed again with FACS buffer and analyzed by flow cytometry. Data were analyzed with Prism (GraphPad Software, San Diego, CA) using non-linear regression, and IC_{50} values were calculated. The competition assays demonstrated the ability of most anti-PD-L1 HCAbs in efficiently inhibiting PD-L1-PD-1 interactions at low concentrations (1-10 μg/ml), the IC_{50} of most HCAbs are comparable to Tecentriq®.

PD-L1-based blockade assay

[0166] CHO-K1 stable expressing PD-L1 cells and Jurkat effector cells are used to assess PD-1 blockade for anti-PD-L1 sdAbs and HCAbs evaluation. The effector cells contain a luciferase construct that is induced upon disruption of the PD-1/PD-L1 receptor-ligand interaction, such as when the PD-L1 cells are mixed with effector cells expressing PD-1. Thus, efficacy of inhibiting PD-L1 on CHO-K1 stable cells by anti-PD-L1 sdAbs and HCAbs can be assessed by measuring luciferase reporter activity. The assay is performed as follows.

[0167] On day one, PD-L1 cells are thawed in a 37°C water bath until cells are just thawed (about 3-4 minutes), and 0.5 mL of thawed cells is transferred to 14.5 mL cell recovery medium (10% FBS/F-12). The cell suspension is mixed well by gently inverting the tube 1-2 times. The cell suspension is then transferred to a sterile reagent reservoir, and dispensed into assay plates with 25 μL of cell suspension per well. 100 μL of assay medium is added per well as blank control. 100 μL of cell recovery medium is added per well for wells serving as blank control. The plates are then lidded and incubated overnight in a CO_2 incubator at 37°C.

[0168] On the day of assay, fresh assay buffer (RPMI 1640 + 1% FBS) is prepared. An eight-point serial dilution is performed in assay buffer for each of the control anti-PD-L1 antibody (e.g., Tecentriq®), sdAbs or HCAbs. The starting concentration and dilution scheme is optimized to achieve full dose-response curves. The assay plates containing PD-L1 cells are retrieved from the CO_2 incubator. 95µl of medium is removed per well from all the wells. 40 µL of serial dilutions of the control anti-PD-L1 antibody, or the antigen binding protein, is added per well to wells containing PD-L1 cells. 80 µL assay buffer is added per well to the blank control wells for each plate.

[0169] Next, PD-1 effector Cells are thawed in a 37°C water bath until cells are just thawed (about 3-4 minutes). The cell suspension is gently mixed in the vial by pipetting up and down, and 0.5 mL of the cells is added to 5.9 mL assay buffer. The cell suspension is mixed well by gently inverting the tube 1-2 times. The cell suspension is then transferred to a sterile reagent reservoir, and 40 µL of the cell suspension is dispensed to each well containing the PD-1 cells and control antibody or bispecific antigen binding protein. The plates are lidded and incubated for six hours at 37°C in a CO_2 incubator.

[0170] The Luciferase Assay System is reconstituted by transferring one bottle of Buffer to the bottle containing Substrate. The system is stored at room temperature and shielded from light for same day use. After 6 hours induction, assay plates are removed from the CO_2 incubator and equilibrated at ambient temperature for 5-10 min. 80 µL of reagent is added to each well. The plates are incubated for 5-10 min at ambient temperature. Luminescence is measured in GloMax® Discover System (Promega, Madison, WI) or a plate reader with glow-type luminescence reading capabilities.

[0171] Luminescence is expressed as Relative Light Unit (RLU). The RLU values of wells having diluted antibody or bispecific antigen binding protein is normalized to the RLU of no antibody or bispecific antigen binding protein control to provide Fold of Luciferase Induction. Data is graphed as RLU versus Log_{10} of concentration of antibody or bispecific antigen binding protein and as Fold of Induction versus Log_{10} concentration of antibody or bispecific antigen binding protein. The data is fitted to a curve and EC_{50} of each bispecific antigen binding proteins and the control anti-PD-1 antibody is determined using curve fitting software such as GraphPad Prism (Tables 14 and 15).

Table 14. EC_{50} of PD-L1- based blockade assay for sdAbs

Sample	AS06617 sdAb	AS06618 sdAb	AS06628 sdAb	AS06682 sdAb	AS06686 sdAb
EC_{50} (M)	6.38E-08	4.45E-08	2.42E-08	4.20E-08	3.52E-07
Sample	AS06703 sdAb	AS06730 sdAb	AS06750 sdAb	AS06775 sdAb	AS06778 sdAb
EC_{50} (M)	1.50E-08	1.72E-08	3.36E-08	7.55E-08	2.01E-08
Sample	AS06791 sdAb	AS11947 sdAb	AS11948 sdAb	AS12003 sdAb	Tecentriq
EC_{50} (M)	3.65E-08	7.72E-08	2.52E-08	9.62E-08	5.92E-09

Table 15. EC_{50} of PD-L1- based blockade assay for HCAbs

Sample	AS06617 HCAb	AS06618 HCAb	AS06628 HCAb	AS06682 HCAb	AS06686 HCAb
EC_{50} (M)	6.20E-09	6.45E-09	5.88E-09	6.66E-09	7.37E-09
Sample	AS06703 HCAb	AS06730 HCAb	AS06750 HCAb	AS06775 HCAb	AS06778 HCAb
EC_{50} (M)	5.59E-09	6.791E-09	9.98E-09	5.8E-09	5.54E-09
Sample	AS06791 HCAb	AS11947 HCAb	AS11948 HCAb	AS12003 HCAb	Tecentriq
EC_{50} (M)	5.82E-09	7.55E-09	5.80E-09	9.34E-09	5.92E-09

Example 10: anti-PD-L1 sdAb humanization

[0172] Five anti-PD-L1 sdAbs (AS06730, AS06750, AS11948, AS06617 and AS06675) were selected for humanization. Protein sequences of wildtype camelid sdAb was aligned with the 5 closest human germline sequences sharing the highest degree of homology. The best human germline sequence was selected as human acceptor. Homology model was made. According to the model analysis data, residues potentially critical for antigen binding or antibody scaffold formation were left untouched while the rest were selected for conversion into the human counterpart. Initially a panel of four sequence optimized variants was generated (stage 1). These variants were analyzed for a number of parameters and the results obtained were used to design a second set of sdAbs (stage 2). For each wildtype sdAb, 1-9 humanized sdAbs were designed for binding, stability and functional evaluation.

Humanized HCAb production

[0173] The HCAb constructs were generated by fusing sdAbs with the human Fc region. The maxiprep of the HCAb constructs were prepared for CHO-K1 cell transient expression and purification. The expressed HCAbs were purified by chromatography through a column containing Protein A agarose resin followed by a size exclusion column.

Affinity ranking of humanized HCAbs

[0174] Binding kinetics of each humanized HCAb to PD-L1 are determined using recombinant human PD-L1 His protein (R&D System) coated on a CM5 (Biacore) sensor chip. Each antigen binding protein is flowed over the antigen-coated chip, using surface plasmon resonance. Alternatively, each antigen binding protein is captured on a CM5 sensor chip, over which human PD-1-His protein is applied. Only the binding affinity of humanized clones comparable to that of the parent HCAbs were selected for further characterization (Tables 16-20).

Table 16. Affinity ranking of humanized sdAbs (AS06730)

Ligand	Analyte	k_a (1/Ms)	k_d (1/s)	K_D (M)
PD-L1/His	AS06730	2.34E+05	3.35E-04	1.43E-09
	AS06730S	2.29E+05	5.73E-04	2.51E-09
	AS06730A	6.96E+05	2.41E-02	3.46E-08
	AS06730Q	3.68E+11	1.18E+03	3.21E-09
	AS06730QVH1	2.58E+06	5.79E-03	2.24E-09
	AS06730QVH2	5.30E+05	2.19E-03	4.14E-09
	AS06730QVH3a	2.32E+06	2.12E-01	9.14E-08
	AS06730SVH12	3.0E+05	1.8E-03	6.1E-09
	AS06730SVH12M8	3.1E+05	6.5E-03	2.1E-08
	AS06730SVH12M9	3.2E+05	1.2E-02	3.7E-08

Table 17. Affinity ranking of humanized sdAbs (AS06750)

Ligand	Analyte	k_a (1/Ms)	k_d (1/s)	K_D (M)
PD-L1/His	AS06750	1.49E+05	3.30E-04	2.21E-09
	AS06750VH2	2.12E+05	3.29E-04	1.55E-09
	AS06750VH3	2.02E+05	3.55E-04	1.75E-09
	AS06750VHa	1.89E+05	3.08E-04	1.63E-09
	AS06750VH1	7.08E+04	2.53E-03	3.57E-08
	AS06750VH11	1.40E+05	2.70E-04	1.90E-09

Table 18. Affinity ranking of humanized sdAbs (AS11948)

Ligand	Analyte	k_a (1/Ms)	k_d (1/s)	K_D (M)
PD-L1/His	AS11948	2.97E+05	5.82E-04	1.96E-09
	AS11948Q	3.01E+05	4.65E-02	1.55E-07
	AS11948QVH1	3.32E+05	7.99E-04	2.41E-09
	AS11948QVH2	2.17E+06	1.95E-01	8.98E-08
	AS11948A	2.05E+06	1.96E-01	9.55E-08
	AS11948QVHa	6.41E+10	6.77E+02	1.06E-08
	AS11948S	2.26E+05	8.61E-04	3.80E-09

	AS11948SVH12	4.0E+05	1.5E-03	3.7E-09
	AS11948SVH12M8	4.3E+05	9.5E-03	2.2E-08
	AS11948SVH12M9	4.0E+05	6.3E-03	1.6E-08

Table 19. Affinity ranking of humanized sdAbs (AS06617)

Ligand	Analyte	k_a (1/Ms)	k_d (1/s)	K_D (M)
PD-L1/His	AS06617	3.10E+05	4.30E-04	1.40E-09
	AS06617VH11	4.30E+05	1.20E-03	2.70E-09

Table 20. Affinity ranking of humanized sdAbs (AS06775)

Ligand	Analyte	k_a (1/Ms)	k_d (1/s)	K_D (M)
PD-L1/His	AS0775	2.10E+05	3.60E-04	1.70E-09
	AS06775VH11	3.20E+05	5.00E-04	1.60E-09
	AS06775VH4	2.46E+05	7.16E-04	2.92E-09

Affinity determination

[0175] AS06730S, AS06730SVH3a, AS06730SVH12, AS06730AVH12M8, AS06730SVH12M9, AS06750VH2, AS06750VH11, AS06750VH4, AS11948S, AS11948SVH12, AS11948SV12M8, AS11948SV12M9, AS06617VH11, AS06775VH11 and AS06775VH4 were selected for affinity determination. Affinity constant (K_d) of each HCAbs was determined by surface plasmon resonance (SPR) on a BIAcore T200 instrument. Briefly, for most of HCAbs affinity determination, PD-L1 His was amine-coupled to a CM5 sensor chip at a density of no higher than 100 RU. Anti-PD-L1 HCAbs were injected at 5 different concentrations between 0.11 nM and 27 nM. Flow rate was 30 µl/min in all experiments. Association and dissociation phases were 5 and 10 min, respectively. The chip was regenerated using Glycine/HCl pH 1.5. For AS06730SVH12, AS06730SVH12M8, AS06730VH12M9, AS11948SV12, AS11948SV12M8 and AS11948SV12M9 HCAbs affinity determination, anti-PD-L1 HCAbs were captured on a CM5 sensor chip at a density of no higher than 100 RU by anti-human IgG antibody. Anti-PD-L1 His was injected at 5 different concentrations between 0.33 and 27 nM. Flow rate was 30 µl/min in all experiments. Association and dissociation phases were 5 min. Binding curves at different concentrations of HCAbs were used to calculate the kinetic parameters k_{on}, k_{off} and K_d. The kinetics data were summarized in Table 21 and Table 22.

Table 21. Affinity parameters of humanized HCAbs

Ligand	**Analyte**	**k_a (1/Ms)**	**k_d (1/s)**	**K_D (M)**
PD-L1 His	AS06730 HCAb	7.6E+04	1.7E-04	2.2E-09
	AS06730S HCAb	7.1E+04	3.5E-04	4.9E-09

	AS06730SVH3a HCAb	6.2E+04	3.7E-04	5.9E-09
	AS06750 HCAb	2.0E+05	3.6E-04	1.8E-09
	AS06750VH2 HCAb	2.0E+05	3.3E-04	1.7E-09
	AS06750VH11 HCAb	9.5E+04	3.0E-04	3.2E-09
	AS06750VH4 HCAb	1.4E+05	2.7E-04	1.9E-09
	AS11948 HCAb	3.5E+05	6.2E-04	1.8E-09
	AS11948S HCAb	2.9E+05	1.1E-03	3.8E-09
	AS06617 HCAb	4.8E+05	1.0E-03	2.1E-09
	AS06617VH11 HCAb	4.3E+05	1.2E-03	2.7E-09
	AS06775 HCAb	3.1E+05	4.3E-04	1.4E-09
	AS06775VH4 HCAb	2.1E+05	3.6E-04	1.7E-09
	AS06775VH11 HCAb	3.2E+05	5.0E-04	1.6E-09

Table 22. Affinity parameters of humanized HCAbs

Ligand	Analyte	k_a (1/Ms)	k_d (1/s)	KD (M)
AS06730SVH12 HCAb	PD-L1 His	3.0E+05	1.8E-03	6.1E-09
AS06730SVH12M8 HCAb		3.6E+05	6.5E-03	1.8E-08
AS06730SVH12M9 HCAb		4.0E+05	1.2E-02	3.0E-08
AS11948SVH12 HCAb		4.0E+05	1.5E-03	3.7E-09
AS11948SVH12M8 HCAb		4.3E+05	9.5E-03	2.2E-08
AS11948SVH12M9 HCAb		4.0E+05	6.3E-03	1.6E-08

PD-L1 based blockade assay

[0176] PD-L1 based blockade assay was performed as described in Example 9. All the selected humanized anti-PD-L1 HCAbs are comparable to Tecentriq® in inhibiting the binding between PD-L1 and PD-1. The EC_{50} data was summarized in Table 23.

