MULTIVALENT AND MULTISPECIFIC OX40-BINDING FUSION PROTEINS

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/277,027, filed January 11, 2016; the contents of each of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] This invention relates generally to molecules that specifically engage OX40, a member of the TNF receptor superfamily (TNFRSF). More specifically this invention relates to multivalent and multispecific molecules that bind at least OX40.

BACKGROUND OF THE INVENTION

[0003] The tumor necrosis factor receptor superfamily consists of several structurally related cell surface receptors. Activation by multimeric ligands is a common feature of many of these receptors. Many members of the TNFRSF have therapeutic utility in numerous pathologies if activated properly. Agonism of this receptor family often requires higher order clustering and conventional bivalent antibodies are not ideal for this. Therefore, there exists a therapeutic need for more potent agonist molecules of the TNFRSF.

SUMMARY OF THE INVENTION

[0004] The disclosure provides multivalent and multispecific TNF receptor superfamily (TNFRSF) binding fusion polypeptides that bind at least OX40 (also known as tumor necrosis factor receptor superfamily, member 4 (TNFRSF4) and/or CD134)). The use of the term "OX40" is intended to cover any variation thereof, such as, by way of non-limiting example, OX-40, and all variations are used herein interchangeably. These molecules that bind at least OX40 are referred to herein as "OX40-targeting molecules" or "OX40-targeting fusions" or "OX40-targeting proteins" or "OX40-targeting fusion polypeptides" or "OX40-targeting fusion proteins." In some embodiments, the OX40-

targeting molecule is a multivalent molecule, for example, a multivalent OX40-targeting fusion protein. In some embodiments, the OX40-targeting molecule is a multispecific molecule, for example, a multispecific OX40-targeting fusion protein. In some embodiments, the OX40-targeting molecule is a multivalent and multispecific molecule, for example, a multivalent and multispecific OX40-targeting fusion protein. As used herein, the term "fusion protein" or "fusion polypeptide" or "OX40-targeting fusion protein" or "OX40-targeting fusion polypeptide," unless otherwise specifically denoted, refers to any fusion protein embodiment of the disclosure, including, but not limited to, multivalent fusion proteins, multispecific fusion proteins, or multivalent and multispecific fusion proteins.

[0005] The disclosure also provides multivalent and multispecific fusion polypeptides that bind at least programmed death ligand 1 (PDL1), also known as PD-L1, CD274, B7 homolog 1 and/or B7-H1. The use of the term "PDL1" is intended to cover any variation thereof, such as, by way of non-limiting example, PD-L1 and/or PDL-1, all variations are used herein interchangeably. These molecules that bind at least PDL1 are referred to herein as "PDL1-targeting molecules" or "PDL1-targeting fusions" or "PDL1-targeting proteins" or "PDL1-targeting fusion polypeptides" or "PDL1-targeting fusion proteins." In some embodiments, the PDL1-targeting molecule is a multivalent molecule, for example, a multivalent PDL1-targeting fusion protein. In some embodiments, the PDL1-targeting molecule is a multispecific molecule, for example, a multispecific PDL1-targeting fusion protein. In some embodiments, the PDL1-targeting molecule is a multivalent and multispecific molecule, for example, a multivalent and multispecific PDL1-targeting fusion protein. As used herein, the term "fusion protein" or "fusion polypeptide" or "PDL1-targeting fusion protein" or "PDL1-targeting fusion polypeptide," unless otherwise specifically denoted, refers to any fusion protein embodiment of the disclosure, including, but not limited to, multivalent fusion proteins, multispecific fusion proteins, or multivalent and multispecific fusion proteins.

[0006] The disclosure also provides multivalent and multispecific fusion polypeptides that bind at least PDL1 and OX40. These molecules that bind at least PDL1 are referred to herein as "PDL1xOX40-targeting molecules" or "PDL1xOX40-targeting fusions" or "PDL1xOX40-targeting proteins" or "PDL1xOX40-targeting fusion polypeptides" or "PDL1xOX40-targeting fusion proteins." In some embodiments, the PDL1xOX40-targeting molecule is a multivalent molecule, for example, a multivalent

PDL1xOX40-targeting fusion protein. In some embodiments, the PDL1xOX40-targeting molecule is a multispecific molecule, for example, a multispecific PDL1xOX40-targeting fusion protein. In some embodiments, the PDL1xOX40-targeting molecule is a multivalent and multispecific molecule, for example, a multivalent and multispecific PDL1-targeting fusion protein. As used herein, the term "fusion protein" or "fusion polypeptide" or "PDL1xOX40-targeting fusion protein" or "PDL1xOX40-targeting fusion polypeptide," unless otherwise specifically denoted, refers to any fusion protein embodiment of the disclosure, including, but not limited to, multivalent fusion proteins, multispecific fusion proteins, or multivalent and multispecific fusion proteins.

[0007] Conventional antibodies targeting members of the TNF receptor superfamily (TNFRSF) have been shown to require exogenous crosslinking to achieve sufficient agonist activity, as evidenced by the necessity for Fc-gamma Receptor (FcγRs) for the activity of antibodies to DR4, DR5, GITR and OX40 (Ichikawa *et al* 2001 al Nat. Med. 7, 954–960, Li *et al* 2008 Drug Dev. Res. 69, 69–82; Pukac et al 2005 Br. J. Cancer 92, 1430–1441; Yanda *et al* 2008 Ann. Oncol. 19, 1060–1067; Yang *et al* 2007 Cancer Lett. 251:146–157; Bulliard *et al* 2013 JEM 210(9): 1685; Bulliard *et al* 2014 Immunol and Cell Biol 92: 475-480). In addition to crosslinking via FcγRs other exogenous agents including addition of the oligomeric ligand or antibody binding entities (e.g. protein A and secondary antibodies) have been demonstrated to enhance anti-TNFRSF antibody clustering and downstream signaling. For example, the addition of the DR5 ligand TRAIL enhanced the apoptosis inducing ability of an anti-DR5 antibody (Graves *et al* 2014 Cancer Cell 26: 177-189). These findings suggest the need for clustering of TNFRSFs beyond a dimer.

[0008] The present disclosure provides multivalent TNFRSF binding fusion proteins, which comprise 2 or more TNFRSF binding domains (TBDs) where at least one TBD binds OX40. In some embodiments, the fusion proteins of the present disclosure have utility in treating neoplasms.

[0009] In some embodiments, the fusion protein contains two or more different TBDs, where each TBD binds OX40. In some embodiments, the fusion protein contains multiple copies of a TBD that binds OX40. For example, in some embodiments, the fusion protein contains at least two copies of a TBD that binds OX40. In some embodiments, the fusion protein contains at least three copies of a TBD that binds OX40. In some embodiments, the fusion protein contains at least four copies of a TBD that binds OX40. In some embodiments, the fusion protein contains at least five copies of a TBD that binds

OX40. In some embodiments, the fusion protein contains at least six copies of a TBD that binds OX40. In some embodiments, the fusion protein contains six or more copies of a TBD that binds OX40.

[0010] In other embodiments, the fusion proteins of the present disclosure bind OX40 and a second TNFRSF member such as, for example, GITR, CD137, CD27, TNFR2, and/or CD40. In these embodiments, the fusion proteins of the present disclosure modulate immune cells leading to enhanced tumor destruction. In other embodiments, the fusion proteins of the present disclosure have utility in treating inflammatory conditions. In these embodiments, the fusion proteins of the present disclosure modulate immune cells leading to dampening of the inflammatory insult. For example, specifically agonizing TNFR2 can enhance Treg proliferation leading to immune suppression.

[0011] The fusion proteins of the present disclosure are capable of enhanced clustering of TNFRSF members compared to non-cross-linked bivalent antibodies. The enhanced clustered of TNFRSF members mediated by the fusion proteins of the present disclosure induce enhanced TNFRSF-dependent signaling compared to non-cross-linked bivalent antibodies. In most embodiments, the fusion protein will incorporate more than 2 TBDs, for example, three, four, five, or six.

[0012] In some embodiments, the fusion proteins are multispecific containing a TBD and a binding domain directed toward a second antigen. In these, embodiments, the binding to the second antigen is capable of providing the additional crosslinking function and TNFRSF activation can be achieved with only one or two TBDs. In these embodiments, the TNFRSF signaling is enhanced and focused by the presence of the second antigen. These multispecific TBD containing fusion proteins are useful means to achieve conditional signaling of a given TNFRSF member.

[0013] The present disclosure provides isolated polypeptides that specifically bind OX40. In some embodiments, the isolated polypeptide is derived from antibodies or antibody fragments including scFv, Fabs, single domain antibodies (sdAb), V_{NAR}, or VHHs. In some embodiments, the isolated polypeptide is human or humanized sdAb. The sdAb fragments can be derived from VHH, V_{NAR}, engineered VH or VK domains. VHHs can be generated from camelid heavy chain only antibodies. V_{NARS} can be generated from cartilaginous fish heavy chain only antibodies. Various methods have been implemented to generate monomeric sdAbs from conventionally heterodimeric VH and VK domains, including interface engineering and selection of specific germline families. In other

embodiments, the isolated polypeptides are derived from non-antibody scaffold proteins for example but not limited to designed ankyrin repeat proteins (darpins), avimers, anticalin/lipocalins, centyrins and fynomers.

[0014] In some embodiments, the isolated polypeptide includes an amino acid sequence selected from the group consisting of SEQ ID NO: 16-29 and 377-386.

[0015] In some embodiments, the isolated polypeptide includes an amino acid sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 16-29 and 377-386.

[0016] In some embodiments, the isolated polypeptide comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 30, 36, and 44; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 31, 35, and 37; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 32-34, 48-43, and 45.

[0017] The present disclosure provides multivalent fusion proteins, which comprise two or more binding domains (BDs) where at least one BD binds PDL1. In some embodiments, the fusion proteins of the present disclosure have utility in treating neoplasms.

[0018] In some embodiments, the fusion protein contains two or more different BDs, where each BD binds PDL1. In some embodiments, the fusion protein contains multiple copies of a BD that binds PDL1. For example, in some embodiments, the fusion protein contains at least two copies of a BD that binds PDL1. In some embodiments, the fusion protein contains at least three copies of a BD that binds PDL1. In some embodiments, the fusion protein contains at least four copies of a BD that binds PDL1. In some embodiments, the fusion protein contains at least five copies of a BD that binds PDL1. In some embodiments, the fusion protein contains at least six copies of a BD that binds PDL1. In some embodiments, the fusion protein contains six or more copies of a BD that binds PDL1.