Table 23. EC_{50} of PD-L1- based blockade assay for HCAbs

Sample	AS0730 HCAb	AS06730S HCAb	AS06730SVH3a HCAb	AS06730SVH12 HCAb	AS06750 HCAb
EC_{50} (M)	2.15E-09	2.54E-09	1.58E-09	4.60E-09	3.60E-09
Sample	AS06750VH2 HCAb	AS06750VH11 HCAb	AS06750H4 HCAb	AS11948 HCAb	AS11948S HCAb
EC_{50} (M)	4.44E-09	3.61E-09	5.77E-09	5.00E-09	3.28E-09
Sample	AS11948VH12 HCAb	AS11948VH12M8 HCAb	AS06617 HCAb	AS06617VH11 HCAb	AS06775 HCAb
EC_{50} (M)	2.91E-09	7.86E-09	3.86E-09	4.28E-09	3.08E-09
Sample	AS06775VH11 HCAb	AS06775VH4 HCAb	Tecentriq		
EC_{50} (M)	5.26E-09	3.43E-09	5.92E-09		

In vivo activity of humanized HCAbs

[0177] In the studies presented here, the efficacy of PD-L1 HCAb blockade against murine tumor model was investigated. Inhibition of the PD-L1 interaction is proposed to exert a

therapeutic effect by restoring anti-tumor CD8+ T cell responses, thus the preclinical efficacy study was conducted in syngeneic murine tumor model in which the immune system of the host is fully intact. The human PD-1 transgenic mice were used.

[0178] In this study, mice were inoculated subcutaneously in the right flank with 1×10^6 human PD-L1 overexpression MC38 colon carcinoma cells. When tumors reached a mean volume of ~100 mm^3, mice were sorted into treatment groups (n=5) (defined as study day 0). 6 humanized HCAbs tested in this study were listed: AS06730QVH1, AS06750VH11, AS11948SVH12, AS06617VH11, AS06617VH11, AS11948QVH1 and AS06775VH11. Groups were administered benchmark antibody MEDI4736 (10 mg/kg) or humanized HCAbs (5.33 mg/kg) intravenously days 0, 2, 5, 7, 9 and 12. A control group was treated with 10 ml/kg of PBS. Tumors were measured twice weekly for the study duration. All treatment groups demonstrated significant efficacy ($P<0.050$) when compared to the control group. These observations support that anti-PD-L1 therapy as an effective strategy for driving anti-tumor CD8+ T cell responses.

Table 24. CDRs of isolated sdAbs

sdAb	ID	CDR1	ID	CDR2	ID	CDR3
AS06617	169	GRTFISYAVG	269	GIRWNGIHTDYADSVKG	369	HRTIATIPEKYEYEY
AS06618	170	GRTFLSYAVG	270	GIRWSGGYTDYAEAVKG	370	HRTIATIPEKYEYEY
AS06624	171	GRTFLTYALG	271	GVSWSGSGTKYADSVKG	371	QISAIVPISAHEYEY
AS06628	172	GRTFITYAIG	272	AINWSGSMTSYADSVKG	372	HRGAIAPMTQSVYDY
AS06639	173	GRTFITYAIG	273	AINWSGSMTSYADSVKG	373	HRGAIAPMTQSVYDT
AS06682	174	GRTFLSYAVG	274	GIRWSGEHTDYAASVKG	374	HTTIATIPKKYEYEY
AS06686	175	GRTFLTYALG	275	GVSWSGSSTKYADSVKG	375	QISAIVPISAHEYQY
AS06703	176	GRTFITYAIG	276	AINWSGSMTSYADSVKG	376	HLGAIAPMSQSVYDY
AS06709	177	GRTFLSYAVG	277	GIRWSGGSTDYSDSVKG	377	HRTIATIPEKYEYEY
AS06730	178	GRTFITYAIG	278	AINWSGSMTSYADSVKG	378	HRGAIAPIAQSVYTN
AS06750	179	GRTFLTYAVG	279	GIRWSGGYTDYADSVKG	379	HRTIATIPEKYEYEY
AS06752	180	GRTFLTYAVG	280	GIRWSGESTDYAESVKG	380	HRTIATIPEKYYYEY
AS06763	181	GRPVSSAVMG	281	RLTSSATSTFYAESVKG	381	DVPGTKIWSIQTPDRYNY
AS06766	182	GRTLIGLLIG	282	IISWTYGSTNYADSVKG	382	RDVAVAKYDS
AS06775	183	GRTFLTLAVG	283	GIRWSGSGTDYADSVKG	383	HTTIATIPEKYEYEY
AS06778	184	GRTFITYAMG	284	AISWSGSSTYSADSVKG	384	EVSARTGEHLPKLMGDY
AS06786	185	GRTFLTLAVG	285	GIRWSGSGTDYADSVKG	385	HTTIATIPEKYEYEY
AS06791	186	GRTFITYAIG	286	AINWSGSMTSYADSVKG	386	HRGAIAPMTQSVYDY
AS06808	187	GRTFSRYAMG	287	TSIGSGGLTSYANSVKG	387	NRYNSDSRYMSSYDW
AS06810	188	GRTFLSYAVG	288	GIRWSGLHTDYADSVKG	388	HRTIATIPEKYEYEY
AS11947	189	GRTFISYAVG	289	GIRWNGISTDYTDSVKG	389	HRTIATIPNKYEYDH
AS11948	190	GRTFVTYGMG	290	AINWSGSMTSYGDSVKG	390	ALGAVVYTTREPYTY
AS12003	191	GRTFLSYAVG	291	GIRWSGGSTDYADSVKG	391	HRTIATVPNKYEYDT
AL22863	192	VSSFSINDMG	292	TIAS-GGSTNYADSVKG	392	DFRDWTRRRYSY
AL23474	193	GRTFSNYTMA	293	VVSRGGGATDYADSVKG	393	GTDLSYYYSTKKWAY
AS06730S	194	GRTFITYAIG	294	AISWSGSMTSYADSVKG	394	HRGAIAPIAQSVYTN
AS06730Q	195	GRTFITYAIG	295	AIQWSGSMTSYADSVKG	395	HRGAIAPIAQSVYTN
AS06730QVH1	196	GRTFITYAIG	296	AIQWSGSMTSYADSVKG	396	HRGAIAPIAQSVYTN
AS06730QVH2	197	GRTFITYAIG	297	AIQWSGSMTSYADSVKG	397	HRGAIAPIAQSVYTN
AS06730QVH3a	198	GRTFITYAIG	298	AIQWSGSMTSYADSVKG	398	HRGAIAPIAQSVYTN
AS06730SVH12	199	GRTFITYAIG	299	AISWSGSMTSYADSVKG	399	HRGAIAPIAQSVYTN
AS06730SVH12M8	200	GRTFITYAIG	300	AISWSGSITSYADSVKG	400	HRGAIAPIAQSVYTN

AS06730SVH12M9	201	GRTFITYAIG	301	AISWSGSLTSYADSVKG	401	HRGAIAPIAQSVYTN
AS06750VH1	202	GRTFLTYAVG	302	GIRWSGGYTDYADSVKG	402	HRTIATIPEKYEYEY
AS06750VH2	203	GRTFLTYAVG	303	GIRWSGGYTDYADSVKG	403	HRTIATIPEKYEYEY
AS06750VH3	204	GRTFLTYAVG	304	GIRWSGGYTDYADSVKG	404	HRTIATIPEKYEYEY
AS06750VHa	205	GRTFLTYAVG	305	GIRWSGGYTDYADSVKG	405	HRTIATIPEKYEYEY
AS06750VH11	206	GRTFLTYAVG	306	GIRWSGGYTDYADSVKG	406	HRTIATIPEKYEYEY
AS11948A	207	GRTFVTYGMG	307	AIAWSGSMTSYGDSVKG	407	ALGAVVYTTREPYTY
AS11948S	208	GRTFVTYGMG	308	AISWSGSMTSYGDSVKG	408	ALGAVVYTTREPYTY
AS11948Q	209	GRTFVTYGMG	309	AIQWSGSMTSYGDSVKG	409	ALGAVVYTTREPYTY
AS11948QVH1	210	GRTFVTYGMG	310	AIQWSGSMTSYGDSVKG	410	ALGAVVYTTREPYTY
AS11948QVH2	211	GRTFVTYGMG	311	AIQWSGSMTSYGDSVKG	411	ALGAVVYTTREPYTY
AS11948QVHa	212	GRTFVTYGMG	312	AIQWSGSMTSYGDSVKG	412	ALGAVVYTTREPYTY
AS11948SVH12	213	GRTFVTYGMG	313	AISWSGSMTSYGDSVKG	413	ALGAVVYTTREPYTY
AS11948SVH12M8	214	GRTFVTYGMG	314	AISWSGSITSYGDSVKG	414	ALGAVVYTTREPYTY
AS11948SVH12M9	215	GRTFVTYGMG	315	AISWSGSLTSYGDSVKG	415	ALGAVVYTTREPYTY
AS06617VH11	216	GRTFISYAVG	316	GIRWSGIHTDYADSVKG	416	HRTIATIPEKYEYEY
AS06775VH11	217	GRTFLTLAVG	317	GIRWSGSGTDYADSVKG	417	HTTIATIPEKYEYEY
AS06775VH4	218	GRTFLTYAVG	318	GIRWSGGYTDYADSVKG	418	HRTIATIPEKYEYEY

ID: SEQ ID NO

Table 25. Framework Regions 1 and 2

sdAb	ID	FR-1	ID	FR-2
AS06617	119	DVQLVESGGGLVQAGDSLRLSCAAS	219	WFRQAPGSEREFVA
AS06618	120	EVQLVESGGRLVRAGDSLRLSCAAS	220	WFRQAPGTEREFVA
AS06624	121	QVQLVESGGGLVQAGGSLRLACSAS	221	WFRQAPGKEREFVA
AS06628	122	QVQLVESGGGLVQAGGSLRLSCAAS	222	WFRQAPGKEREFVT
AS06639	123	AVQLVESGGGLVQAGGSLRLSCAAS	223	WFRQAPGKEREFVS
AS06682	124	AVQLVESGGGLVQAGDSLRLSCTAS	224	WFRQAPGTEREFVA
AS06686	125	AVQLVESGGGLVQAGDSLRLACAAS	225	WFRQAPGKEREFVA
AS06703	126	EVQLVESGGGLVRAGGSLRLSCAAS	226	WFRQAPGKEREFVT
AS06709	127	EVQLVESGGGLVQAGDSLRLSCTAS	227	WFRQAPGTEREFVA
AS06730	128	QVQLVESGGGLVQAGGSLRLSCAAS	228	WFRQAPGKEREFVS
AS06750	129	AVQLVESGGGLVQAGDSLRLSCTAS	229	WFRQAPGTEREFVA
AS06752	130	EVQLVESGGGLVQAGDSLRLSCAAS	230	WFRQAPGTEREFVA
AS06763	131	QVQLVESGGGLVQAGGSLRLSCAVS	231	WFRQAPGKEREFVG
AS06766	132	EVQLVESGGGLVQAGGSLSLSCAVS	232	WFRQAPGKERELVA
AS06775	133	QVQLVESGGGLVQAGDSLRLSCAAS	233	WFRQAPGTEREFVA
AS06778	134	QVQLVESGGGLVQAGGSLKLSCAAS	234	WFRQAPGKERELVA
AS06786	135	QVQLVESGGGLVQAGDSLRLSCAAS	235	WFRQAPGTEREFVA
AS06791	136	QVQLVESGGGLVQAGGSLRLSCAAS	236	WFRQAPGKEREFVT
AS06808	137	QVKLEESGGGLVQAGGSLRLSCVAS	237	WFRQAPGKEREFVS
AS06810	138	AVQLVESGGGLVQAGDSLRLSCAAS	238	WFRQAPGTEREFVA
AS11947	139	DVQLVESGGGLVQAGDSLRLTCSAS	239	WFRQAPGTEREFVA
AS11948	140	EVQLVESGGGLVQAGDSLRLSCVAS	240	WFRQAPGKEREFVA
AS12003	141	EVQLVESGGGLVQAGDSLRLSCAAS	241	WFRQAPGTEREFVA
AL22863	142	QVKLEESGGGLVQVGDSLRLSCAAS	242	WYRQAPGKQRELVA
AL23474	143	QVKLEESGGGLVQVGDSLRLSCAAS	243	WFRQFPGKEREFVA
AS06730S	144	QVQLVESGGGLVQAGGSLRLSCAAS	244	WFRQAPGKEREFVS
AS06730Q	145	QVQLVESGGGLVQAGGSLRLSCAAS	245	WFRQAPGKEREFVS
AS06730QVH1	146	EVQLVESGGGLVQPGGSLRLSCAAS	246	WVRQAPGKGLEWVS
AS06730QVH2	147	EVQLVESGGGLVQPGGSLRLSCAAS	247	WFRQAPGKGLEWVS
AS06730QVH3a	148	EVQLVESGGGLVQPGGSLRLSCAAS	248	WFRQAPGKGLEFVS
AS06730SVH12	149	EVQLVESGGGLVQPGGSLRLSCAAS	249	WFRQAPGKGREFVS
AS06730SVH12M8	150	EVQLVESGGGLVQPGGSLRLSCAAS	250	WFRQAPGKGREFVS
AS06730SVH12M9	151	EVQLVESGGGLVQPGGSLRLSCAAS	251	WFRQAPGKGREFVS
AS06750VH1	152	EVQLVESGGGLVQPGGSLRLSCAAS	252	WVRQAPGKGLEWVS
AS06750VH2	153	EVQLVESGGGLVQPGGSLRLSCAAS	253	WFRQAPGKGLEWVA
AS06750VH3	154	EVQLVESGGGLVQPGGSLRLSCAAS	254	WFRQAPGKGLEFVA
AS06750VHa	155	EVQLVESGGGLVQPGGSLRLSCTAS	255	WFRQAPGKGLEFVA
AS06750VH11	156	EVQLVESGGGLVQPGGSLRLSCAAS	256	WFRQAPGKGREFVS
AS11948A	157	EVQLVESGGGLVQAGDSLRLSCVAS	257	WFRQAPGKEREFVA
AS11948S	158	EVQLVESGGGLVQAGDSLRLSCVAS	258	WFRQAPGKEREFVA