[0019] The present disclosure provides isolated polypeptides that specifically bind OX40. In some embodiments, the isolated polypeptide is derived from antibodies or antibody fragments including scFv, Fabs, single domain antibodies (sdAb), V_{NAR}, or VHHs. In some embodiments, the isolated polypeptide is human or humanized sdAb. The sdAb

fragments can be derived from VHH, V_{NAR}, engineered VH or VK domains. VHHs can be generated from camelid heavy chain only antibodies. V_{NARS} can be generated from cartilaginous fish heavy chain only antibodies. Various methods have been implemented to generate monomeric sdAbs from conventionally heterodimeric VH and VK domains, including interface engineering and selection of specific germline families. In other embodiments, the isolated polypeptides are derived from non-antibody scaffold proteins for example but not limited to designed ankyrin repeat proteins (darpins), avimers, anticalin/lipocalins, centyrins and fynomers.

[0020] In some embodiments, the isolated polypeptide includes an amino acid sequence selected from the group consisting of SEQ ID NO: 46-57. In some embodiments, the isolated polypeptide includes an amino acid sequence selected from the group consisting of SEQ ID NO: 52-57.

[0021] In some embodiments, the isolated polypeptide includes an amino acid sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 46-57. In some embodiments, the isolated polypeptide includes an amino acid sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 52-57.

[0022] In some embodiments, the isolated polypeptide comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 58, 61, and 64; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 59, 62, 65, and 69; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 60, 63, 66-68, and 70.

[0023] In some embodiments, the present disclosure provides isolated polypeptides that specifically bind at least OX40 and PDL1. In some embodiments, each binding domain (BD) in the isolated polypeptide is derived from antibodies or antibody fragments including scFv, Fabs, single domain antibodies (sdAb), V_{NAR}, or VHHs. In some embodiments, each BD is human or humanized sdAb. The sdAb fragments can be derived from VHH, V_{NAR}, engineered VH or VK domains. VHHs can be generated from camelid heavy chain only antibodies. V_{NARS} can be generated from cartilaginous fish heavy chain only antibodies. Various methods have been implemented to generate monomeric sdAbs from

conventionally heterodimeric VH and VK domains, including interface engineering and selection of specific germline families. In other embodiments, the isolated polypeptides are derived from non-antibody scaffold proteins for example but not limited to designed ankyrin repeat proteins (darpins), avimers, anticalin/lipocalins, centyrins and fynomers.

[0024] In some embodiments, the isolated polypeptide includes a first amino acid sequence that binds 4B11 selected from the group consisting of SEQ ID NO: 16-29 and 377-386, and a second amino acid sequence that binds PDL1 selected from the group consisting of SEQ ID NO: 46-57.

[0025] In some embodiments, the isolated polypeptide includes a first amino acid sequence that binds 4B11 selected from the group consisting of SEQ ID NO: 16-29 and 377-386, and a second amino acid sequence that binds PDL1 selected from the group consisting of SEQ ID NO: 52-57.

[0026] In some embodiments, the isolated polypeptide includes a first amino acid sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence that binds 4B11 selected from the group consisting of SEQ ID NO: 16-29 and 377-386, and a second amino acid sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence that binds PDL1 selected from the group consisting of SEQ ID NO: 46-57.

[0027] In some embodiments, the isolated polypeptide includes a first amino acid sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence that binds 4B11 selected from the group consisting of SEQ ID NO: 16-29 and 377-386, and a second amino acid sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence that binds PDL1 selected from the group consisting of SEQ ID NO: 52-57.

[0028] In some embodiments, the isolated polypeptide includes (i) a first amino acid sequence that binds 4B11 and comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 30, 36, and 44; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 31, 35, and 37; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 32-34, 48-43, and 45; and (ii) a second amino

acid sequence that binds PDL1 and comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 58, 61, and 64; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 59, 62, 65, and 69; and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 60, 63, 66-68, and 70.

[0029] In some embodiments, the binding domains (BDs) of the present disclosure are derived from antibodies or antibody fragments including scFv, Fabs, single domain antibodies (sdAb), V_{NAR}, or VHHs. In some embodiments, the BDs are human or humanized sdAb. The sdAb fragments, can be derived from VHH, V_{NAR}, engineered VH or VK domains. VHHs can be generated from camelid heavy chain only antibodies. V_{NAR}s can be generated from cartilaginous fish heavy chain only antibodies. Various methods have been implemented to generate monomeric sdAbs from conventionally heterodimeric VH and VK domains, including interface engineering and selection of specific germline families. In other embodiments, the DBs are derived from non-antibody scaffold proteins for example, but not limited to designed ankyrin repeat proteins (darpins), avimer, anticalin/lipocalins, centyrins and fynomers.

[0030] Generally, the fusion proteins of the present disclosure consist of at least two or more BDs operably linked via a linker polypeptide. The utilization of sdAb fragments as the specific BD within the fusion protein of the present disclosure has the benefit of avoiding the heavy chain : light chain mis-pairing problem common to many bi/multispecific antibody approaches. In addition, the fusion proteins of the present disclosure avoid the use of long linkers necessitated by many bispecific antibodies.

[0031] In some embodiments, all of the TBDs of the fusion protein recognize the same epitope on the given TNFRSF member. For example, the fusion proteins of present disclosure may incorporate 2, 3, 4, 5, or 6 TBDs with identical specificity to OX40. In other embodiments, the fusion protein incorporates TBDs that recognize distinct epitopes on the given TNFRSF member. For example, the fusion proteins of present disclosure may incorporate 2, 3, 4, 5, or 6 TBDs with distinct recognition specificities toward various epitopes on OX40. In these embodiments, the fusion proteins of the present disclosure contain multiple TBDs that target distinct regions of the particular TNFRSF member. In some embodiments, the TBDs may recognize different epitopes on the same TNFRSF member or recognize epitopes on distinct TNFRSF members. For example, the present

disclosure provides multispecific fusion proteins incorporating TBDs that bind GITR and OX40 or CD137 and OX40.

[0032] In some embodiments, all of the BDs of the fusion protein recognize the same epitope on PDL1. For example, the fusion proteins of present disclosure may incorporate 2, 3, 4, 5, or 6 BDs with identical specificity to PDL1. In other embodiments, the fusion protein incorporates BDs that recognize distinct epitopes on PDL1. For example, the fusion proteins of present disclosure may incorporate 2, 3, 4, 5, or 6 BDs with distinct recognition specificities toward various epitopes on PDL1. In these embodiments, the fusion proteins of the present disclosure with contain multiple BDs that target distinct regions of the PDL1. In some embodiments, the BDs may recognize different epitopes on PDL1.

[0033] In some embodiments, the multispecific fusion protein is a bispecific molecule that targets OX40 and PDL1 and comprises an amino acid sequence that is selected from the group consisting of SEQ ID NO: 387-394.

[0034] In some embodiments, the multispecific fusion protein is a bispecific molecule that targets OX-40 and PDL1 and comprises an amino acid sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 387-394.

[0035] In some embodiments, the fusion protein of the present disclosure is composed of a single polypeptide. In other embodiments, the fusion protein of the present disclosure is composed of more than one polypeptide. For example, wherein a heterodimerization domain is incorporated into the fusion protein so as the construct an asymmetric fusion protein. For example, if an immunoglobulin Fc region is incorporated into the fusion protein the CH3 domain can be used as a homodimerization domain, or the CH3 dimer interface region can be mutated so as to enable heterodimerization.

[0036] In some embodiments, the fusion protein contains the BDs at opposite ends. For example, the BDs are located on both the amino-terminal (N-terminal) portion of the fusion protein and the carboxy-terminal (C-terminal) portion of the fusion protein. In other embodiments, all the TBDs reside on the same end of the fusion protein. For example, BDs reside on either the amino- or carboxy-terminal portions of the fusion protein.

[0037] In some embodiments, the linker polypeptide contains an immunoglobulin Fc region. In some embodiments, the immunoglobulin Fc region is an IgG isotype selected from the group consisting of IgG1 isotype, IgG2 isotype, IgG3 isotype, and IgG4 isotype.

[0038] In some embodiments, the immunoglobulin Fc region or immunologically active fragment thereof is an IgG isotype. For example, the immunoglobulin Fc region of the fusion protein is of human IgG1 isotype, having an amino acid sequence:

PAP**ELL**GGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV
EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
EALHNHYTQK SLSLSPGK (SEQ ID NO: 1)

[0039] In some embodiments, the immunoglobulin Fc region or immunologically active fragment thereof comprises a human IgG1 polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 1.

[0040] In some embodiments, the human IgG1 Fc region is modified at amino acid Asn297 (Boxed in SEQ ID NOs: 1-4, Kabat Numbering) to prevent to glycosylation of the fusion protein, *e.g.*, Asn297Ala (N297A) or Asn297Asp (N297D). In some embodiments, the Fc region of the fusion protein is modified at amino acid Leu235 (Bold in SEQ ID NO: 1, Kabat Numbering) to alter Fc receptor interactions, *e.g.*, Leu235Glu (L235E) or Leu235Ala (L235A). In some embodiments, the Fc region of the fusion protein is modified at amino acid Leu234 (Bold in SEQ ID NO: 1, Kabat Numbering) to alter Fc receptor interactions, *e.g.*, modified at amino acid Leu234 (Boxed, Kabat Numbering) to alter Fc receptor interactions, *e.g.*, Leu235Glu (L235E). In some embodiments, the Fc region of the fusion protein is Leu234Ala (L234A). In some embodiments, the Fc region of the fusion protein is altered at both amino acid 234 and 235, *e.g.*, Leu234Ala and Leu235Ala (L234A/L235A) or Leu234Val and Leu235Ala (L234V/L235A). In some embodiments, the Fc region of the fusion protein is lacking an amino acid at one or more of the following positions to reduce Fc receptor binding: Glu233 (E233, Bold in SEQ ID NO: 1), Leu234 (L234), or Leu235 (L235). In some embodiments, the Fc region of the fusion protein is altered at Gly235 to reduce Fc receptor binding. For example, wherein Gly235 is deleted from the fusion protein. In some embodiments, the human IgG1 Fc region is modified at amino acid Gly236 (Boxed in SEQ ID NO: 1) to enhance the interaction with CD32A, e.g.,

Gly236Ala (G236A). In some embodiments, the human IgG1 Fc region lacks Lys447 (EU index of *Kabat et al* 1991 *Sequences of Proteins of Immunological Interest*).

[0041] In some embodiments, the Fc region of the fusion protein is altered at one or more of the following positions to reduce Fc receptor binding: Leu 234 (L234), Leu235 (L235), Asp265 (D265), Asp270 (D270), Ser298 (S298), Asn297 (N297), Asn325 (N325) orAla327 (A327). For example, Leu 234Ala (L234A), Leu235Ala (L235A), Asp265Asn (D265N), Asp270Asn (D270N), Ser298Asn (S298N), Asn297Ala (N297A), Asn325Glu (N325E) orAla327Ser (A327S). In preferred embodiments, modifications within the Fc region reduce binding to Fc-receptor-gamma receptors while have minimal impact on binding to the neonatal Fc receptor (FcRn).

[0042] In some embodiments, the Fc region of the fusion protein is lacking an amino acid at one or more of the following positions to reduce Fc receptor binding: Glu233 (E233), Leu234 (L234), or Leu235 (L235). In these embodiments, Fc deletion of these three amino acids reduces the complement protein C1q binding. These modified Fc region polypeptides are referred to herein as "Fc deletion" polypeptides.

PAPGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
EKTISKAKGQ PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE
SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL
HNHYTQKSLS LSPGK (SEQ ID NO: 2)

[0043] In some embodiments, the immunoglobulin Fc region or immunologically active fragment thereof comprises a human IgG1 polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 2.

[0044] In some embodiments, the immunoglobulin Fc region or immunologically active fragment of the fusion protein is of human IgG2 isotype, having an amino acid sequence:

PAPPVAGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFNWYVD
GVEVHNAKTK PREEQFNSTF RVVSVLTVVH QDWLNGKEYK CKVSNKGLPA
PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDISVE

WESNGQPENN YKTTPPMLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK (SEQ ID NO: 3)

[0045] In some embodiments, the fusion or immunologically active fragment thereof comprises a human IgG2 polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 3.

[0046] In some embodiments, the human IgG2 Fc region is modified at amino acid Asn297 (Boxed in SEQ ID NOs: 1, 3, 4, and 5, to prevent to glycosylation of the antibody, *e.g.*, Asn297Ala (N297A). In some embodiments, the human IgG2 Fc region lacks Lys447, which corresponds to residue 217 of SEQ ID NO: 3 (EU index of *Kabat et al* 1991 *Sequences of Proteins of Immunological Interest*).

[0047] In some embodiments, the immunoglobulin Fc region or immunologically active fragment of the fusion protein is of human IgG3 isotype, having an amino acid sequence:

PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFKWYV DGVEVHNAKT KPREEQYNST FRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESSGQPEN NYNTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNIFSCSVMH EALHNRFTQK SLSLSPGK (SEQ ID NO: 4)

[0048] In some embodiments, the antibody or immunologically active fragment thereof comprises a human IgG3 polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 4.

[0049] In some embodiments, the human IgG3 Fc region is modified at amino acid Asn297 (Boxed in SEQ ID NOs: 1-4, Kabat Numbering) to prevent to glycosylation of the antibody, *e.g.*, Asn297Ala (N297A). In some embodiments, the human IgG3 Fc region is modified at amino acid 435 to extend the half-life, *e.g.*, Arg435His (R435H, Boxed in SEQ ID NO: 3). In some embodiments, the human IgG3 Fc region lacks Lys447, which corresponds to residue of 218 SEQ ID NO: 4 (EU index of *Kabat et al* 1991 *Sequences of Proteins of Immunological Interest*).

[0050] In some embodiments, the immunoglobulin Fc region or immunologically active fragment of the fusion protein is of human IgG4 isotype, having an amino acid sequence:

PAPEF[L]GGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV
DGVEVHNAKT KPREEQF[N]ST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP
SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV
EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH
EALHNHYTQK SLSLSLGK (SEQ ID NO: 5)

[0051] In some embodiments, the antibody or immunologically active fragment thereof comprises a human IgG4 polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 5.

[0052] In other embodiments, the human IgG4 Fc region is modified at amino acid 235 to alter Fc receptor interactions, *e.g.*, Leu235Glu (L235E). In some embodiments, the human IgG4 Fc region is modified at amino acid Asn297 (Boxed in SEQ ID NOs: 1-4, Kabat Numbering) to prevent to glycosylation of the antibody, *e.g.*, Asn297Ala (N297A). In some embodiments, the human IgG4 Fc region lacks Lys447, which corresponds to residue 218 of SEQ ID NO: 4 (EU index of *Kabat et al* 1991 *Sequences of Proteins of Immunological Interest*).

[0053] In some embodiments, the immunoglobulin Fc region or immunologically active fragment of the fusion protein is of human IgG4 isotype, having an amino acid sequence:

PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV
DGVEVHNAKT KPREEQF[N]ST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP
SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV
EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH
EALHNHYTQK SLSLSLGK (SEQ ID NO: 6)

[0054] In some embodiments, the antibody or immunologically active fragment thereof comprises a human IgG4 polypeptide sequence that is at least 50%, 60%, 65%,

70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 6.

[0055] In some embodiments, the human IgG Fc region is modified to enhance FcRn binding. Examples of Fc mutations that enhance binding to FcRn are Met252Tyr, Ser254Thr, Thr256Glu (M252Y, S254T, T256E, respectively) (Kabat numbering, Dall'Acqua *et al* 2006, *J. Biol Chem* Vol. 281(33) 23514–23524), Met428Leu and Asn434Ser (M428L, N434S) (Zalevsky *et al* 2010 *Nature Biotech*, Vol. 28(2) 157-159), or Met252Ile, Thr256Asp, Met428Leu (M252I, T256D, M428L, respectively), (EU index of *Kabat et al* 1991 *Sequences of Proteins of Immunological Interest*). Met252 corresponds to residue 23 in SEQ ID NOs: 1, 4, and 5 and residue 22 in SEQ ID NO: 3. Ser254 corresponds to corresponds to residue 25 in SEQ ID NOs: 1, 4, and 5 and residue 24 in SEQ ID NO: 3. Thr256 corresponds to residue 27 in SEQ ID NOs: 1, 4, and 5 and residue 26 in SEQ ID NO: 3. Met428 corresponds to residue 199 in SEQ ID NOs: 1, 4, and 5 and residue 198 in SEQ ID NO: 3. Asn434 corresponds to residue 205 in SEQ ID NOs: 1, 4, and 5 and residue 204 in SEQ ID NO: 3. In some embodiments where the fusion protein of the disclosure includes an Fc polypeptide, the Fc polypeptide is mutated or modified. In these embodiments, the mutated or modified Fc polypeptide includes the following mutations: Met252Tyr and Met428Leu (M252Y, M428L) using the Kabat numbering system.

[0056] In some embodiments, the human IgG Fc region is modified to alter antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC), *e.g.*, the amino acid modifications described in Natsume et al., 2008 Cancer Res, 68(10): 3863-72; Idusogie et al., 2001 J Immunol, 166(4): 2571-5; Moore et al., 2010 mAbs, 2(2): 181-189; Lazar et al., 2006 PNAS, 103(11): 4005–4010, Shields et al., 2001 JBC, 276(9): 6591–6604; Stavenhagen et al., 2007 Cancer Res, 67(18): 8882-8890; Stavenhagen et al., 2008 Advan. Enzyme Regul., 48: 152-164; Alegre et al, 1992 J Immunol, 148: 3461-3468; Reviewed in Kaneko and Niwa, 2011 Biodrugs, 25(1):1-11. Examples of mutations that enhance ADCC include modification at Ser239 and Ile332, for example Ser239Asp and Ile332Glu (S239D, I332E). Examples of mutations that enhance CDC include modifications at Lys326, which corresponds to residue 97 of SEQ ID NOs: 1, 4, and 5 and residue 96 of SEQ ID NO: 3, and Glu333, which corresponds to residue 104 of SEQ ID NOs: 1, 4, and 5 and residue 103 of SEQ ID NO: 3. In some embodiments, the Fc region is modified at one or both of these positions, for example, Lys326Ala and/or Glu333Ala (K326A and E333A).

[0057] In some embodiments, the human IgG Fc region is modified to induce heterodimerization. For example, having an amino acid modification within the CH3 domain at Thr366, which when replaced with a more bulky amino acid, *e.g.*, Trp (T366W), is able to preferentially pair with a second CH3 domain having amino acid modifications to less bulky amino acids at positions Thr366, which corresponds to residue 137 of SEQ ID NOs: 1, 4, and 5 and residue 136 of SEQ ID NO: 3, Leu368, which corresponds to residue 139 of SEQ ID NOs: 1, 4, and 5 and residue 138 of SEQ ID NO: 3, and Tyr407, which corresponds to residue 178 of SEQ ID NOs: 1, 4, and 5 and residue 177 of SEQ ID NO: 3, *e.g.*, Ser, Ala and Val, respectively (T366S/L368A/Y407V). Heterodimerization via CH3 modifications can be further stabilized by the introduction of a disulfide bond, for example, by changing Ser354, which corresponds to residue 125 of SEQ ID NOs: 1, 4, and 5 and residue 124 of SEQ ID NO: 3, to Cys (S354C) and Tyr349, which corresponds to residue 120 of SEQ ID NOs: 1, 4, and 5 and residue 119 of SEQ ID NO: 3, to Cys (Y349C) on opposite CH3 domains (Reviewed in Carter, 2001 Journal of Immunological Methods, 248: 7–15). In some of these embodiments, the Fc region may be modified at the protein-A binding site on one member of the heterodimer so as to prevent protein-A binding and thereby enable more efficient purification of the heterodimeric fusion protein. An exemplary modification within this binding site is Ile253, which corresponds to residue 24 of SEQ ID NOs: 1, 4, and 5 and residue 23 of SEQ ID NO: 3, for example, Ile253Arg (I253R). For example, the I253R modification maybe combined with either the T366S/L368A/Y407V modifications or with the T366W modifications. The T366S/L368A/Y407V modified Fc is capable of forming homodimers as there is no steric occlusion of the dimerization interface as there is in the case of the T336W modified Fc. Therefore, in some embodiments, the I253R modification is combined with the T366S/L368A/Y407V modified Fc to disallow purification any homodimeric Fc that may have formed.