AS11948Q	159	EVQLVESGGGLVQAGDSLRLSCVAS	259	WFRQAPGKEREFVA
AS11948QVH1	160	EVQLVESGGGLVQPGGSLRLSCAAS	260	WVRQAPGKGLEWVS
AS11948QVH2	161	EVQLVESGGGLVQPGGSLRLSCAAS	261	WFRQAPGKGLEFVA
AS11948QVHa	162	EVQLVESGGGLVQPGGSLRLSCVAS	262	WFRQAPGKGREFVS
AS11948SVH12	163	EVQLVESGGGLVQPGGSLRLSCAAS	263	WFRQAPGKGREFVS
AS11948SVH12M8	164	EVQLVESGGGLVQPGGSLRLSCAAS	264	WFRQAPGKGREFVS
AS11948SVH12M9	165	EVQLVESGGGLVQPGGSLRLSCAAS	265	WFRQAPGKGREFVS
AS06617VH11	166	EVQLVESGGGLVQPGGSLRLSCAAS	266	WFRQAPGKGREFVS
AS06775VH11	167	EVQLVESGGGLVQPGGSLRLSCAAS	267	WFRQAPGKGREFVS
AS06775VH4	168	EVQLVESGGGLVQPGGSLRLSCAAS	268	WFRQAPGKGLEFVS

ID: SEQ ID NO; FR: Framework region

Table 26: Framework Regions 3 and 4

sdAb	ID	FR-3	ID	FR-4
AS06617	319	RFTISRDNAKNTVTLEMTSLKPEDTAVYYCAA	419	WGQGTQVTVSS
AS06618	320	RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA	420	WGQGTQVTVSS
AS06624	321	RFTISRDNAKNTVYMQMNSLKPEDTAVYYCAA	421	WGQGTQVTVSS
AS06628	322	RFTISRDNNKNMVYLQMNSLKPEDTAVYYCAA	422	WGQGTQVTVSS
AS06639	323	RFTISRDNAKNTVYLQMNGLKPEDTAVYYCAA	423	WGQGTQVTVSS
AS06682	324	RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA	424	WGQGTQVTVSS
AS06686	325	RFTISRDNAKNTVYMQMNSLKPEDTAVYYCAA	425	WGQGTQVTVSS
AS06703	326	RFTISRDNNKNTVYLQMNSLKPEDTALYYCAA	426	WGQGTQVTVSS
AS06709	327	RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA	427	WGQGTQVTVSS
AS06730	328	RFTISRDNAKNTVYLQMNGLKPEDTAVYYCAA	428	WGQGTQVTVSS
AS06750	329	RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA	429	WGQGTQVTVSS
AS06752	330	RFTISRDDTKNTVYLQMNSLKPEDTAVYYCAA	430	WGQGTQVTVSS
AS06763	331	RFTISRDNAKNTVYLQMNNLKPEDTAVYYCAA	431	WGQGTQVTVSS
AS06766	332	RFTISRDNAKNTVLLQMNSLKPEDTAVYYCSA	432	WGQGTQVTVSS
AS06775	333	RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA	433	WGRGTQVTVSS
AS06778	334	RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA	434	WGQGTQVTVSS
AS06786	335	RFTISRDNAANTVYLQMNSLKPEDTAVYYCAA	435	WGQGTQVTVSS
AS06791	336	RFTISRDNAKNTVYLQINGLKSEDTAVYYCAA	436	WGQGTQVTVSS
AS06808	337	RFTISRDNAKNTVYLQMNNLKPEDTAIYYCAA	437	WGQGTQVTVSS
AS06810	338	RFTISRDNAKNTVYLQMNSLKPEDTAIYYCAA	438	WGQGTQVTVSS
AS11947	339	RFTISRDNAKNTVYLQMNSLKPEDTAIYYCAA	439	WGQGTQVTVSS
AS11948	340	RFAISRDNAKNTVYLQMNSLKPEDTAVYYCAA	440	WGRGTQVTVSS
AS12003	341	RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA	441	WGQGTQVTVSS
AL22863	342	RFTISRDNVKNTVYLQMNGLKPEDTAVYYCNA	442	WGQGTQVTVSS
AL23474	343	RFTISRDNAKNTMYLQMNSLKTEDTAVYYCAA	443	WGQGTQVTVSS
AS06730S	344	RFTISRDNAKNTVYLQMNGLKPEDTAVYYCAA	444	WGQGTQVTVSS
AS06730Q	345	RFTISRDNAKNTVYLQMNGLKPEDTAVYYCAA	445	WGQGTQVTVSS
AS06730QVH1	346	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	446	WGQGTLVTVSS
AS06730QVH2	347	RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAA	447	WGQGTLVTVSS
AS06730QVH3a	348	RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAA	448	WGQGTLVTVSS
AS06730SVH12	349	RFTISRDNAKNTLYLQMNSLRPEDTAVYYCAA	449	WGQGTLVTVSS
AS06730SVH12M8	350	RFTISRDNAKNTLYLQMNSLRPEDTAVYYCAA	450	WGQGTLVTVSS
AS06730SVH12M9	351	RFTISRDNAKNTLYLQMNSLRPEDTAVYYCAA	451	WGQGTLVTVSS
AS06750VH1	352	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	452	WGQGTLVTVSS
AS06750VH2	353	RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAA	453	WGQGTLVTVSS
AS06750VH3	354	RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAA	454	WGQGTLVTVSS
AS06750VHa	355	RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAA	455	WGQGTLVTVSS
AS06750VH11	356	RFTISRDNAKNTLYLQMNSLRPEDTAVYYCAA	456	WGQGTLVTVSS
AS11948A	357	RFAISRDNAKNTVYLQMNSLKPEDTAVYYCAA	457	WGRGTQVTVSS
AS11948S	358	RFAISRDNAKNTVYLQMNSLKPEDTAVYYCAA	458	WGRGTQVTVSS
AS11948Q	359	RFAISRDNAKNTVYLQMNSLKPEDTAVYYCAA	459	WGRGTQVTVSS
AS11948QVH1	360	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	460	WGQGTLVTVSS
AS11948QVH2	361	RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAA	461	WGQGTLVTVSS
AS11948QVHa	362	RFTISRDNSKNTVYLQMNSLRAEDTAVYYCAA	462	WGQGTLVTVSS
AS11948SVH12	363	RFTISRDNAKNTLYLQMNSLRPEDTAVYYCAA	463	WGQGTLVTVSS
AS11948SVH12M8	364	RFTISRDNAKNTLYLQMNSLRPEDTAVYYCAA	464	WGQGTLVTVSS
AS11948SVH12M9	365	RFTISRDNAKNTLYLQMNSLRPEDTAVYYCAA	465	WGQGTLVTVSS
AS06617VH11	366	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAA	466	WGQGTLVTVSS

AS06775VH11	367	RFTISRDNAKNTLYLQMNSLRPEDTAVYYCAA	467	WGQGTLVTVSS
AS06775VH4	368	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAA	468	WGQGTLVTVSS

ID: SEQ ID NO; FR: Framework region

Table 27. sdAbs

sdAb	ID	Sequence
AS06617	469	DVQLVESGGGLVQAGDSLRLSCAASGRTFISYAVGWFRQAPGSEREFVAGIRWNGIH TDYADSVKGRFTISRDNAKNTVTLEMTSLKPEDTAVYYCAAHRTIATIPEKYEYEYW GQGTQVTVSS
AS06618	470	EVQLVESGGRLVRAGDSLRLSCAASGRTFLSYAVGWFRQAPGTEREFVAGIRWSGGY TDYAEAVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAHRTIATIPEKYEYEYW GQGTQVTVSS
AS06624	471	QVQLVESGGGLVQAGGSLRLACSASGRTFLTYALGWFRQAPGKEREFVAGVSWSGSG TKYADSVKGRFTISRDNAKNTVYMQMNSLKPEDTAVYYCAAQISAIVPISAHEYEYW GQGTQVTVSS
AS06628	472	QVQLVESGGGLVQAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVTAINWSGSM TSYADSVKGRFTISRDNNKNMVYLQMNSLKPEDTAVYYCAAHRGAIAPMTQSVYDYW GQGTQVTVSS
AS06639	473	AVQLVESGGGLVQAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVSAINWSGSM TSYADSVKGRFTISRDNAKNTVYLQMNGLKPEDTAVYYCAAHRGAIAPMTQSVYDTW GQGTQVTVSS
AS06682	474	AVQLVESGGGLVQAGDSLRLSCTASGRTFLSYAVGWFRQAPGTEREFVAGIRWSGEH TDYAASVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAHTTIATIPKKYEYEYW GQGTQVTVSS
AS06686	475	AVQLVESGGGLVQAGDSLRLACAASGRTFLTYALGWFRQAPGKEREFVAGVSWSGSS TKYADSVKGRFTISRDNAKNTVYMQMNSLKPEDTAVYYCAAQISAIVPISAHEYQYW GQGTQVTVSS
AS06703	476	EVQLVESGGGLVRAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVTAINWSGSM TSYADSVKGRFTISRDNNKNTVYLQMNSLKPEDTALYYCAAHLGAIAPMSQSVYDYW GQGTQVTVSS
AS06709	477	EVQLVESGGGLVQAGDSLRLSCTASGRTFLSYAVGWFRQAPGTEREFVAGIRWSGGS TDYSDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAHRTIATIPEKYEYEYW GQGTQVTVSS
AS06730	478	QVQLVESGGGLVQAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVSAINWSGSM TSYADSVKGRFTISRDNAKNTVYLQMNGLKPEDTAVYYCAAHRGAIAPIAQSVYTNW GQGTQVTVSS
AS06750	479	AVQLVESGGGLVQAGDSLRLSCTASGRTFLTYAVGWFRQAPGTEREFVAGIRWSGGY TDYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAHRTIATIPEKYEYEYW GQGTQVTVSS
AS06752	480	EVQLVESGGGLVQAGDSLRLSCAASGRTFLTYAVGWFRQAPGTEREFVAGIRWSGES TDYAESVKGRFTISRDDTKNTVYLQMNSLKPEDTAVYYCAAHRTIATIPEKYYYEYW GQGTQVTVSS
AS06763	481	QVQLVESGGGLVQAGGSLRLSCAVSGRPVSSAVMGWFRQAPGKEREFVGRLTSSATS TFYAESVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCAADVPGTKIWSIQTPDRY NYWGQGTQVTVSS
AS06766	482	EVQLVESGGGLVQAGGSLSLSCAVSGRTLTGLLIGWFRQAPGKERELVAIISWTYGS TNYADSVKGRFTISRDNAKNTVLLQMNSLKPEDTAVYYCSARDVAVAKYDSWGQGTQ VTVSS
AS06775	483	QVQLVESGGGLVQAGDSLRLSCAASGRTFLTLAVGWFRQAPGTEREFVAGIRWSGSG TDYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAHTTIATIPEKYEYEYW GRGTQVTVSS
AS06778	484	QVQLVESGGGLVQAGGSLKLSCAASGRTFITYAMGWFRQAPGKERELVAAISWSGSS TYSADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAEVSARTGEHLPKLMGD YWGQGTQVTVSS
AS06786	485	QVQLVESGGGLVQAGDSLRLSCAASGRTFLTLAVGWFRQAPGTEREFVAGIRWSGSG TDYADSVKGRFTISRDNAANTVYLQMNSLKPEDTAVYYCAAHTTIATIPEKYEYEYW GQGTQVTVSS
AS06791	486	QVQLVESGGGLVQAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVTAINWSGSM TSYADSVKGRFTISRDNAKNTVYLQINGLKSEDTAVYYCAAHRGAIAPMTQSVYDYW GQGTQVTVSS

AS06808	487	QVKLEESGGGLVQAGGSLRLSCVASGRTFSRYAMGWFRQAPGKEREFVSTSTGSGGL TSYANSVKGRFTISRDNAKNTVYLQMNNLKPEDTAIYYCAANRYNSDSRYMSSYDWW GQGTQVTVSS
AS06810	488	AVQLVESGGGLVQAGDSLRLSCAASGRTFLSYAVGWFRQAPGTEREFVAGIRWSGLH TDYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCAAHRTIATIPEKYEYEYW GQGTQVTVSS
AS11947	489	DVQLVESGGGLVQAGDSLRLTCSASGRTFISYAVGWFRQAPGTEREFVAGIRWNGIS TDYTDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCAAHRTIATIPNKYEYDHW GQGTQVTVSS
AS11948	490	EVQLVESGGGLVQAGDSLRLSCVASGRTFVTYGMGWFRQAPGKEREFVAAINWSGSM TSYGDSVKGRFAISRDNAKNTVYLQMNSLKPEDTAVYYCAAALGAVVYTTREPYTYW GRGTQVTVSS
AS12003	491	EVQLVESGGGLVQAGDSLRLSCAASGRTFLSYAVGWFRQAPGTEREFVAGIRWSGGS TDYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAHRTIATVPNKYEYDTW GQGTQVTVSS
AL22863	492	QVKLEESGGGLVQVGDSLRLSCAASVSSFSINDMGWYRQAPGKQRELVATIASGGST NYADSVKGRFTISRDNVKNTVYLQMNGLKPEDTAVYYCNADFRDWTRRRYSYWGQGT QVTVSS
AL23474	493	QVKLEESGGGLVQVGDSLRLSCAASGRTFSNYTMAWFRQFPGKEREFVAVVSRGGGA TDYADSVKGRFTISRDNAKNTMYLQMNSLKTEDTAVYYCAAGTDLSYYYSTKKWAYW GQGTQVTVSS
AS06730S	494	QVQLVESGGGLVQAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVSAISWSGSM TSYADSVKGRFTISRDNAKNTVYLQMNGLKPEDTAVYYCAAHRGAIAPIAQSVYTNW GQGTQVTVSS
AS06730Q	495	QVQLVESGGGLVQAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVSAIQWSGSM TSYADSVKGRFTISRDNAKNTVYLQMNGLKPEDTAVYYCAAHRGAIAPIAQSVYTNW GQGTQVTVSS
AS06730QVH1	496	EVQLVESGGGLVQPGGSLRLSCAASGRTFITYAIGWVRQAPGKGLEWVSAIQWSGSM TSYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHRGAIAPIAQSVYTNW GQGTLVTVSS
AS06730QVH2	497	EVQLVESGGGLVQPGGSLRLSCAASGRTFITYAIGWFRQAPGKGLEWVSAIQWSGSM TSYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAHRGAIAPIAQSVYTNW GQGTLVTVSS
AS06730QVH3a	498	EVQLVESGGGLVQPGGSLRLSCAASGRTFITYAIGWFRQAPGKGLEFVSAIQWSGSM TSYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAHRGAIAPIAQSVYTNW GQGTLVTVSS
AS06730SVH12	499	EVQLVESGGGLVQPGGSLRLSCAASGRTFITYAIGWFRQAPGKGREFVSAISWSGSM TSYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHRGAIAPIAQSVYTNW GQGTLVTVSS
AS06730SVH12M8	500	EVQLVESGGGLVQPGGSLRLSCAASGRTFITYAIGWFRQAPGKGREFVSAISWSGSI TSYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHRGAIAPIAQSVYTNW GQGTLVTVSS
AS06730SVH12M9	501	EVQLVESGGGLVQPGGSLRLSCAASGRTFITYAIGWFRQAPGKGREFVSAISWSGSL TSYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHRGAIAPIAQSVYTNW GQGTLVTVSS
AS06750VH1	502	EVQLVESGGGLVQPGGSLRLSCAASGRTFLTYAVGWVRQAPGKGLEWVSGIRWSGGY TDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHRTIATIPEKYEYEYW GQGTLVTVSS
AS06750VH2	503	EVQLVESGGGLVQPGGSLRLSCAASGRTFLTYAVGWFRQAPGKGLEWVAGIRWSGGY TDYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAHRTIATIPEKYEYEYW GQGTLVTVSS
AS06750VH3	504	EVQLVESGGGLVQPGGSLRLSCAASGRTFLTYAVGWFRQAPGKGLEFVAGIRWSGGY TDYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAHRTIATIPEKYEYEYW GQGTLVTVSS
AS06750VHa	505	EVQLVESGGGLVQPGGSLRLSCTASGRTFLTYAVGWFRQAPGKGLEFVAGIRWSGGY TDYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAHRTIATIPEKYEYEYW GQGTLVTVSS
AS06750VH11	506	EVQLVESGGGLVQPGGSLRLSCAASGRTFLTYAVGWFRQAPGKGREFVSGIRWSGGY TDYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHRTIATIPEKYEYEYW GQGTLVTVSS

AS11948A	507	EVQLVESGGGLVQAGDSLRLSCVASGRTFVTYGMGWFRQAPGKEREFVAAIAWSGSM TSYGDSVKGRFAISRDNAKNTVYLQMNSLKPEDTAVYYCAAALGAVVYTTREPYTYW GRGTQVTVSS
AS11948S	508	EVQLVESGGGLVQAGDSLRLSCVASGRTFVTYGMGWFRQAPGKEREFVAAISWSGSM TSYGDSVKGRFAISRDNAKNTVYLQMNSLKPEDTAVYYCAAALGAVVYTTREPYTYW GRGTQVTVSS
AS11948Q	509	EVQLVESGGGLVQAGDSLRLSCVASGRTFVTYGMGWFRQAPGKEREFVAAIQWSGSM TSYGDSVKGRFAISRDNAKNTVYLQMNSLKPEDTAVYYCAAALGAVVYTTREPYTYW GRGTQVTVSS
AS11948QVH1	510	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWVRQAPGKGLEWVSAIQWSGSM TSYGDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKALGAVVYTTREPYTYW GQGTLVTVSS
AS11948QVH2	511	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGLEFVAAIQWSGSM TSYGDSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAALGAVVYTTREPYTYW GQGTLVTVSS
AS11948QVHa	512	EVQLVESGGGLVQPGGSLRLSCVASGRTFVTYGMGWFRQAPGKGREFVSAIQWSGSM TSYGDSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAALGAVVYTTREPYTYW GQGTLVTVSS
AS11948SVH12	513	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGREFVSAISWSGSM TSYGDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYW GQGTLVTVSS
AS11948SVH12M8	514	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGREFVSAISWSGSI TSYGDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYW GQGTLVTVSS
AS11948SVH12M9	515	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGREFVSAISWSGSL TSYGDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYW GQGTLVTVSS
AS06617VH11	516	EVQLVESGGGLVQPGGSLRLSCAASGRTFISYAVGWFRQAPGKGREFVSGIRWSGIH TDYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHRTIATIPEKYEYEYW GQGTLVTVSS
AS06775VH11	517	EVQLVESGGGLVQPGGSLRLSCAASGRTFLTLAVGWFRQAPGKGREFVSGIRWSGSG TDYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHTTIATIPEKYEYEYW GQGTLVTVSS
AS06775VH4	518	EVQLVESGGGLVQPGGSLRLSCAASGRTFLTYAVGWFRQAPGKGLEFVSGIRWSGGY TDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAHRTIATIPEKYEYEYW GQGTLVTVSS

ID: SEQ ID NO

Table 28. HCAbs

HCAb	ID	Sequence
AS06617	519	DVQLVESGGGLVQAGDSLRLSCAASGRTFISYAVGWFRQAPGSEREFVAGIRWNG IHTDYADSVKGRFTISRDNAKNTVTLEMTSLKPEDTAVYYCAAHRTIATIPEKYE YEYWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06617VH11	520	EVQLVESGGGLVQPGGSLRLSCAASGRTFISYAVGWFRQAPGKGREFVSGIRWSG IHTDYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHRTIATIPEKYE YEYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06618	521	EVQLVESGGRLVRAGDSLRLSCAASGRTFLSYAVGWFRQAPGTEREFVAGIRWSG GYTDYAEAVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAHRTIATIPEKYE YEYWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK

AS06628	522	QVQLVESGGGLVQAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVTAINWSG SMTSYADSVKGRFTISRDNNKNMVYLQMNSLKPEDTAVYYCAAHRGAIAPMTQSV YDYWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06682	523	AVQLVESGGGLVQAGDSLRLSCTASGRTFLSYAVGWFRQAPGTEREFVAGIRWSG EHTDYAASVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAHTTIATIPKKYE YEYWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06686	524	AVQLVESGGGLVQAGDSLRLACAASGRTFLTYALGWFRQAPGKEREFVAGVSWSG SSTKYADSVKGRFTISRDNAKNTVYMQMNSLKPEDTAVYYCAAQISAIVPISAHE YQYWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06703	525	EVQLVESGGGLVRAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVTAINWSG SMTSYADSVKGRFTISRDNNKNTVYLQMNSLKPEDTALYYCAAHLGAIAPMSQSV YDYWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06730	526	QVQLVESGGGLVQAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVSAINWSG SMTSYADSVKGRFTISRDNAKNTVYLQMNGLKPEDTAVYYCAAHRGAIAPIAQSV YTNWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06750	527	AVQLVESGGGLVQAGDSLRLSCTASGRTFLTYAVGWFRQAPGTEREFVAGIRWSG GYTDYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAHRTIATIPEKYE YEYWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06775	528	QVQLVESGGGLVQAGDSLRLSCAASGRTFLTLAVGWFRQAPGTEREFVAGIRWSG SGTDYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAHTTIATIPEKYE YEYWGRGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06775VH4	529	EVQLVESGGGLVQPGGSLRLSCAASGRTFLTLAVGWFRQAPGKGREFVSGIRWSG SGTDYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHTTIATIPEKYE YEYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06775VH11	530	EVQLVESGGGLVQPGGSLRLSCAASGRTFLTYAVGWFRQAPGKGLEFVSGIRWSG GYTDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAHRTIATIPEKYE YEYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06778	531	QVQLVESGGGLVQAGGSLKLSCAASGRTFITYAMGWFRQAPGKERELVAAISWSG SSTYSADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAEVSARTGEHLPK LMGDYWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL

		HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
AS06791	532	QVQLVESGGGLVQAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVTAINWSG SMTSYADSVKGRFTISRDNAKNTVYLQINGLKSEDTAVYYCAAHRGAIAPMTQSV YDYWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS11947	533	DVQLVESGGGLVQAGDSLRLTCSASGRTFISYAVGWFRQAPGTEREFVAGIRWNG ISTDYTDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCAAHRTIATIPNKYE YDHWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS11948	534	EVQLVESGGGLVQAGDSLRLSCVASGRTFVTYGMGWFRQAPGKEREFVAAINWSG SMTSYGDSVKGRFAISRDNAKNTVYLQMNSLKPEDTAVYYCAAALGAVVYTTREP YTYWGRGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS12003	535	EVQLVESGGGLVQAGDSLRLSCAASGRTFLSYAVGWFRQAPGTEREFVAGIRWSG GSTDYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAHRTIATVPNKYE YDTWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06730A	536	QVQLVESGGGLVQAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVSAIAWSG SMTSYADSVKGRFTISRDNAKNTVYLQMNGLKPEDTAVYYCAAHRGAIAPIAQSV YTNWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06730S	537	QVQLVESGGGLVQAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVSAISWSG SMTSYADSVKGRFTISRDNAKNTVYLQMNGLKPEDTAVYYCAAHRGAIAPIAQSV YTNWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06730Q	538	QVQLVESGGGLVQAGGSLRLSCAASGRTFITYAIGWFRQAPGKEREFVSAIQWSG SMTSYADSVKGRFTISRDNAKNTVYLQMNGLKPEDTAVYYCAAHRGAIAPIAQSV YTNWGQGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06730QVH1	539	EVQLVESGGGLVQPGGSLRLSCAASGRTFITYAIGWVRQAPGKGLEWVSAIQWSG SMTSYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHRGAIAPIAQSV YTNWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06730QVH2	540	EVQLVESGGGLVQPGGSLRLSCAASGRTFITYAIGWFRQAPGKGLEWVSAIQWSG SMTSYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAHRGAIAPIAQSV YTNWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK

AS06730QVH3a	541	EVQLVESGGGLVQPGGSLRLSCAASGRTFITYAIGWFRQAPGKGLEFVSAIQWSG SMTSYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAHRGAIAPIAQSV YTNWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06730SVH12	542	EVQLVESGGGLVQPGGSLRLSCAASGRTFITYAIGWFRQAPGKGREFVSAISWSG SMTSYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHRGAIAPIAQSV YTNWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06730SVH12M8	543	EVQLVESGGGLVQPGGSLRLSCAASGRTFITYAIGWFRQAPGKGREFVSAISWSG SITSYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHRGAIAPIAQSV YTNWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06730SVH12M9	544	EVQLVESGGGLVQPGGSLRLSCAASGRTFITYAIGWFRQAPGKGREFVSAISWSG SLTSYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHRGAIAPIAQSV YTNWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06750VH1	545	EVQLVESGGGLVQPGGSLRLSCAASGRTFLTYAVGWVRQAPGKGLEWVSGIRWSG GYTDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHRTIATIPEKYE YEYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06750VH2	546	EVQLVESGGGLVQPGGSLRLSCAASGRTFLTYAVGWFRQAPGKGLEWVAGIRWSG GYTDYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAHRTIATIPEKYE YEYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06750VH3	547	EVQLVESGGGLVQPGGSLRLSCAASGRTFLTYAVGWFRQAPGKGLEFVAGIRWSG GYTDYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAHRTIATIPEKYE YEYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06750VHa	548	EVQLVESGGGLVQPGGSLRLSCTASGRTFLTYAVGWFRQAPGKGLEFVAGIRWSG GYTDYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAHRTIATIPEKYE YEYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS06750VH11	549	EVQLVESGGGLVQPGGSLRLSCAASGRTFLTYAVGWFRQAPGKGREFVSGIRWSG GYTDYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHRTIATIPEKYE YEYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS11948A	550	EVQLVESGGGLVQAGDSLRLSCVASGRTFVTYGMGWFRQAPGKEREFVAAIAWSG SMTSYGDSVKGRFAISRDNAKNTVYLQMNSLKPEDTAVYYCAAALGAVVYTTREP YTYWGRGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ

		DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS11948S	551	EVQLVESGGGLVQAGDSLRLSCVASGRTFVTYGMGWFRQAPGKEREFVAAISWSG SMTSYGDSVKGRFAISRDNAKNTVYLQMNSLKPEDTAVYYCAAALGAVVYTTREP YTYWGRGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS11948Q	552	EVQLVESGGGLVQAGDSLRLSCVASGRTFVTYGMGWFRQAPGKEREFVAAIQWSG SMTSYGDSVKGRFAISRDNAKNTVYLQMNSLKPEDTAVYYCAAALGAVVYTTREP YTYWGRGTQVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS11948QVH1	553	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWVRQAPGKGLEWVSAIQWSG SMTSYGDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKALGAVVYTTREP YTYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS11948QVH2	554	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGLEFVAAIQWSG SMTSYGDSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAALGAVVYTTREP YTYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS11948QVHa	555	EVQLVESGGGLVQPGGSLRLSCVASGRTFVTYGMGWFRQAPGKGREFVSAIQWSG SMTSYGDSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAAALGAVVYTTREP YTYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS11948SVH12	556	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGREFVSAISWSG SMTSYGDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREP YTYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS11948SVH12M8	557	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGREFVSAISWSG SITSYGDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREP YTYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK
AS11948SVH12M9	558	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGREFVSAISWSG SLTSYGDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREP YTYWGQGTLVTVSSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK

ID: SEQ ID NO

Example 11. Anti-SIRPα anti-PD-L1 Bispecific Antibodies

[0179] In this example, a few anti-SIRPα anti-PD-L1 bispecific antibodies were generated and tested, and showed potent activities in enhancing both T cell function and macrophage phagocytosis.

[0180] Bispecific antibodies of four different formats (FIG. 6A-D) were prepared with two anti-SIRPα antibodies (HSP210-02-hz52, a humanized version from 248G3F6, and HSP210-03-hz51, a humanized version from 300A6A6) and an anti-PD-L1 sdAb (AS11948SVH12, a humanized version of AS11948).

[0181] In the format illustrated in **FIG. 6A**, the anti-PD-L1 sdAb is located at the N-terminus of the Fc fragment of the anti-SIRPα antibody (**Format I**). In the format illustrated in **FIG. 6B**, the anti-PD-L1 sdAb is located at the C-terminus of the heavy chains of the anti-SIRPα antibody (**Format II**). In the format illustrated in **FIG. 6C**, the anti-PD-L1 sdAb is located at the C-terminus of the light chains of the anti-SIRPα antibody (**Format III**). In the format illustrated in **FIG. 6D**, the anti-PD-L1 sdAb is located at the N-terminus of the light chains of the anti-SIRPα antibody (**Format IV**).