[0058] In some embodiments, the human IgG Fc region is modified to prevent dimerization. In these embodiments, the fusion proteins of the present disclosure are monomeric. For example, modification at residue Thr366 to a charged residue, e.g. Thr366Lys, Thr366Arg, Thr366Asp, or Thr366Glu (T366K, T366R, T366D, or T366E, respectively), prevents CH3-CH3 dimerization.

[0059] In some embodiments, the Fc region of the fusion protein is altered at one or more of the following positions to reduce Fc receptor binding: Leu 234 (L234), Leu235

(L235), Asp265 (D265), Asp270 (D270), Ser298 (S298), Asn297 (N297), Asn325 (N325) orAla327 (A327). For example, Leu 234Ala (L234A), Leu235Ala (L235A), Asp265Asn (D265N), Asp270Asn (D270N), Ser298Asn (S298N), Asn297Ala (N297A), Asn325Glu (N325E) orAla327Ser (A327S). In preferred embodiments, modifications within the Fc region reduce binding to Fc-receptor-gamma receptors while have minimal impact on binding to the neonatal Fc receptor (FcRn).

[0060] In some embodiments, the fusion protein contains a polypeptide derived from an immunoglobulin hinge region. The hinge region can be selected from any of the human IgG isotypes. For example, the fusion protein may contain a modified IgG1 hinge having the sequence of EPKSSDKTHTCPPC (SEQ ID NO: 7), where in the Cys220 that forms a disulfide with the C-terminal cysteine of the light chain is mutated to serine, e.g., Cys220Ser (C220S). In other embodiments, the fusion protein contains a truncated hinge having a sequence DKTHTCPPC (SEQ ID NO: 8).

[0061] In some embodiments, the fusion protein has a modified hinge from IgG4, which is modified to prevent or reduce strand exchange, *e.g.*, Ser228Pro (S228P), having the sequence ESKYGPPCPPC (SEQ ID NO: 9). In some embodiments, the fusion protein contains one or more linker polypeptides. In other embodiments, the fusion protein contains one or more linker and hinge polypeptides.

[0062] In some embodiments, the fusion proteins of the present disclosure lack or have reduced Fucose attached to the N-linked glycan-chain at N297. There are numerous ways to prevent fucosylation, including but not limited to production in a FUT8 deficient cell line; addition inhibitors to the mammalian cell culture media, for example, Castanospermine, 2-deoxy-fucose, 2-flurofucose; the use of production cell lines with naturally reduced fucosylation pathways, and metabolic engineering of the production cell line.

[0063] In some embodiments, the single domain antibody, VHH, or humanized single domain antibody, or human single domain antibody is engineered to eliminate recognition by pre-existing antibodies found in humans. In some embodiments, single domain antibodies of the present disclosure are modified by mutation of position Leu11, for example, Leu11Glu (L11E) or Leu11Lys (L11K). In other embodiments, single domain antibodies of the present disclosure are modified by changes in carboxy-terminal region, for example, the terminal sequence consists of GQGTLVTVKPGG (SEQ ID NO: 14) or GQGTLVTVEPGG (SEQ ID NO: 15) or modification thereof. In some embodiments, the

single domain antibodies of the present disclosure are modified by mutation of position 11 and by changes in carboxy-terminal region.

[0064] In some embodiments, the TBDs of the fusion proteins of the present disclosure are operably linked via amino acid linkers. In some embodiments, these linkers are composed predominately of the amino acids Glycine and Serine, denoted as GS-linkers herein. The GS-linkers of the fusion proteins of the present disclosure can be of various lengths, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 amino acids in length.

[0065] In some embodiments, the GS-linker comprises an amino acid sequence selected from the group consisting of GGSGGS, i.e., $(GGS)_2$ (SEQ ID NO: 10); GGSGGSGGS, i.e., $(GGS)_3$ (SEQ ID NO: 11); GGSGGSGGSGGS, i.e., $(GGS)_4$ (SEQ ID NO: 12); and GGSGGSGGSGGSGGS, i.e., $(GGS)_5$ (SEQ ID NO: 13).

[0066] In some embodiments, the multivalent binding fusion protein is tetravalent. In some embodiments, the tetravalent fusion protein has the following structure: BD-Linker-BD-Linker-Hinge-Fc. In some embodiments, the tetravalent fusion protein has the following structure: BD-Linker-Hinge-Fc-Linker-BD.

[0067] In some embodiments, the BD of the tetravalent fusion protein is a single domain antibody or VHH. In some embodiments, each BD of the tetravalent fusion protein is a single domain antibody or VHH. In some embodiments, the tetravalent fusion protein has the following structure: VHH-Linker-VHH-Linker-Hinge-Fc, where the VHH is a humanized or fully human VHH sequence. In some embodiments, the tetravalent fusion protein has the following structure: VHH-Linker-Hinge-Fc-Linker-VHH, where the VHH is a humanized or fully human VHH sequence.

[0068] In some embodiments, the multivalent TNFRSF binding fusion protein is tetravalent. In some embodiments, the tetravalent TNFRSF binding fusion protein has the following structure: TBD-Linker-TBD-Linker-Hinge-Fc. In some embodiments, the tetravalent TNFRSF binding fusion protein has the following structure: TBD-Linker-Hinge-Fc-Linker-TBD.

[0069] In some embodiments, the TBD of the tetravalent TNFRSF binding fusion protein is a single domain antibody or VHH. In some embodiments, each TBD of the multivalent TNFRSF binding fusion protein is single domain antibody or VHH. In some embodiments, the multivalent TNFRSF binding fusion protein is tetravalent. In some embodiments, the tetravalent TNFRSF binding fusion protein has the following structure:

VHH-Linker-VHH-Linker-Hinge-Fc, where the VHH is a humanized or fully human VHH sequence. In some embodiments, the tetravalent TNFRSF binding fusion protein has the following structure: VHH-Linker-Hinge-Fc-Linker-VHH, where the VHH is a humanized or fully human VHH sequence.

[0070] In some embodiments, the GS-linker comprises an amino acid sequence selected from the group consisting of GGSGGS, i.e., $(GGS)_2$ (SEQ ID NO: 10); GGSGGSGGS, i.e., $(GGS)_3$ (SEQ ID NO: 11); GGSGGSGGSGGS, i.e., $(GGS)_4$ (SEQ ID NO: 12); and GGSGGSGGSGGSGGS, i.e., $(GGS)_5$ (SEQ ID NO: 13).

[0071] In some embodiments, the TBD of the tetravalent TNFRSF binding fusion protein is a single domain antibody or VHH. In some embodiments, each TBD of the multivalent TNFRSF binding fusion protein is single domain antibody or VHH. In some embodiments, the multivalent fusion protein is hexavalent. In some embodiments, the hexavalent fusion protein has the following structure: BD-Linker-TBD-Linker-BD-Linker-Hinge-Fc. In some embodiments, the hexavalent fusion protein has the following structure: BD-Linker-BD-Linker-Hinge-Fc-Linker-BD, or BD-Linker-Hinge-Fc-Linker-BD-Linker-BD.

[0072] In some embodiments, the BD of the hexavalent fusion protein is a single domain antibody or VHH. In some embodiments, each BD of the hexavalent fusion protein is a single domain antibody or VHH. In some embodiments, the hexavalent fusion protein has the following structure: VHH-Linker-VHH-Linker-VHH-Linker-Hinge-Fc, where the VHH is a humanized or fully human VHH sequence. In some embodiments, the hexavalent fusion protein has the following structure: VHH-Linker-VHH-Linker-Hinge-Fc-Linker-VHH, or VHH-Linker-Hinge-Fc-Linker-VHH-Linker-VHH where the VHH is a humanized or fully human VHH sequence.

[0073] In some embodiments, the multivalent TNFRSF binding fusion protein is hexavalent. In some embodiments, the hexavalent TNFRSF binding fusion protein has the following structure: TBD-Linker-TBD-Linker-TBD-Linker-Hinge-Fc. In some embodiments, the hexavalent TNFRSF binding fusion protein has the following structure: TBD-Linker-TBD-Linker-Hinge-Fc-Linker-TBD, or TBD-Linker-Hinge-Fc-Linker-TBD-Linker-TBD.

[0074] In some embodiments, the multivalent TNFRSF binding fusion protein is hexavalent. In some embodiments, the hexavalent TNFRSF binding fusion protein has the following structure: VHH-Linker-VHH-Linker-VHH-Linker-Hinge-Fc, where the VHH is a

humanized or fully human VHH sequence. In some embodiments, the hexavalent TNFRSF binding fusion protein has the following structure: VHH-Linker-VHH-Linker-Hinge-Fc-Linker-VHH, or VHH-Linker-Hinge-Fc-Linker-VHH-Linker-VHH where the VHH is a humanized or fully human VHH sequence.

[0075] In some embodiments, the multivalent fusion protein lacks an Fc region. In some of these embodiments, the fusion protein is tetravalent and has the following structure BD-Linker-BD-Linker-BD-Linker-BD-Linker. In some of these embodiments, the fusion protein is pentavalent and has the following structure BD-Linker-BD-Linker-BD-Linker-BD-Linker-BD. In some of these embodiments, the fusion protein is hexavalent and has the following structure BD-Linker-BD-Linker-BD-Linker-BD-Linker-BD-Linker-BD.

[0076] In some embodiments, the multivalent TNFRSF binding fusion protein lacks an Fc region. In some of these embodiments, the TNFRSF binding fusion protein is tetravalent and has the following structure TBD-Linker-TBD-Linker-TBD-Linker-TBD-Linker. In some of these embodiments, the TNFRSF binding fusion protein is pentavalent and has the following structure TBD-Linker-TBD-Linker-TBD-Linker-TBD-Linker-TBD. In some of these embodiments, the TNFRSF binding fusion protein is hexavalent and has the following structure TBD-Linker-TBD-Linker-TBD-Linker-TBD-Linker-TBD-Linker-TBD.

[0077] In some embodiments, the BD of a multivalent fusion protein is a single domain antibody or VHH. In some embodiments, the multivalent fusion protein lacks an Fc region. In some of these embodiments, the fusion protein is tetravalent and has the following structure VHH-Linker-VHH-Linker-VHH-Linker-VHH-Linker. In some of these embodiments, the fusion protein is pentavalent and has the following structure VHH-Linker-VHH-Linker-VHH-Linker-VHH-Linker-VHH. In some of these embodiments, the fusion protein is hexavalent and has the following structure VHH-Linker-VHH-Linker-VHH-Linker-VHH-Linker-VHH-Linker-VHH. In any of these embodiments, the VHH is a humanized or fully human VHH sequence.

[0078] In some embodiments, the TBD of the a multivalent TNFRSF binding fusion protein is a single domain antibody or VHH. In some embodiments, the multivalent TNFRSF binding fusion protein lacks an Fc region. In these embodiments, the TNFRSF binding fusion protein is tetravalent and has the following structure VHH-Linker-VHH-Linker-VHH-Linker-VHH-Linker. In these embodiments, the TNFRSF binding fusion protein is pentavalent and has the following structure VHH-Linker-VHH-Linker-VHH-

Linker-VHH-Linker-VHH. In these embodiments, the TNFRSF binding fusion protein is hexavalent and has the following structure VHH-Linker-VHH-Linker-VHH-Linker-VHH-Linker-VHH-Linker-VHH. In these embodiments, the VHH is a humanized or fully human VHH sequence.

[0079] In some embodiments, the GS-linker comprises an amino acid sequence selected from the group consisting of GGSGGS, i.e., $(GGS)_2$ (SEQ ID NO: 10); GGSGGSGGS, i.e., $(GGS)_3$ (SEQ ID NO: 11); GGSGGSGGSGGS, i.e., $(GGS)_4$ (SEQ ID NO: 12); and GGSGGSGGSGGSGGS, i.e., $(GGS)_5$ (SEQ ID NO: 13).

[0080] In some embodiments, the fusion proteins are multispecific containing a TBD and a binding domain directed toward a second antigen. In these embodiments, the second antigen binding domain can be positioned at numerous positions within the molecule relative to the TBD. In some embodiments, the second antigen binding domain is located N-terminal TBD. In other embodiments, the second antigen binding domain is located to C-terminal to the TBD. In other embodiments, the second antigen binding domain is located on a distinct polypeptide that associates with a first polypeptide containing the TBD.

[0081] In some embodiments, the fusion proteins are multispecific containing an anti-OX40 binding domain and a binding domain directed toward a second antigen. In these embodiments, the second antigen binding domain can be positioned at numerous positions within the molecule relative to the an anti-OX40 binding domain. In some embodiments, the second antigen binding domain is located N-terminal an anti-OX40 binding domain. In other embodiments, the second antigen binding domain is located to C-terminal to the an anti-OX40 binding domain. In other embodiments, the second antigen binding domain is located on a distinct polypeptide that associates with a first polypeptide containing the an anti-OX40 binding domain.

[0082] In some embodiments, the fusion proteins are multispecific containing an anti-PDL1 binding domain and a binding domain directed toward a second antigen. In these embodiments, the second antigen binding domain can be positioned at numerous positions within the molecule relative to the an anti-PDL1 binding domain. In some embodiments, the second antigen binding domain is located N-terminal an anti-PDL1 binding domain. In other embodiments, the second antigen binding domain is located to C-terminal to the an anti-PDL1 binding domain. In other embodiments, the second antigen binding domain is located on a distinct polypeptide that associates with a first polypeptide containing the an anti-PDL1 binding domain.

[0083] In some embodiments, the TBD within the multispecific TNFRSF binding fusion protein is a single domain antibody or VHH. In some embodiments, the TBD within the multispecific TNFRSF binding fusion protein is a composed of antibody variable heavy (VH) chain and variable light (VL) chain region. In some embodiments, the VH and VL of the TBD are formatted as a single chain variable fragment (scFv) connected via a linker region. In some embodiments, the VH and VL of the TBD are formatted as a FAB fragment that associates via a constant heavy 1 (CH1) domain and a constant light chain (CL) domain. In some embodiments, non-antibody heterodimerization domains are utilized to enable the proper association of the VH and VL of the TBD. In some embodiments, the TBD within the multispecific TNFRSF binding fusion protein is derived from non-antibody scaffold proteins for example but not limited to designed ankyrin repeat proteins (darpins), avimer, anticalin/lipocalins, centyrins and fynomers.

[0084] In some embodiments, the TBD within the multispecific TNFRSF binding fusion protein is a single domain antibody or VHH that binds OX40. In some embodiments, the anti-OX40 binding domain within the multispecific TNFRSF binding fusion protein is a composed of antibody variable heavy (VH) chain and variable light (VL) chain region. In some embodiments, the VH and VL of the anti-OX40 binding domain are formatted as a single chain variable fragment (scFv) connected via a linker region. In some embodiments, the VH and VL of the anti-OX40 binding domain are formatted as a Fab fragment that associates via a constant heavy 1 (CH1) domain and a constant light chain (CL) domain. In some embodiments, non-antibody heterodimerization domains are utilized to enable the proper association of the VH and VL of the anti-OX40 binding domain. In some embodiments, the anti-OX40 binding domain within the multispecific TNFRSF binding fusion protein is derived from non-antibody scaffold proteins for example but not limited to designed ankyrin repeat proteins (darpins), avimer, anticalin/lipocalins, centyrins and fynomers.

[0085] In some embodiments, the binding domain within the multispecific fusion protein is a single domain antibody or VHH that binds PDL1. In some embodiments, the anti-PDL1 binding domain within the multispecific TNFRSF binding fusion protein is a composed of antibody variable heavy (VH) chain and variable light (VL) chain region. In some embodiments, the VH and VL of the anti-PDL1 binding domain are formatted as a single chain variable fragment (scFv) connected via a linker region. In some embodiments, the VH and VL of the anti-PDL1 binding domain are formatted as a Fab fragment that

associates via a constant heavy 1 (CH1) domain and a constant light chain (CL) domain. In some embodiments, non-antibody heterodimerization domains are utilized to enable the proper association of the VH and VL of the anti-PDL1 binding domain. In some embodiments, the anti-PDL1 binding domain within the multispecific fusion protein is derived from non-antibody scaffold proteins for example but not limited to designed ankyrin repeat proteins (darpins), avimer, anticalin/lipocalins, centyrins and fynomers.

[0086] In some embodiments, the anti-OX40 binding domain of the multispecific TNFRSF binding fusion protein is a bispecific antibody or antigen-binding fragment thereof.

[0087] In some embodiments, the anti-PDL1 binding domain of the multispecific fusion protein is a bispecific antibody or antigen-binding fragment thereof.

[0088] In any of these embodiments, the bispecific antibody or antigen-fragment thereof can be any suitable bispecific format known in the art, including, by way of non-limiting example, formats based on antibody fragments such as, e.g., X-Link Fab, cross-linked Fab fragments; tascFv/BiTE, tandem-scFv/Bispecific T cell Engager; Db, diabody; taDb, tandem diabody; formats based on Fc-fusions such as, e.g., Db-Fc, diabody-Fc fusion; taDb-Fc fusion, tandem diabody-Fc fusion; taDb-CH3, tandem diabody-CH3 fusion; (scFv)4-Fc, tetra scFv-Fc fusion; DVD-Ig, dual variable domain immunoglobulin; IgG formats such as, e.g., knob-hole and SEED, strand exchange engineered domain; CrossMab, knob-hole combined with heavy and light chain domain exchange; bsAb, quadroma derived bispecific antibody; sdAb, single domain based antibody; and kappa-lambda bodies such as those described in PCT Publication No. WO 2012/023053.

[0089] In any of the above embodiments, at least one TBD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 16-29 and 377-386.

[0090] In any of the above embodiments, at least one TBD comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 30, 36, and 44; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 31, 35, and 37; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 32-34, 48-43, and 45.

[0091] In any of the above embodiments, at least one BD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 46-57.

[0092] In any of the above embodiments, at least one BD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 52-57.

[0093] In any of the above embodiments, at least one BD comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 58, 61, and 64; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 59, 62, 65, and 69; and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 60, 63, 66-68, and 70.

[0094] In any of the above embodiments, at least one TBD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 16-29 and 377-386, and at least one BD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 46-57.

[0095] In any of the above embodiments, at least one TBD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 16-29 and 377-386, and at least one BD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 52-57.

[0096] In any of the above embodiments, at least one TBD comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 30, 36, and 44; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 31, 35, and 37; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 32-34, 48-43, and 45, and at least one BD comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 58, 61, and 64; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 59, 62, 65, and 69; and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 60, 63, 66-68, and 70.

BRIEF DESCRIPTION OF THE DRAWINGS

[0097] Figure 1 is schematic of exemplary multivalent and multispecific fusion proteins of the present disclosure.

[0098] Figures 2A and 2B are graphs demonstrating the ability of OX40 single domain antibodies (VHHs) to bind cell surface OX40. Binding was assessed by flow

cytometry using OX40 expressing CHO cells and data is presented as median fluorescence intensity.

[0099] Figure 3 is a graph demonstrating the capacity of OX40 single domain antibodies (VHHs) to block the interaction between OX40 and OX40L. 2E4 blocks the interaction between OX40 and OX40L, while the other single domain antibodies do not. A single domain antibody that binds GITR was included as a negative control. Blocking was assessed by flow cytometry using a OX40L-citrine fusion protein and data is presented as median fluorescence intensity.

[00100] Figure 4 is a graph depicting the ability of OX40 single domain antibodies to bind cynomolgus monkey OX40. Binding was assessed by flow cytometry using cynoOX40 expressing CHO cells and data is presented as median fluorescence intensity.

[00101] Figure 5A is schematic depicting the format of multivalent OX40 binding fusion proteins and estimated molecular weights.