Table 29. Listing of bispecific antibodies

Name	**Location of anti-PD-L1 sdAb**
PD-L1/SIRPa-HC1	Format I – C-terminus of IgG4 Fc (248G3F6)
PD-L1/SIRPa-HC2	Format I – C-terminus of IgG4 Fc (300A6A6)
PD-L1/SIRPa-HN1	Format II – N-terminus of heavy chain (248G3F6)
PD-L1/SIRPa-HN2	Format II – N-terminus of heavy chain (300A6A6)
PD-L1/SIRPa-LC1	Format III – C-terminus of light chain (248G3F6)
PD-L1/SIRPa-LC2	Format III – C-terminus of light chain (300A6A6)
PD-L1/SIRPa-LN1	Format IV – N-terminus of light chain (248G3F6)
PD-L1/SIRPa-LN2	Format IV – N-terminus of light chain (300A6A6)

[0182] The sequences of these antibodies are provided in the table below.

Table 30. Sequences of the bispecific antibodies

Name	Sequence	SEQ ID NO:
PD-L1 sdAb	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGREFVSA ISWSGSMTSYGDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAAL GAVVYTTREPYTYWGQGTLVTVSS	513
SIRPa IgG#1 02-hz52 VH1	QVQLVQSGAEVKKPGASVKVSCKASGFNFEDTYMHWVRQAPGQGLEWMGR IDPADADTKYNPKFQDRVTITVDTSTNTAYMELSSLRSEDTAVYYCVRGN YVNWGQGTTVTVSS	27
SIRPa IgG#1 02-hz52 VL1	EIVLTQSPGTLSLSPGERATLSCRASSSVSSSYLYWYQQKPGQAPRLLIY STSNLASGIPDRFSGSGSGTDYTLTISRLEPEDAAVYFCHQWYSYPRTFG GGTKVEIK	29

SIRPa IgG#2 03-hz51 VH2	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPGDADTNYNQKFNGRVTLTADKSTSTAYMELSSLRSEDTAVYYCAINY GGIWFAYWGQGTLVTVSS	43
SIRPa IgG#2 03-hz51 VL2	DIQMTQSPSSLSASVGDRVTITCQASQDIGNKLIWYQQKPGKAPKLLIYY VTNLPGGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQYKQNPLTFGQ GTKLEIK	45
PD-L1/SIRPa-HC1 heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGFNFEDTYMHWVRQAPGQGLEWMGR IDPADADTKYNPKFQDRVTITVDTSTNTAYMELSSLRSEDTAVYYCVRGN YVNWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK***GGGGSGGGG SGGGGS***EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKG REFVSAISWSGSMTSYGDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVY YCAAALGAVVYTTREPYTYWGQGTLVTVSS	559
PD-L1/SIRPa-HC1 light chain	EIVLTQSPGTLSLSPGERATLSCRASSSVSSSYLYWYQQKPGQAPRLLIY STSNLASGIPDRFSGSGSGTDYTLTISRLEPEDAAVYFCHQWYSYPRTFG GGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC	560
PD-L1/SIRPa-HC2 heavy chain	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPGDADTNYNQKFNGRVTLTADKSTSTAYMELSSLRSEDTAVYYCAINY GGIWFAYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYT CNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLP PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK***GGGGS GGGGSGGGGS***EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQA PGKGREFVSAISWSGSMTSYGDSVKGRFTISRDNAKNTLYLQMNSLRPED TAVYYCAAALGAVVYTTREPYTYWGQGTLVTVSS	561
PD-L1/SIRPa-HC2 light chain	DIQMTQSPSSLSASVGDRVTITCQASQDIGNKLIWYQQKPGKAPKLLIYY VTNLPGGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQYKQNPLTFGQ GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC	562
PD-L1/SIRPa-HN1 heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGREFVSA ISWSGSMTSYGDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAAL GAVVYTTREPYTYWGQGTLVTVSS***GGGGSGGGGSGGGGS***QVQLVQSGAEV KKPGASVKVSCKASGFNFEDTYMHWVRQAPGQGLEWMGRIDPADADTKYN PKFQDRVTITVDTSTNTAYMELSSLRSEDTAVYYCVRGNYVNWGQGTTVT VSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKR VESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMHEALHNHYTQKSLSLSLGK	563
PD-L1/SIRPa-HN1 light chain	EIVLTQSPGTLSLSPGERATLSCRASSSVSSSYLYWYQQKPGQAPRLLIY STSNLASGIPDRFSGSGSGTDYTLTISRLEPEDAAVYFCHQWYSYPRTFG GGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC	560
PD-L1/SIRPa-HN2 heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGREFVSA ISWSGSMTSYGDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAAL GAVVYTTREPYTYWGQGTLVTVSS***GGGGSGGGGSGGGGS***QVQLVQSGAEV KKPGSSVKVSCKASGYTFTSNYIHWVRQAPGQGLEWMGWIYPGDADTNYN QKFNGRVTLTADKSTSTAYMELSSLRSEDTAVYYCAINYGGIWFAYWGQG	564

	TLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVD KSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	
PD-L1/SIRPa-HN2 light chain	DIQMTQSPSSLSASVGDRVTITCQASQDIGNKLIWYQQKPGKAPKLLIYY VTNLPGGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQYKQNPLTFGQ GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC	562
PD-L1/SIRPa-LC1 heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGFNFEDTYMHWVRQAPGQGLEWMGR IDPADADTKYNPKFQDRVTITVDTSTNTAYMELSSLRSEDTAVYYCVRGN YVNWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	565
PD-L1/SIRPa-LC1 light chain	EIVLTQSPGTLSLSPGERATLSCRASSSVSSSYLYWYQQKPGQAPRLLIY STSNLASGIPDRFSGSGSGTDYTLTISRLEPEDAAVYFCHQWYSYPRTFG GGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC***GGGGSGGGGSGGGGS***EVQLVESGGGLVQPGGSLRL SCAASGRTFVTYGMGWFRQAPGKGREFVSAISWSGSMTSYGDSVKGRFTI SRDNAKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYWGQGTLV TVSS	566
PD-L1/SIRPa-LC2 heavy chain	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPGDADTNYNQKFNGRVTLTADKSTSTAYMELSSLRSEDTAVYYCAINY GGIWFAYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYT CNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLP PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	567
PD-L1/SIRPa-LC2 light chain	DIQMTQSPSSLSASVGDRVTITCQASQDIGNKLIWYQQKPGKAPKLLIYY VTNLPGGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQYKQNPLTFGQ GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC***GGGGSGGGGSGGGGS***EVQLVESGGGLVQPGGSLRLS CAASGRTFVTYGMGWFRQAPGKGREFVSAISWSGSMTSYGDSVKGRFTIS RDNAKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYWGQGTLVT VSS	568
PD-L1/SIRPa-LN1 heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGFNFEDTYMHWVRQAPGQGLEWMGR IDPADADTKYNPKFQDRVTITVDTSTNTAYMELSSLRSEDTAVYYCVRGN YVNWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	565
PD-L1/SIRPa-LN1 light chain	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGREFVSA ISWSGSMTSYGDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAAL GAVVYTTREPYTYWGQGTLVTVSS***GGGGSGGGGSGGGGS***EIVLTQSPGTL SLSPGERATLSCRASSSVSSSYLYWYQQKPGQAPRLLIYSTSNLASGIPD RFSGSGSGTDYTLTISRLEPEDAAVYFCHQWYSYPRTFGGGTKVEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ	569

	ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC	
PD-L1/SIRPa-LN2 heavy chain	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSNYIHWVRQAPGQGLEWMGW IYPGDADTNYNQKFNGRVTLTADKSTSTAYMELSSLRSEDTAVYYCAINY GGIWFAYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYT CNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLP PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	567
PD-L1/SIRPa-LN2 light chain	EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGREFVSA ISWSGSMTSYGDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAAL GAVVYTTREPYTYWGQGTLVTVSS***GGGGSGGGGSGGGGS***DIQMTQSPSSL SASVGDRVTITCQASQDIGNKLIWYQQKPGKAPKLLIYYVTNLPGGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCLQYKQNPLTFGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	570

[0183] The bispecific antibodies were first tested for their ability to bind to PD-L1 expressed on cells. The results are summarized in **FIG. 7**, which shows that all of the tested bispecific antibodies had stronger affinity than IgG and anti-PD-L1 sdAb alone (fused to IgG4 Fc).

[0184] The bispecific antibodies were also tested for their ability to bind to SIRP-α expressed on cells. As shown in **FIG. 8**, the tested bispecific antibodies had comparable affinity to the monospecific counterparts (03HZ51: HSP210-03-hz51, 02HZ52: HSP210-02-hz52).

[0185] The bispecific antibodies' activity in blocking PD-1/PD-L1 interaction was also tested and the results are shown in **FIG. 9**. All of them exhibited superior biological activities, which are comparable to Tecentriq®.

[0186] The bispecific antibodies' activity in blocking CD47/SIRPa interaction was further tested and the results are shown in **FIG. 10**. All of them exhibited superior biological activities, which are comparable to parent monocolonal SIRPa antibodies.Four of the bispecific antibodies (PD-L1/SIRPa-HC1, PD-L1/SIRPa-HC2, PD-L1/SIRPa-LC2, PD-L1/SIRPa-LN1) were selected for testing with respect their ability to induce phagocytosis. The procedure is similar to Example 7, and the results are summarized in **FIG. 11**. All of these bispecific antibodies exhibited similar activities as the monospecific counterparts, and PD-L1/SIRPa-HC1 and PD-L1/SIRPa-HC2, wherein the sdAb was fused to the C-terminus of the Fc fragment, showed slightly stronger activity.

* * *

[0187] The present disclosure is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the disclosure, and any compositions or methods which are functionally equivalent are within the scope of this disclosure. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present disclosure without departing from the spirit or scope of the disclosure. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

[0188] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS

What is claimed is:

1. An antibody, comprising an anti-signal regulatory protein alpha (SIRPα) unit and an anti-programmed death-ligand 1 (PD-L1) unit, wherein the anti-SIRPα unit comprises an Fab fragment having binding specificity to a human SIRPα protein, and the anti-PD-L1 unit comprises a single-domain antibody (sdAb) having binding specificity to a human PD-L1 protein.

2. The antibody of claim 1, further comprising an Fc fragment.

3. The antibody of claim 1 or 2, wherein the sdAb is fused to the heavy chain of the Fab fragment.

4. The antibody of any one of claims 1-3, wherein the sdAb is fused to the C-terminus of the heavy chain.

5. The antibody of any preceding claim, wherein the Fab fragment comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein:

(a) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16, 21 or 22, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20;

(b) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 31, 36, 37 or 38, the

CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 33, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35;

(c) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 48, 53 or 54, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52;

(d) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 63, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 64, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 65, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 66, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 67, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 68;

(e) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 77, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 78, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 79, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 80, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 81, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 82;

(f) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 91, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 92, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 93, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 94, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 95, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 96; or

(g) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 103, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 104, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 105, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 106, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 107, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 108.

6. The antibody of claim 5, wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 15, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 16, 21 or 22, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 17, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 18, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 19, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 20.

7. The antibody of claim 6, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1 and 23-27 and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 and 28-29.

8. The antibody of claim 6, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:27 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:29.

9. The antibody of claim 5, wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 30, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 31, 36, 37 or 38, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 32, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 33, the CDRL2 comprises the amino acid

sequence of SEQ ID NO: 34, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 35.

10. The antibody of claim 9, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:3 and 39-44 and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:4 and 45-46.

11. The antibody of claim 9, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:43 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:45.

12. The antibody of claim 5, wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 47, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 48, 53 or 54, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 49, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 50, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 51, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 52;

13. The antibody of claim 12, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:5 and 55-60 and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:6 and 61-62.

14. The antibody of any one of claims 1-13, wherein the sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NO:169-218, a CDR2 comprising the amino acid sequence of any one of SEQ ID NO:169-318, and a CDR3 comprising the amino acid sequence of any one of SEQ ID NO:369-418.

15. The antibody of any one of claims 1-13, wherein the sdAb comprises the CDR1, CDR2 and CDR3 of the amino acid sequences of SEQ ID NO:469-518.

16. The antibody of claim 14, wherein the CDR1 comprises the amino acid sequence of SEQ ID NO:213, the CDR2 comprises the amino acid sequence of SEQ ID NO:313, and the CDR3 comprises the amino acid sequence of SEQ ID NO:413.

17. The antibody of claim 14, wherein sdAb comprises the amino acid sequence of SEQ ID NO:513.

18. The antibody of claim 1, which comprises:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO:559 and a light chain comprising the amino acid sequence of SEQ ID NO:560;

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO:561 and a light chain comprising the amino acid sequence of SEQ ID NO:562;

(c) a heavy chain comprising the amino acid sequence of SEQ ID NO:563 and a light chain comprising the amino acid sequence of SEQ ID NO:560;

(d) a heavy chain comprising the amino acid sequence of SEQ ID NO:564 and a light chain comprising the amino acid sequence of SEQ ID NO:562;

(e) a heavy chain comprising the amino acid sequence of SEQ ID NO:565 and a light chain comprising the amino acid sequence of SEQ ID NO:566;

(f) a heavy chain comprising the amino acid sequence of SEQ ID NO:567 and a light chain comprising the amino acid sequence of SEQ ID NO:568;

(g) a heavy chain comprising the amino acid sequence of SEQ ID NO:565 and a light chain comprising the amino acid sequence of SEQ ID NO:569; or

(h) a heavy chain comprising the amino acid sequence of SEQ ID NO:567 and a light chain comprising the amino acid sequence of SEQ ID NO:570.

19. A composition comprising the antibody of any one of claims 1-18 and a pharmaceutically acceptable carrier.

20. A method of treating cancer in a patient in need thereof, comprising administering to the patient the antibody of any one of claims 1-18.