[00102] Figure 5B is a photograph of a Coomassie blue stain SDS-PAGE gel of multivalent OX40 binding fusion proteins under reducing and non-reducing conditions.

[00103] Figure 6 is a graph demonstrating enhanced OX40 signaling is mediated by higher valency binding. Shown here is the comparison between tetravalent and bivalent binding of OX40 using the fusion proteins of the present disclosure. OX40 signaling was monitored using an NF-kB reporter 293 cell line expressing OX40. Fusion proteins incorporating 1D10 as the TBD were used in these assays.

[00104] Figures 7A and 7B are graphs demonstrating enhanced OX40 signaling is mediated by higher valency binding. Shown in Figure 7A is the comparison between tetravalent and hexavalent binding of OX40 using the fusion proteins of the present disclosure. Shown in Figure 7B is the comparison between hexavalent binding of OX40 using a fusion protein of the present disclosure and a hexameric OX40L-fusion protein (described in US_7959925). The multivalent OX40 binding fusion proteins of the present disclosure display equivalent or enhanced OX40 agonistic activity compared to the hexameric OX40L fusion protein. OX40 signaling was monitored using a NF-kB reporter 293 cell line expressing OX40. Fusion proteins incorporating 1D10 as the TBD were used in these assays.

[00105] Figures 8A and 8B are a series of graphs depicting the elution profile from a size exclusion chromatography (SEC) column (Superdex 200) for tetravalent (Fig. 8A) and

hexavalent (Fig. 8B) 1D10. Calculated molecular weights based on the retention volume (standard curve) are also shown.

[00106] Figure 9 is a graph demonstrating OX40 signaling is mediated by various multivalent formats of the fusion proteins of the present disclosure, including hexavalent with three tandem OX40 VHHs linked to an Fc region, tetravalent with two tandem OX40 VHHs linked to an Fc region and tetravalent with an N-terminal and C-terminal OX40 VHH separated by an Fc region. OX40 signaling was monitored using an NF-kB reporter 293 cell line expressing OX40. Fusion proteins incorporating 1D10 as the TBD were used in for these assays.

[00107] Figures 10A, 10B, and 10C depict PDL1-dependent OX40 agonism mediated by bispecific PDL1-OX40 targeting fusion proteins of the present disclosure. Figures 10 A and B are conceptual schematics wherein the bispecific fusion protein have minimal OX40 agonistic properties (Figure 10A) unless bound by a PDL1 expressing cell (Figure 10B). Figure 10C is graph demonstrating the ability of a PDL1-positive cell (here PDL1 transfected CHO cells) to mediate OX40 signaling and the inability of PDL1-negative cell (here untransfected CHO cells) to mediate OX40 signaling. OX40 signaling was monitored using a NF-kB reporter 293 cell line expressing OX40. Numerous distinct bispecific fusion proteins are shown in this figure, each containing a distinct OX40 binding VHH (e.g., G3, 2E4, 3G9, 1D10) and the same PDL1 VHH, 28A10.

[00108] Figure 11 depicts FRα-dependent OX40 agonists signaling mediated by bispecific FRa-OX40 targeting fusion proteins of the present disclosure. An FRα expressing ovarian cancer cell line, SKOV3, was used in these assays. OX40 signaling was monitored using a NF-kB reporter 293 cell line expressing OX40. Numerous distinct bispecific fusion proteins are shown in this figure, each containing a distinct FRα binding VHH (e.g., 1G10, 1A3, 57, 5) and the same OX40 VHH, 1D10.

[00109] Figures 12A and 12B are a series of graphs depicting the ability of humanized OX40 single domain antibodies to bind human OX40 and cynomolgus OX40. Binding was assessed by flow cytometry using OX40 expressing CHO cells and data is presented as median fluorescence intensity.

[00110] Figures 13A and 13B are a pair of graphs depicting the ability of humanized OX40 single domain antibodies to bind various TNFRSF members. Various humanized sdAbs of the disclosure as the TBD were used in the tetravalent and hexavalent molecules for these assays.

[00111] Figures 14A and 14B are a pair of graphs demonstrating OX40 signaling is mediated by various multivalent formats of the fusion proteins of the present disclosure, including hexavalent with three tandem OX40 VHHs linked to an Fc region, tetravalent with two tandem OX40 VHHs linked to an Fc region and tetravalent with an N-terminal and C-terminal OX40 VHH separated by an Fc region. OX40 signaling was monitored using an NF-kB reporter 293 cell line expressing OX40. Various humanized sdAbs of the disclosure as the TBD were used in the tetravalent and hexavalent molecules for these assays.

DETAILED DESCRIPTION OF THE INVENTION

[00112] All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

Definitions

[00113] Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well-known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. *See e.g.,* Sambrook *et al.* Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the

art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[00114] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[00115] As used herein, the terms "dual-targeting fusion protein" and "antibody" can be synonyms. As used herein, the term "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. By "specifically bind" or "immunoreacts with" "or directed against" is meant that the antibody reacts with one or more antigenic determinants of the desired antigen and does not react with other polypeptides or binds at much lower affinity ($K_d > 10^{-6}$). Antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, dAb (domain antibody), single chain, Fab, Fab' and $F(ab')_2$ fragments, F_v, scFvs, a Fab expression library, and single domain antibody (sdAb) fragments, for example, V_HH, V_{NAR}, engineered V_H or V_K.

[00116] The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. In general, antibody molecules obtained from humans relate to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses (also known as isotypes) as well, such as IgG_1, IgG_2, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain.

[00117] The term "monoclonal antibody" (MAb) or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

[00118] The term "antigen-binding site" or "binding portion" refers to the part of the immunoglobulin molecule that participates in antigen binding. The antigen binding site is

formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains, referred to as "hypervariable regions," are interposed between more conserved flanking stretches known as "framework regions," or "FRs". Thus, the term "FR" refers to amino acid sequences which are naturally found between, and adjacent to, hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three-dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as "complementarity-determining regions," or "CDRs." The assignment of amino acids to each domain is in accordance with the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk J. Mol. Biol. 196:901-917 (1987), Chothia *et al.* Nature 342:878-883 (1989).

[00119] The single domain antibody (sdAb) fragments portions of the fusion proteins of the present disclosure are referred to interchangeably herein as targeting polypeptides herein.

[00120] As used herein, the term "epitope" includes any protein determinant capable of specific binding to/by an immunoglobulin or fragment thereof, or a T-cell receptor. The term "epitope" includes any protein determinant capable of specific binding to/by an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. An antibody is said to specifically bind an antigen when the dissociation constant is $\leq$ 1 mM, for example, in some embodiments, $\leq$ 1 μM; *e.g.*, $\leq$ 100 nM, $\leq$ 10 nM or $\leq$ 1 nM.

[00121] As used herein, the terms "immunological binding," and "immunological binding properties" refer to the non-covalent interactions of the type which occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using

methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and geometric parameters that equally influence the rate in both directions. Thus, both the "on rate constant" (k_{on}) and the "off rate constant" (k_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. (*See* Nature 361:186-87 (1993)). The ratio of k_{off} /k_{on} enables the cancellation of all parameters not related to affinity, and is equal to the dissociation constant K_d. (*See, generally*, Davies et al. (1990) Annual Rev Biochem 59:439-473). An antibody of the present disclosure is said to specifically bind to an antigen, when the equilibrium binding constant (K_d) is $\leq$ 1 mM, in some embodiments, $\leq$1 μM, $\leq$ 100 nM, $\leq$ 10 nM, or $\leq$ 100 pM to about 1 pM, as measured by assays such as radioligand binding assays, surface plasmon resonance (SPR), flow cytometry binding assay, or similar assays known to those skilled in the art.

[00122] The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated polynucleotide" (1) is not associated with all or a portion of a polynucleotide in which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.

[00123] The term "isolated protein" referred to herein means a protein of cDNA, recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the "isolated protein" (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, *e.g.*, free of marine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

[00124] The term "polypeptide" is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein fragments, and analogs are species of the polypeptide genus.

[00125] The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.

[00126] The term "operably linked" as used herein refers to positions of components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

[00127] The term "control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. The term "polynucleotide," as referred to herein, refers to a polymeric boron of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

[00128] The term "oligonucleotide" referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. In some embodiments, oligonucleotides are 10 to 60 bases in length and in some embodiments, 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, *e.g.,* for probes, although oligonucleotides may be double stranded, *e.g.,* for use in the construction of a gene mutant. Oligonucleotides of the disclosure are either sense or antisense oligonucleotides.

[00129] The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselerloate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate, phosphoronmidate, and the like. *See e.g.,* LaPlanche *et al.* Nucl. Acids Res. 14:9081 (1986); Stec *et al.* J. Am. Chem. Soc. 106:6077 (1984), Stein *et al.* Nucl. Acids Res. 16:3209 (1988), Zon *et al.* Anti Cancer Drug Design

6:539 (1991); Zon *et al.* Oligonucleotides and Analogues: A Practical Approach, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec *et al.* U.S. Patent No. 5,151,510; Uhlmann and Peyman Chemical Reviews 90:543 (1990). An oligonucleotide can include a label for detection, if desired.