21. Use of the antibody of any one of claims 1-18 for the manufacture of a medicament for treating cancer.

22. The method of claim 20 or the use of claim 21, wherein the cancer is selected from the group consisting of bladder cancer, liver cancer, colon cancer, rectal cancer, endometrial cancer, leukemia, lymphoma, pancreatic cancer, small cell lung cancer, non-small cell lung cancer, breast cancer, urethral cancer, head and neck cancer, gastrointestinal cancer, stomach cancer, oesophageal cancer, ovarian cancer, renal cancer, melanoma, prostate cancer and thyroid cancer.

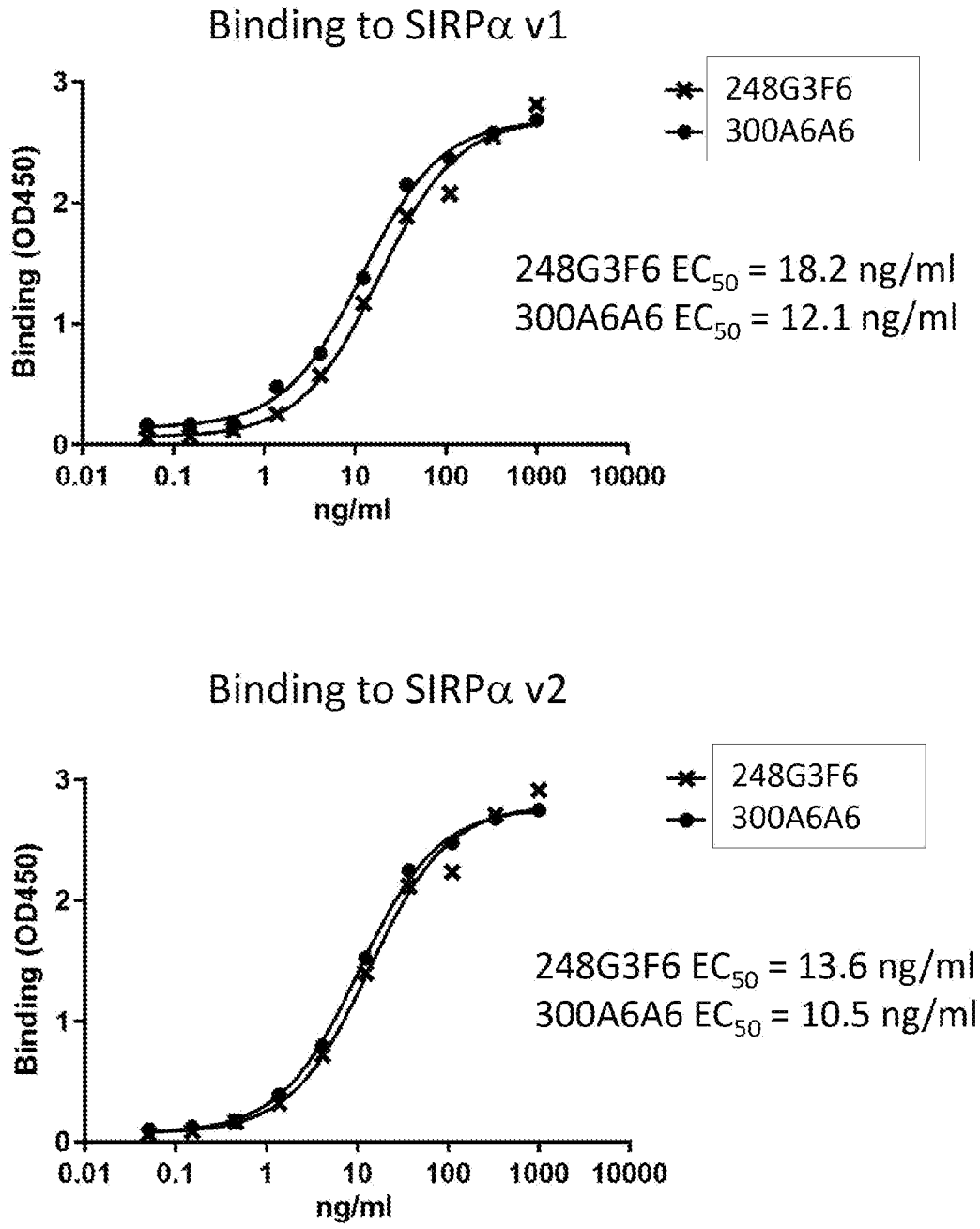

Binding to SIRPα v1
248G3F6
300A6A6
Binding (OD450)
ng/ml
0.01
0.1
1
10
100
1000
10000
248G3F6 EC50 = 18.2 ng/ml
300A6A6 EC50 = 12.1 ng/ml
Binding to SIRPα v2
248G3F6
300A6A6
Binding (OD450)
ng/ml
248G3F6 EC50 = 13.6 ng/ml
300A6A6 EC50 = 10.5 ng/ml

FIG. 1

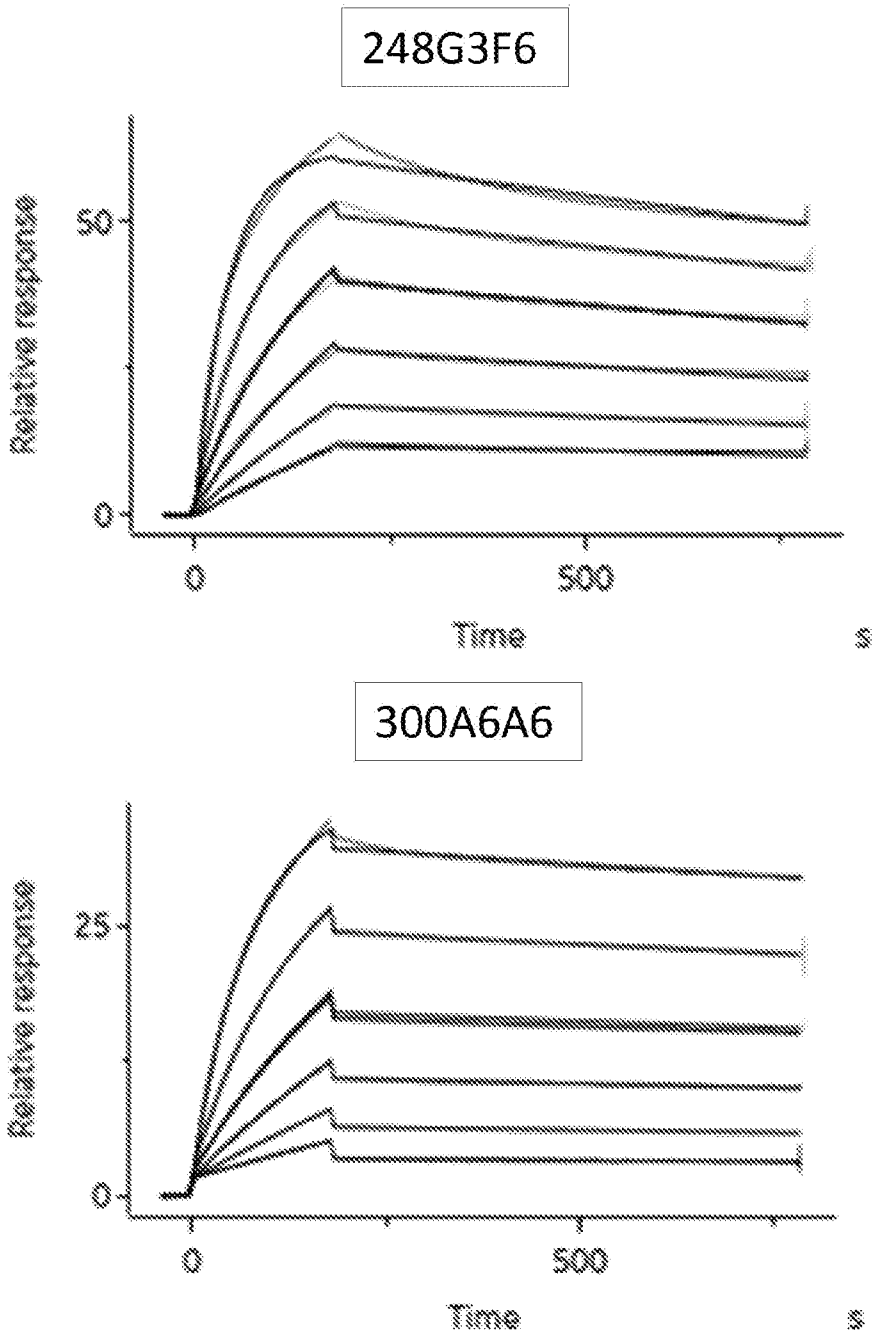
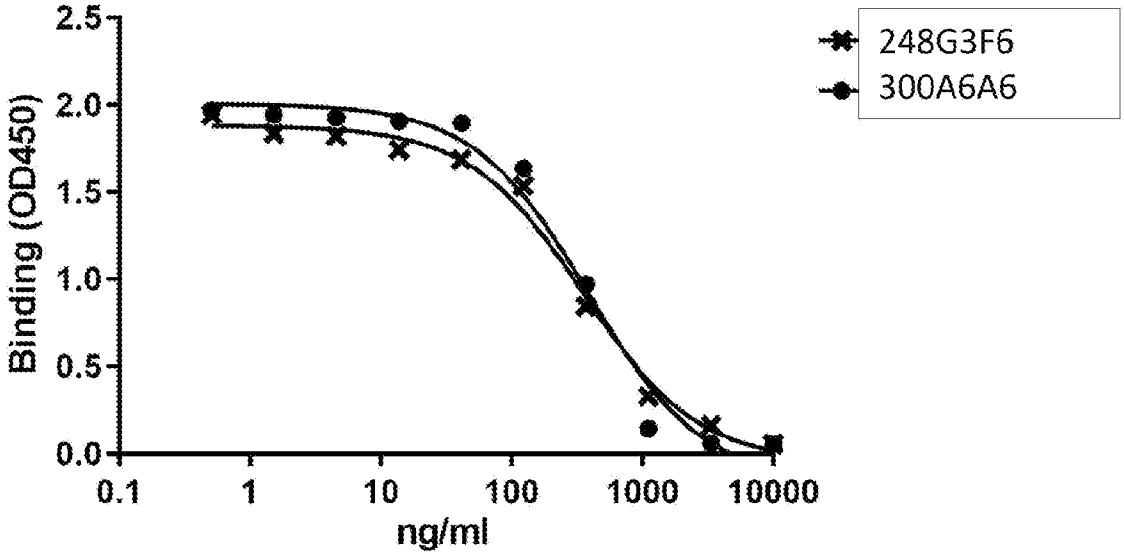

248G3F6
Relative response
50
0
0
500
Time
s
300A6A6
Relative response
25
0
0
500
Time
s

FIG. 2



248G3F6 IC_{50} = 355.8 ng/ml
300A6A6 IC_{50} = 383.1 ng/ml

FIG. 3

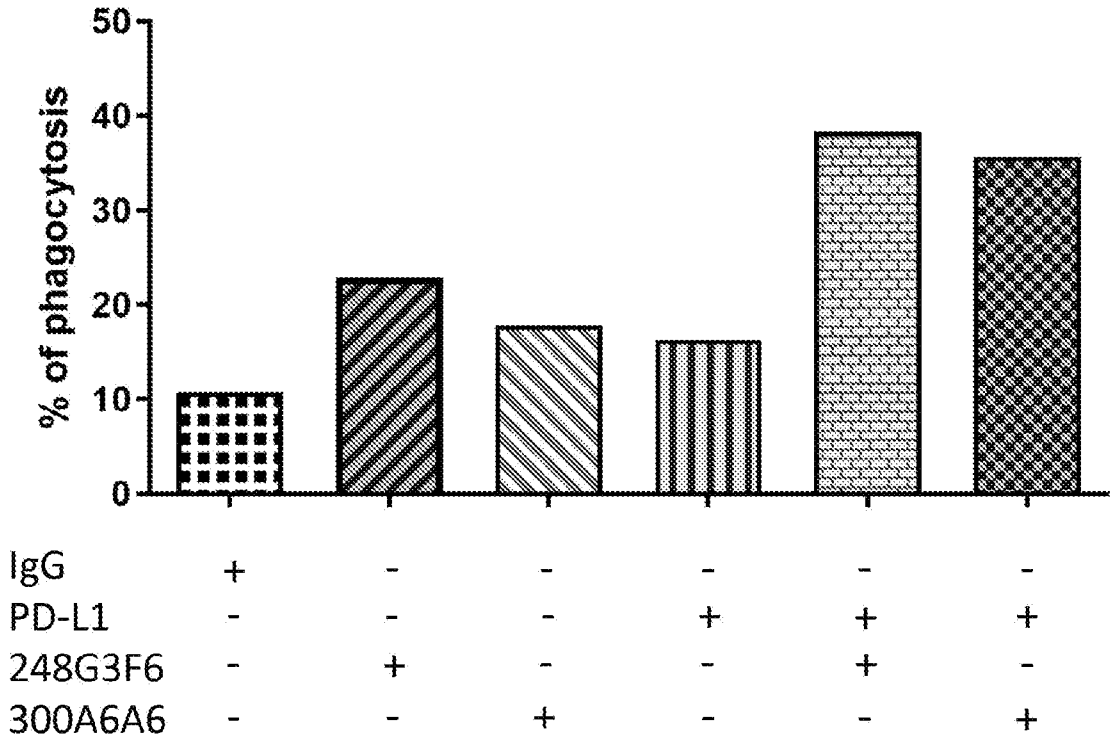


FIG. 4

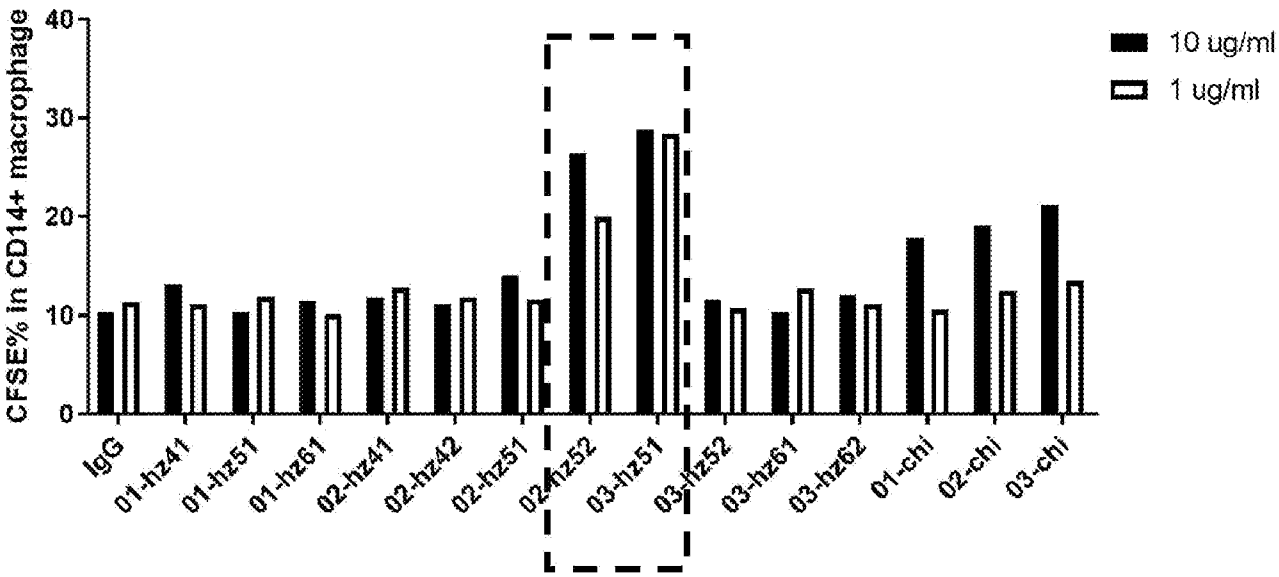

CFSE% in CD14+ macrophage
40
30
20
10
0
IgG
01-hz41
01-hz51
01-hz61
02-hz41
02-hz42
02-hz51
02-hz52
03-hz51
03-hz52
03-hz61
03-hz62
01-chi
02-chi
03-chi
10 ug/ml
1 ug/ml