[00130] The term "selectively hybridize" referred to herein means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof in accordance with the disclosure selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and fragments of the disclosure and a nucleic acid sequence of interest will be at least 80%, and more typically with increasing homologies of at least 85%, 90%, 95%, 99%, and 100%. Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as this term is used herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. *See* Dayhoff, M.O., in Atlas of Protein Sequence and Structure, pp. 101-110 (Volume 5, National Biomedical Research Foundation (1972)) and Supplement 2 to this volume, pp. 1-10. The two sequences or parts thereof are more preferably homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program. The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous (*i.e.,* is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to a reference polypeptide sequence. In contradistinction, the term "complementary to" is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

[00131] The following terms are used to describe the sequence relationships between two or more polynucleotide or amino acid sequences: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 18 nucleotides or 6 amino acids in length, frequently at least 24 nucleotides or 8 amino acids in length, and often at least 48 nucleotides or 16 amino acids in length. Since two polynucleotides or amino acid sequences may each (1) comprise a sequence (*i.e.*, a portion of the complete polynucleotide or amino acid sequence) that is similar between the two molecules, and (2) may further comprise a sequence that is divergent between the two polynucleotides or amino acid sequences, sequence comparisons between two (or more) molecules are typically performed by comparing sequences of the two molecules over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window", as used herein, refers to a conceptual segment of at least 18 contiguous nucleotide positions or 6 amino acids wherein a polynucleotide sequence or amino acid sequence may be compared to a reference sequence of at least 18 contiguous nucleotides or 6 amino acid sequences and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions, deletions, substitutions, and the like (*i.e.*, gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman Proc. Natl. Acad. Sci. (U.S.A.) 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, (Genetics Computer Group, 575 Science Dr., Madison, Wis.), Geneworks, or MacVector software packages), or by inspection, and the best alignment (*i.e.*, resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

[00132] The term "sequence identity" means that two polynucleotide or amino acid sequences are identical (*i.e.*, on a nucleotide-by-nucleotide or residue-by-residue basis) over

the comparison window. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.,* A, T, C, G, U or I) or residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the comparison window (*i.e.,* the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denotes a characteristic of a polynucleotide or amino acid sequence, wherein the polynucleotide or amino acid comprises a sequence that has at least 85 percent sequence identity, for example, at least 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 18 nucleotide (6 amino acid) positions, frequently over a window of at least 24-48 nucleotide (8-16 amino acid) positions, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the comparison window. The reference sequence may be a subset of a larger sequence.

[00133] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. *See* Immunology - A Synthesis (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland7 Mass. (1991)). Stereoisomers (*e.g.,* D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α-, α -disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present disclosure. Examples of unconventional amino acids include: 4 hydroxyproline, γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε -N-acetyllysine, O-phosphoserine, N- acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ-N-methylarginine, and other similar amino acids and imino acids (*e.g.,* 4- hydroxyproline). In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

[00134] Similarly, unless specified otherwise, the left-hand end of single-stranded polynucleotide sequences is the 5' end the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction sequence regions on the DNA strand having the same sequence as the RNA and which are 5' to the 5' end of the

RNA transcript are referred to as "upstream sequences", sequence regions on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

[00135] As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, for example, at least 90 percent sequence identity, at least 95 percent sequence identity, or at least 99 percent sequence identity.

[00136] In some embodiments, residue positions which are not identical differ by conservative amino acid substitutions.

[00137] Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide- containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur- containing side chains is cysteine and methionine. Suitable conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine valine, glutamic- aspartic, and asparagine-glutamine.

[00138] As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the present disclosure, providing that the variations in the amino acid sequence maintain at least 75%, for example, at least 80%, 90%, 95%, or 99%. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families: (1) acidic amino acids are aspartate, glutamate; (2) basic amino acids are lysine, arginine, histidine; (3) non-polar amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and (4) uncharged polar amino acids are glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. The hydrophilic amino acids include arginine, asparagine, aspartate, glutamine, glutamate, histidine, lysine, serine, and threonine. The hydrophobic amino acids include alanine, cysteine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan,

tyrosine and valine. Other families of amino acids include (i) serine and threonine, which are the aliphatic-hydroxy family; (ii) asparagine and glutamine, which are the amide containing family; (iii) alanine, valine, leucine and isoleucine, which are the aliphatic family; and (iv) phenylalanine, tryptophan, and tyrosine, which are the aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Assays are described in detail herein. Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art. Suitable amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. In some embodiments, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie *et al.* Science 253:164 (1991). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the invention.

[00139] Suitable amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (for example, conservative amino acid substitutions) may be made in the naturally- occurring sequence (for example, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (*e.g.,* a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the

parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. Nature 354:105 (1991).

[00140] The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence deduced, for example, from a full length cDNA sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long, for example, at least 14 amino acids long, at least 20 amino acids long, at least 50 amino acids long, or at least 70 amino acids long. The term "analog" as used herein refers to polypeptides which are comprised of a segment of at least 25 amino acids that has substantial identity to a portion of a deduced amino acid sequence and which has specific binding to CD47, under suitable binding conditions. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally- occurring sequence. Analogs typically are at least 20 amino acids long, for example, at least 50 amino acids long or longer, and can often be as long as a full-length naturally-occurring polypeptide.

[00141] Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics". Fauchere, J. Adv. Drug Res. 15:29 (1986), Veber and Freidinger TINS p.392 (1985); and Evans *et al.* J. Med. Chem. 30:1229 (1987). Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (*i.e.,* a polypeptide that has a biochemical property or pharmacological activity), such as human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: -- CH_2NH--, --CH_2S-, --CH_2-CH_2--, --CH=CH--(cis and trans), --$COCH_2$--, $CH(OH)CH_2$--, and -CH_2SO--, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (*e.g.,* D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides comprising a

consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch Ann. Rev. Biochem. 61:387 (1992)); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

[00142] The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, and/or an extract made from biological materials.

[00143] As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, *e.g.,* by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (*e.g.,* streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods). In certain situations, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (*e.g.,* ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), fluorescent labels (*e.g.,* FITC, rhodamine, lanthanide phosphors), enzymatic labels (*e.g.,* horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.,* leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance. The term "pharmaceutical agent or drug" as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient.

[00144] The term "antineoplastic agent" is used herein to refer to agents that have the functional property of inhibiting a development or progression of a neoplasm in a human, particularly a malignant (cancerous) lesion, such as a carcinoma, sarcoma, lymphoma, or leukemia. Inhibition of metastasis is frequently a property of antineoplastic agents.

[00145] As used herein, the terms "treat," treating," "treatment," and the like refer to reducing and/or ameliorating a disorder and/or symptoms associated therewith. By "alleviate" and/or "alleviating" is meant decrease, suppress, attenuate, diminish, arrest, and/or stabilize the development or progression of a disease such as, for example, a cancer. It will be appreciated that, although not precluded, treating a disorder or condition does not

require that the disorder, condition or symptoms associated therewith be completely eliminated.

[00146] Other chemistry terms herein are used according to conventional usage in the art, as exemplified by The McGraw-Hill Dictionary of Chemical Terms (Parker, S., Ed., McGraw-Hill, San Francisco (1985)).

[00147] As used herein, "substantially pure" means an object species is the predominant species present (*i.e.*, on a molar basis it is more abundant than any other individual species in the composition), and in some embodiments, a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present.

[00148] Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, for example, more than about 85%, 90%, 95%, and 99%. In some embodiments, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

[00149] In this disclosure, "comprises," "comprising," "containing," "having," and the like can have the meaning ascribed to them in U.S. Patent law and can mean "includes," "including," and the like; the terms "consisting essentially of" or "consists essentially" likewise have the meaning ascribed in U.S. Patent law and these terms are open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited are not changed by the presence of more than that which is recited, but excludes prior art embodiments.

[00150] By "effective amount" is meant the amount required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount.

[00151] By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, rodent, ovine, primate, camelid, or feline.

[00152] The term "administering," as used herein, refers to any mode of transferring, delivering, introducing, or transporting a therapeutic agent to a subject in need of treatment with such an agent. Such modes include, but are not limited to, oral, topical, intravenous, intraperitoneal, intramuscular, intradermal, intranasal, and subcutaneous administration.

[00153] By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

[00154] Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

[00155] Unless specifically stated or obvious from context, as used herein, the terms "a," "an," and "the" are understood to be singular or plural. Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive.

[00156] Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example, within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from the context, all numerical values provided herein are modified by the term "about."

OX40 (TNFRSF4, CD134) Targeting

[00157] OX40 is a member of the TNF receptor superfamily that is predominantly expressed on activated T-cells and serves as a co-stimulatory molecule. OX40 engagement has been shown to induce down regulation of CTLA-4. OX40 blocking agents are capable of dampening immune response, while agonists of OX40 enhance immune responses. OX40 agonists have potential to enhance anti-tumor immunity. Crystallographic studies reveal that the OX40 ligand (OX40L) exists as a trimer. Furthermore, murine studies comparing the

anti-tumor activity of an OX40 agonist antibody utilizing FcγR expressing and deficient mice, demonstrated the need for FcγR engagement, suggesting a need for antibody crosslinking. OX40 signaling has been suggested to dampen the suppressive capacity of regulatory T-cells and co-stimulate effector T-cells. OX40 Agonism has been shown to be important for driving differentiation toward and protecting memory T-cells.

[00158] Exemplary amino acid sequences of OX40 binding single domain antibodies are shown below:

1A06:

EVQLVQSGGGLVQPGGSLTLSCVAS[GIILSHNE]MRWYRQNPGKPRDLVAG[ITSAAYT]YYGD FVKGRFTISRDNARNTAYLQMNSLNPGDTGNYYC[EVSDGDNQY]WGQGTQATVSS (SEQ ID NO: 16)

CDR1: GIILSHNE (SEQ ID NO: 30)

CDR2: ITSAAYT (SEQ ID NO: 31)

CDR3: EVSDGDNQY (SEQ ID NO: 32)

2H06:

QLQLQESGGGLVQPGGSLTLSCVAS[GIILSHNE]MRWYRQNPGEPRDLVAG[ITSAAYT]YYGD FVKGRFTISRDNAKNTAYLQMNRLSPEDTGNYYC[EVSDGDNQY]WGQGTQVTVSS (SEQ ID NO: 17)

CDR1: GIILSHNE (SEQ ID NO: 30)

CDR2: ITSAAYT (SEQ ID NO: 31)

CDR3: EVSDGDNQY (SEQ ID NO: 32)

2E4:

QVQLQQSGGGLVQPGGSLSLSCVAS[GIILSHNE]MRWYRQNPGKPRDLVAG[ITSAAYT]YYGD FVKGRFTISRDNAKNTAYLQMDRLNPEDTGNYYC[EVSDGDNRY]WGQGTQATV (SEQ ID NO: 18)

CDR1: GIILSHNE (SEQ ID NO: 30)

CDR2: ITSAAYT (SEQ ID NO: 31)

CDR3: EVSDGDNRY (SEQ ID NO: 33)

2C09:

QVQLQQSGGGLVQPGGSLTLSCVAS[GIILSHNE]MRWYRQNPGKPRDLVAG[ITSAAYT]YYGD FFKGRFTISRDNAKNTAYLQMNRLNPEDTGNYYC[EVSDGDIQY]WGQGTQATVSS (SEQ ID NO: 19)