FIG. 5

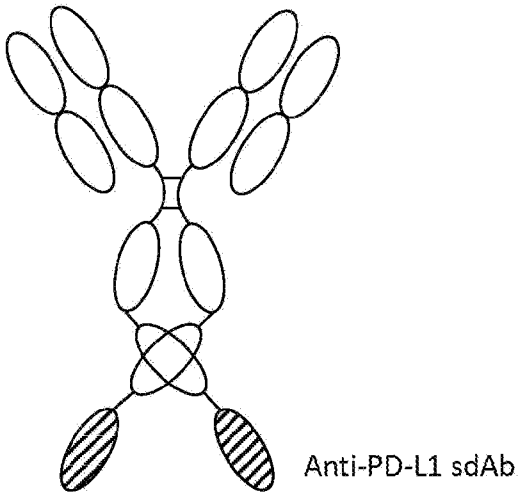


FIG. 6A

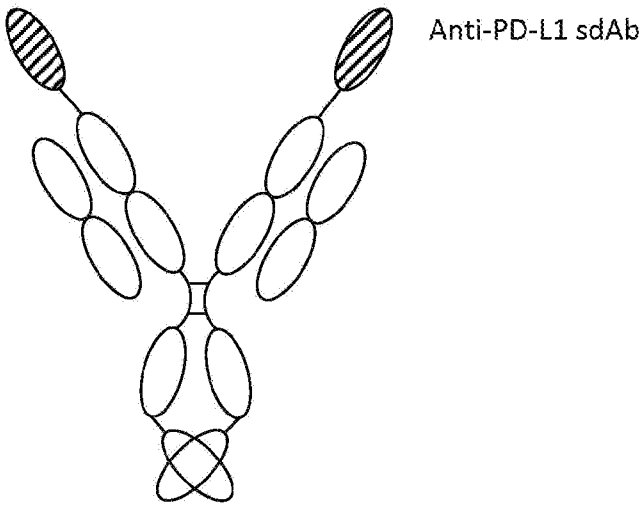


FIG. 6B

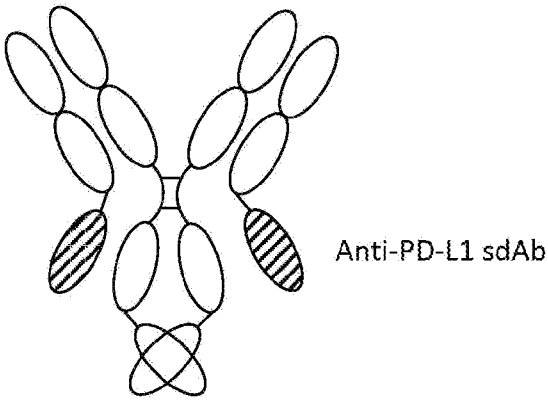


FIG. 6C

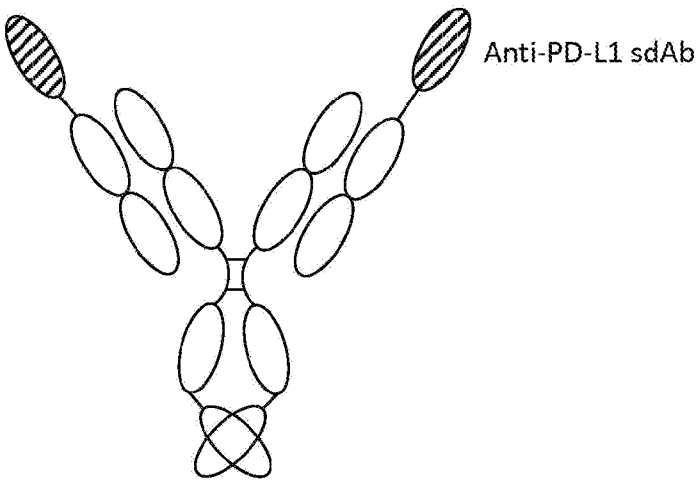
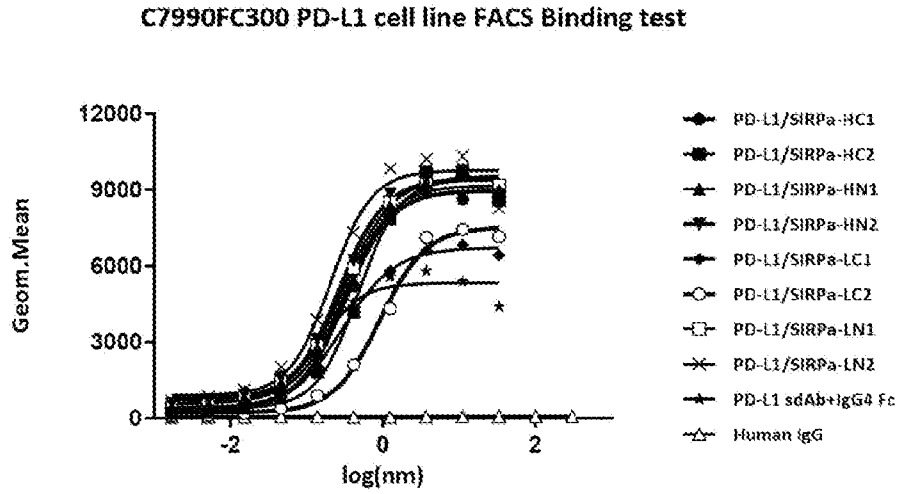


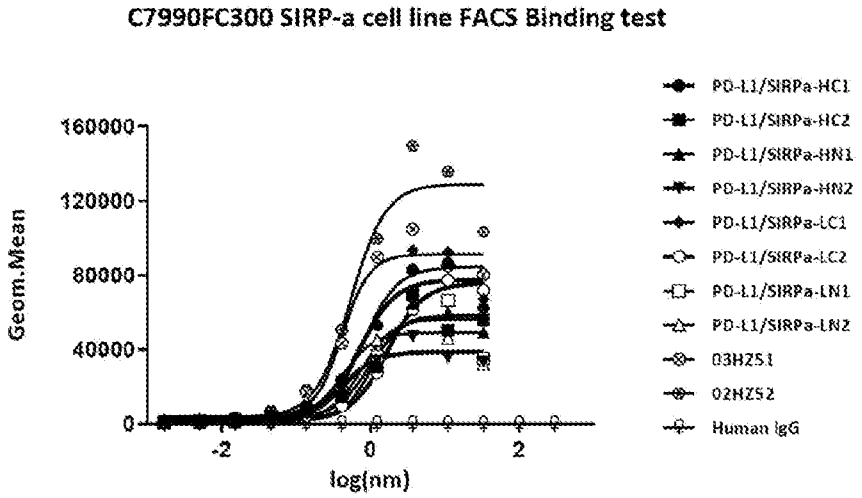
FIG. 6D



	PD-L1/SIRPa-HC1	PD-L1/SIRPa-HC2	PD-L1/SIRPa-HN1	PD-L1/SIRPa-HN2	PD-L1/SIRPa-LC1	PD-L1/SIRPa-LC2
EC50	0.3208	0.4726	0.3110	0.2689	0.2883	0.9118

	PD-L1/SIRPa-LN1	PD-L1/SIRPa-LN2	PD-L1 sdAb+IgG4 Fc	Human IgG
EC50	0.2963	0.2047	0.1545	~ 3.476

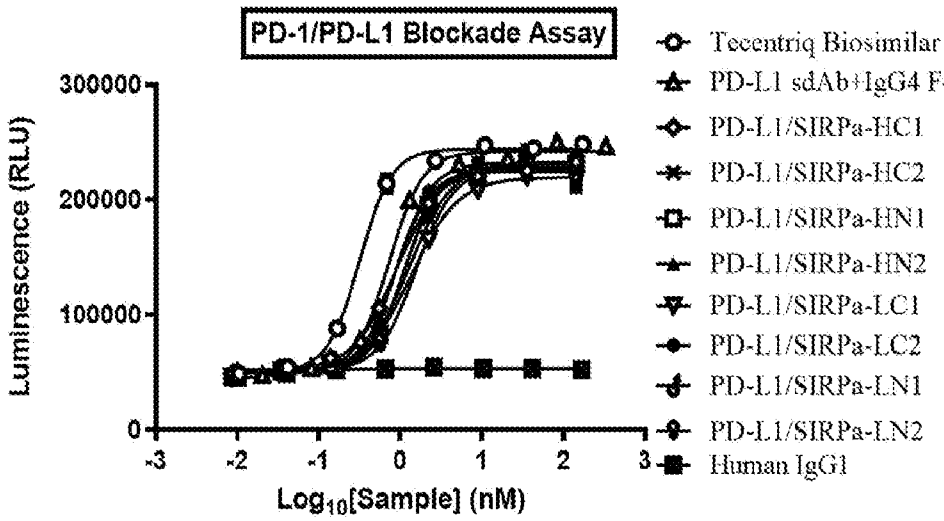
FIG. 7



	PD-L1/SIRPa-HC1	PD-L1/SIRPa-HC2	PD-L1/SIRPa-HN1	PD-L1/SIRPa-HN2	PD-L1/SIRPa-LC1	PD-L1/SIRPa-LC2
EC50	0.7109	1.064	0.9673	0.4335	0.7947	1.674

	PD-L1/SIRPa-LN1	PD-L1/SIRPa-LN2	03HZ51	02HZ52	Human IgG
EC50	0.7728	0.4303	0.4172	0.5398	26.63

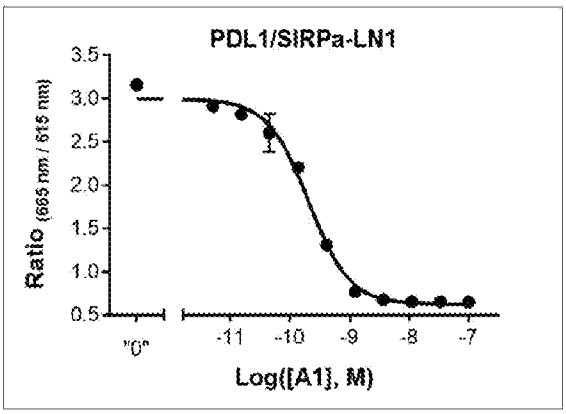
FIG. 8

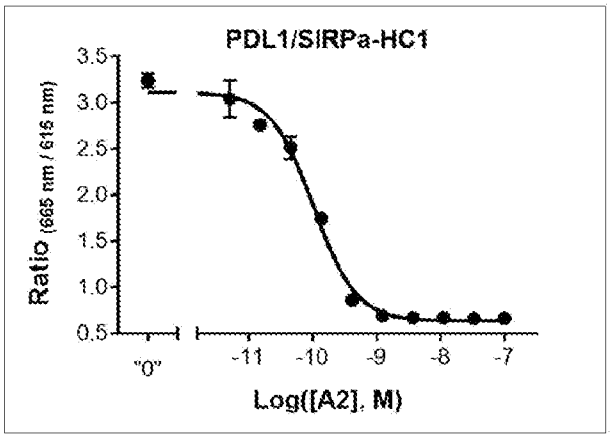


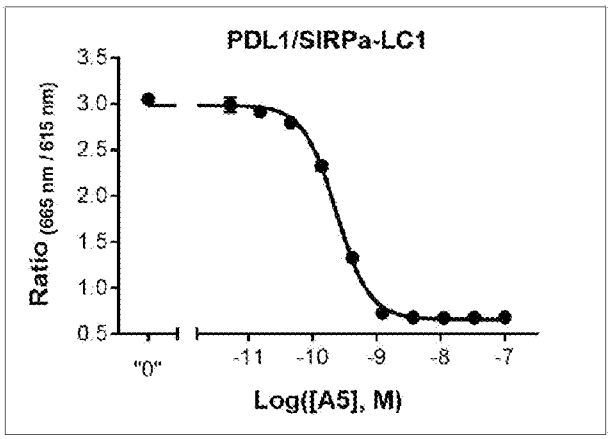
	Tecentriq Biosimilar	PD-L1 sdAb+IgG4 Fc	PD-L1/SIRPa-HC1	PD-L1/SIRPa-HC2	PD-L1/SIRPa-HN1
EC50	0.3179	0.7282	0.8996	1.277	1.130

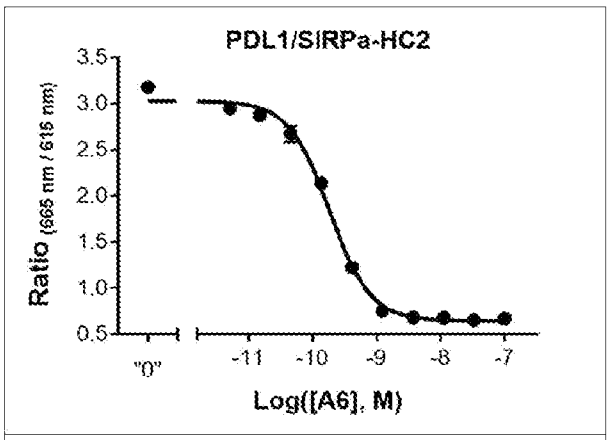
PD-L1/SIRPa-HN2	PD-L1/SIRPa-LC1	PD-L1/SIRPa-LC2	PD-L1/SIRPa-LN1	PD-L1/SIRPa-LN2	Human IgG1
0.9078	1.363	1.555	1.130	0.8727	0.03949

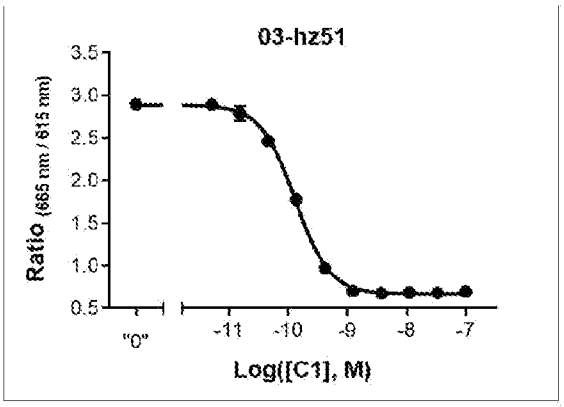
FIG. 9


PDL1/SIRPa-LN1
Ratio (665 nm / 615 nm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
"0"
-11
-10
-9
-8
-7
Log([A1], M)


PDL1/SIRPa-HC1
Ratio (665 nm / 615 nm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
"0"
-11
-10
-9
-8
-7
Log([A2], M)


PDL1/SIRPa-LC1
Ratio (665 nm / 615 nm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
"0"
-11
-10
-9
-8
-7
Log([A5], M)


PDL1/SIRPa-HC2
Ratio (665 nm / 615 nm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
"0"
-11
-10
-9
-8
-7
Log([A6], M)


03-hz51
Ratio (665 nm / 615 nm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
"0"
-11
-10
-9
-8
-7
Log([C1], M)

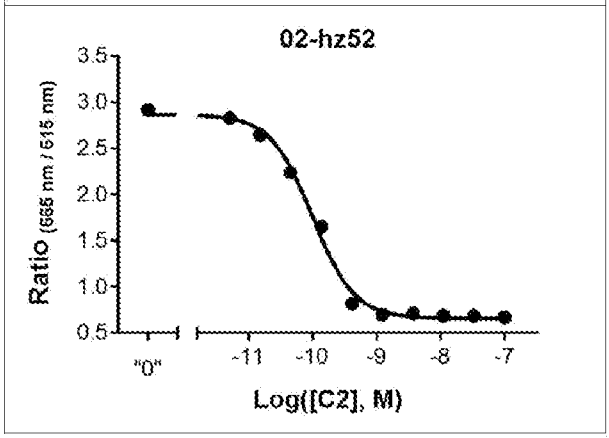

02-hz52
Ratio (665 nm / 615 nm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
"0"
-11
-10
-9
-8
-7
Log([C2], M)

FIG. 10

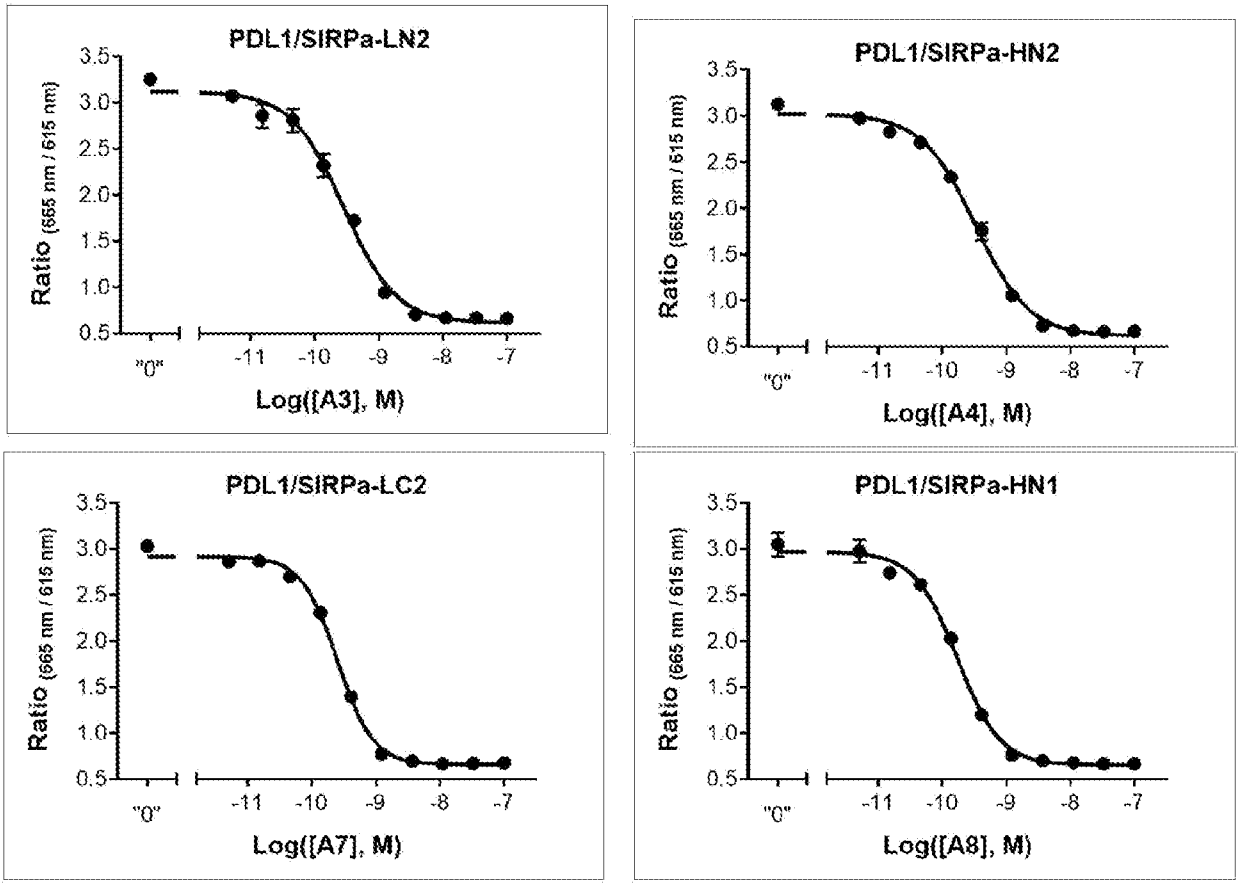

PDL1/SIRPa-LN2
Ratio (665 nm / 615 nm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
"0"
-11
-10
-9
-8
-7
Log([A3], M)
PDL1/SIRPa-HN2
Ratio (665 nm / 615 nm)
Log([A4], M)
PDL1/SIRPa-LC2
Ratio (665 nm / 615 nm)
Log([A7], M)
PDL1/SIRPa-HN1
Ratio (665 nm / 615 nm)
Log([A8], M)

FIG. 10 (cont'd)

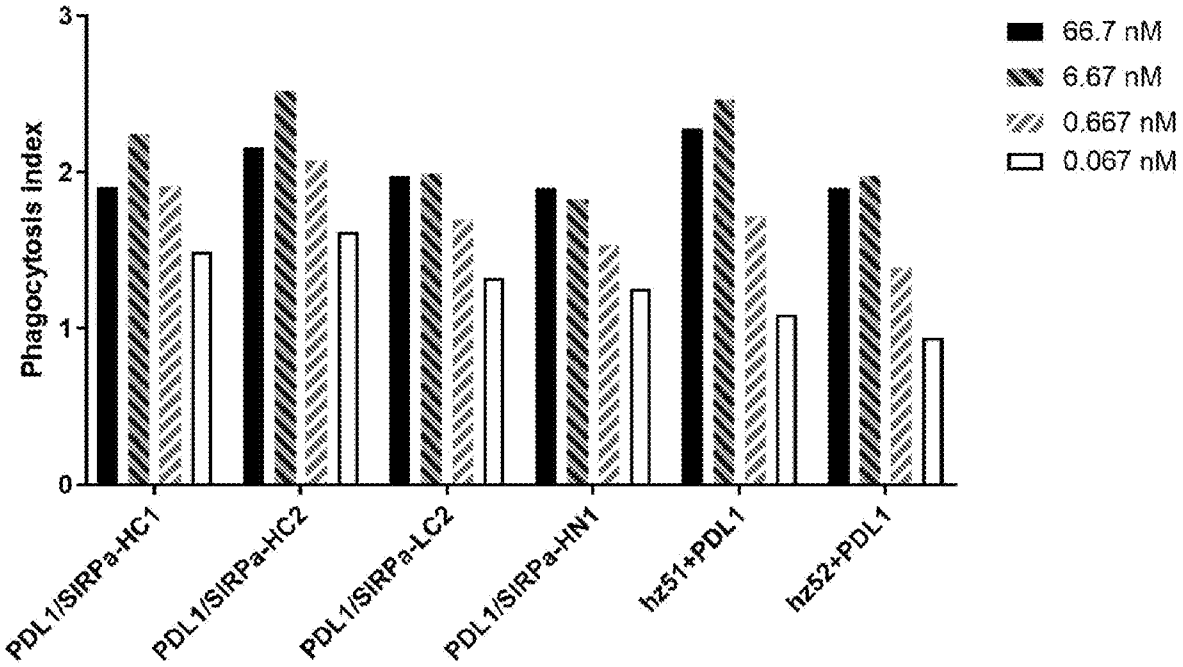

Phagocytosis index
3
2
1
0
66.7 nM
6.67 nM
0.667 nM
0.067 nM
PDL1/SIRPa-HC1
PDL1/SIRPa-HC2
PDL1/SIRPa-LC2
PDL1/SIRPa-HN1
hz51+PDL1
hz52+PDL1

FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2021/139115

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/46(2006.01)i; C12N 15/13(2006.01)i; A61K 39/395(2006.01)i; A61P 35/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; C12N; A61K; A61P

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

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

VEN,CNABS, PubMed, DWPI, CNTXT, WOTXT, USTXT, EPTXT, JPTXT, CNKI, Web of Science, baidu, Patentics, NCBI Genbank, EBI, STN: bispecific, antibod+, sirpα,pd-l, CDR, sdAb, Fab, SEQ ID NO: 1-14

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIU, X.J. et al. "Dual Targeting of Innate and Adaptive Checkpoints on Tumor Cells Limits Immune Evasion" *CELL REPORTS*, Vol. 24, 21 August 2018 (2018-08-21), pages 2101–2111	1-4, 19-22
X	US 2018311348 A1 (LUDWIG-MAXIMILIANS-UNIV. MUNCHEN) 01 November 2018 (2018-11-01) claims 1 and 7	1-4, 19-22
A	WO 2020057225 A1 (SYNBIO TECHNOLOGIES, LLC) 26 March 2020 (2020-03-26) the whole document	1-22
A	US 2020297842 A1 (ARCH ONCOLOGY, INC.) 24 September 2020 (2020-09-24) the whole document	1-22
A	CN 109897111 A (HANGZHOU HANSI BIOLOGICAL MEDICINE CO., LTD.) 18 June 2019 (2019-06-18) the whole document	1-22
A	CN 109970860 A (INNOVENT BIOPHARMACEUTICAL SUZHOU CO., LTD.) 05 July 2019 (2019-07-05) the whole document	1-22

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 February 2022	**15 March 2022**
Name and mailing address of the ISA/CN **National Intellectual Property Administration, PRC** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing** **100088, China** Facsimile No. **(86-10)62019451**	Authorized officer **XI,Jing** Telephone No. **86-(10)-53961974**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2021/139115

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WANG, Y. et al. "Tumor-selective blockade of CD47 signaling with a CD47/PD-L1 bispecific antibody for enhanced anti-tumor activity and limited toxicity" *CANCER IMMUNOLOGY, IMMUNOTHERAPY,* Vol. 70, 06 August 2020 (2020-08-06), pages 365–376	1-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2021/139115

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed:
      ☑ in the form of an Annex C/ST.25 text file.
      ☐ on paper or in the form of an image file.
   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2021/139115

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

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

1. ☑ Claims Nos.: **20 and 22**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Claims 20 and 22(partial) do not warrant an international search according to the criteria set out in PCT Rule 39.1(iv). The search has been carried out and based on the use of the antibody for manufacturing of a medicament for the treatment of cancer.

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

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2021/139115

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
US	2018311348	A1	01 November 2018	EP	3165536	A1	10 May 2017
				EP	3699197	A1	26 August 2020
				US	11229700	B2	25 January 2022
				AU	2016353468	A1	26 April 2018
				CA	3000597	A1	18 May 2017
				WO	2017081101	A1	18 May 2017
				EP	3374394	A1	19 September 2018
				EP	3374394	B1	20 October 2021
WO	2020057225	A1	26 March 2020	CN	109053891	A	21 December 2018
US	2020297842	A1	24 September 2020	CN	113573728	A	29 October 2021
				CA	3119748	A1	22 May 2020
				WO	2020102422	A1	22 May 2020
				US	11202828	B2	21 December 2021
				AU	2019380503	A1	03 June 2021
				EP	3880239	A1	22 September 2021
				BR	112021009325	A2	14 September 2021
CN	109897111	A	18 June 2019	None			
CN	109970860	A	05 July 2019	AU	2018393424	A1	14 May 2020
				CA	3081117	A1	04 July 2019
				TW	201927824	A	16 July 2019
				KR	20200104305	A	03 September 2020
				BR	112020012782	A2	01 December 2020
				JP	2021507720	A	25 February 2021
				EP	3733715	A1	04 November 2020
				EP	3733715	A4	15 September 2021
				IL	275055	D0	30 July 2020
				SG	11202003782X	A	28 May 2020