CDR1: GIILSHNE (SEQ ID NO: 30)

CDR2: ITSAAYT (SEQ ID NO: 31)

CDR3: EVSDGDIQY (SEQ ID NO: 34)

2E10:

QVQLVQSGGGLVQPGGSLTLSCAAS[GIILSHNE]MRWYRQNPGKPRDLVAG[ITGLDYT]YYGD FVKGRFTISRDNAMNTAYLQMDSLNPEDTGNYYC[EVSDGDNQY]WGQGTQVTVSS (SEQ ID NO: 20)

CDR1: GIILSHNE (SEQ ID NO: 30)

CDR2: ITGLDYT (SEQ ID NO: 35)

CDR3: EVSDGDNQY (SEQ ID NO: 32)

13-5:

QVQLQESGGGLVQPGGSLRLSCAAS[GFTFSDAF]MYWVRQAPGKELEWVSS[ISNRGLKT]AYA ESVKGRLTISRDNTKNTLYLEMNSLKPEDTGMYYC[SRDVGGDL]RGQGTQVTVSS (SEQ ID NO: 21)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: SRDVGGDL (SEQ ID NO: 38)

1D10:

QVQLQESGGGLVQPGGSLRLSCAAS[GFTFSDAF]MYWVRQAPGKELEWVSS[ISNRGLKT]AYK ESVKGRFTISRDNTKNMLFLEMNRLEPEDTGLYYC[SRDVDGDF]RGQGTQVTV (SEQ ID NO: 22)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: SRDVDGDF (SEQ ID NO: 39)

3E11:

QVQLQESGGGLVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKELEWVSSISNRGLKTAYA
ESVKGRFTISRDNTKNTLYLQMSSLKPEDTAVYYCATESIGGNGSPYFDLWGQGTQVTVKP
(SEQ ID NO: 23)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: ATESIGGNGSPYFDL (SEQ ID NO: 40)

3G9:

QVQLQESGGGLVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKELEWVSSISNRGLKTAYA
ESVKGRFTISRDNTKNTLYLQMSSLKPEDTAVYYCATESIGSNGSPYFDLWGQGTQVTVKP
(SEQ ID NO: 24)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: ATESIGSNGSPYFDL (SEQ ID NO: 41)

G3:

QVQLQQSGGGLVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKELEWVSSISNRGLKTAYK
ESVKGRFTISRDNTKNMLFLEMNRLEPEDTGLYYCVEGDWNLGPRGQGTQVTVKP (SEQ
ID NO: 25)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: VEGDWNLGP (SEQ ID NO: 42)

hzG3v1:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGLEWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKP
(SEQ ID NO: 377)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: VEGDWNLGP (SEQ ID NO: 42)

hzG3v2:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKELEWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKP
(SEQ ID NO: 378)

CDR1: GFTFSDAF (SEQ ID NO: 36)
CDR2: ISNRGLKT (SEQ ID NO: 37)
CDR3: VEGDWNLGP (SEQ ID NO: 42)

hzG3v3:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKELEWVSSISNRGLKTAYA
ESVKGRFTISRDNTKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKP
(SEQ ID NO: 379)

CDR1: GFTFSDAF (SEQ ID NO: 36)
CDR2: ISNRGLKT (SEQ ID NO: 37)
CDR3: VEGDWNLGP (SEQ ID NO: 42)

hzG3v4:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKELEWVSSISNRGLKTAYA
ESVKGRFTISRDNTKNMLFLEMNRLEPEDTGLYYCVEGDWNLGPRGQGTLVTVKP
(SEQ ID NO: 380)

CDR1: GFTFSDAF (SEQ ID NO: 36)
CDR2: ISNRGLKT (SEQ ID NO: 37)
CDR3: VEGDWNLGP (SEQ ID NO: 42)

hzG3v5:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKELEWVSSISNRGLKTAYA
ESVKGRFTISRDNTKNTLFLEMNRLEPEDTGLYYCVEGDWNLGPRGQGTLVTVKP
(SEQ ID NO: 381)

CDR1: GFTFSDAF (SEQ ID NO: 36)
CDR2: ISNRGLKT (SEQ ID NO: 37)
CDR3: VEGDWNLGP (SEQ ID NO: 42)

hzG3v6:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKELEWVSSISNRGLKTAYK
ESVKGRFTISRDNTKNMLFLEMNRLEPEDTGLYYCVEGDWNLGPRGQGTLVTVKP
(SEQ ID NO: 382)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: VEGDWNLGP (SEQ ID NO: 42)

hzG3v7:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKELEWVSSISNRGLKTAYK
ESVKGRFTISRDNTKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKP (SEQ
ID NO: 383)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: VEGDWNLGP (SEQ ID NO: 42)

hzG3v8:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGREWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKP
(SEQ ID NO: 384)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: VEGDWNLGP (SEQ ID NO: 42)

hzG3v9:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKEREWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKP
(SEQ ID NO: 385)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: VEGDWNLGP (SEQ ID NO: 42)

1G4:

QVQLQESGGGLVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKELEWVSSISNRGLKTAYK ESVKGRFTISRDNTKNMLYLEMNRLEPEDTGLYYCSRDVDGGFRGQGTQVTVKP (SEQ ID NO: 26)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: SRDVDGGF (SEQ ID NO: 43)

A1:

QVQLQESGGGLVQPGGSLRLSCAASGFTFNDAFMYWVRQAPGKELEWVSSISNRGLKTAYK ESXKGLFTISRDNTKNMLFLEMNRLEPEDXGLYYCSRDVDGDFWGRGTHVTVKP (SEQ ID NO: 27)

CDR1: GFTFNDAF (SEQ ID NO: 44)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: SRDVDGDF (SEQ ID NO: 39)

G1:

QVQLQESGGGLVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKELEWVSSISNRGLKTAYA ESVKGRFTISRDNTKNTLYLEMNSLKPEDTGMYYCSRDVGGDLRGQGTQVTVKP (SEQ ID NO: 28)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: SRDVGGDL (SEQ ID NO: 38)

hz1D10v1:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGLEWVSSISNRGLKTAYA ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCSRDVDGDFRGQGTLVTVKP (SEQ ID NO: 29)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: SRDVDGDF (SEQ ID NO: 39)

1D11:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGLEWVSSISNRGLKTAYA ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCSIDVDGDFRGQGTLVTVKP (SEQ ID NO: 386)

CDR1: GFTFSDAF (SEQ ID NO: 36)

CDR2: ISNRGLKT (SEQ ID NO: 37)

CDR3: SIDVDGDF (SEQ ID NO: 45)

[00159] In some embodiments, the fusion proteins are multispecific containing at least a first binding domain, *e.g.*, a TBD, and a second binding domain directed toward Program Death Ligand 1 (PD-L1). In these, embodiments, the binding to PD-L1 is capable of providing the additional crosslinking function and TNFRSF activation is achieved with only one or two TBDs. In these embodiments, the TNFRSF signaling is enhanced and focused by the presence of a PD-L1 expressing cell.

[00160] PDL1 is a 40kDa type I transmembrane protein that forms a complex with its receptor programmed cell death protein 1 (PD1), also known as CD279. Engagement of PDL1 with its receptor PD1 on T cells delivers a signal that inhibits TCR-mediated activation of IL-2 production and T cell proliferation. Aberrant expression and/or activity of PDL1 and PDL1-related signaling has been implicated in the pathogenesis of many diseases and disorders, such as cancer, inflammation, and autoimmunity.

[00161] In some embodiments, the PD-L1 binding portion is single domain antibody. In some embodiments, the PD-L1 binding portion of the fusion blocks or dampens the interaction of PD-L1 and PD-1. Exemplary PD-L1-targeting single domain sequences are shown below:

28A10:

QVQLQESGGGLVQAGGSLRLACTTSGGIFNIRPISWYRQPPGMQREWVATIAFGGATNYAN SIKGRFTASRDNAKNTVYLQMNGLKPEDTAVYYCNAFEIWGQGTQVTV (SEQ ID NO: 46)

CDR1: GGIFNIRP (SEQ ID NO: 58)

CDR2: IAFGGAT (SEQ ID NO: 59)

CDR3: NAFEI (SEQ ID NO: 60)

28A2:

QLQLQESGGGLVRAGGSLRLACTTSGGIFAIKPISWYRQPPGQEREWVTTTTSSGATNYAN SIKGRFTVARDNAKNTVYLQMNDLKLEDTAVYYCNVFEYWGQGTQVTV (SEQ ID NO: 47)

CDR1: GGIFAIKP (SEQ ID NO: 61)

CDR2: TTSSGAT (SEQ ID NO: 62)

CDR3: NVFEY (SEQ ID NO: 63)

B03:

QVQLQESGGDLVQAGSSLRLACATSGGVFNIRPISWYRQPPGKQREWVATIASGGATNYAN SIKGRFTASRDNAKNTVYLQMNGLKPEDTAVYYCNAFEVWGQGTQVTV (SEQ ID NO: 48)

CDR1: GGVFNIRP (SEQ ID NO: 64)

CDR2: IASGGAT (SEQ ID NO: 65)

CDR3: NAFEV (SEQ ID NO: 66)

B10:

QVQLQQSGGGLVQAGGSLRLACTTSGGIFNIRPISWYRQPPGMQREWVATIASGGATNYAN SIKGRFTASRDNAKNTVYLQMNGLKPEDTAVYYCNTLNFWGRGTQVTV (SEQ ID NO: 49)

CDR1: GGIFNIRP (SEQ ID NO: 58)

CDR2: IASGGAT (SEQ ID NO: 65)

CDR3: NTLNF (SEQ ID NO: 67)

D02:

QVQLQESGGGLVQAGGSLRLACTTSGGIFNIRPISWYRQPPGMQREWVATIASGGATNYAN SIKGRFTASRDNAKNTVYLQMNGLKPEDTAVYYCNVFEIWGQGTQVTV (SEQ ID NO: 50)

CDR1: GGIFNIRP (SEQ ID NO: 58)

CDR2: IASGGAT (SEQ ID NO: 65)

CDR3: NVFEI (SEQ ID NO: 68)

A03:

QVQLQQSGGGLVQAGGSLRLACITSGGIFNIRPISWYRQPPGKQREWVATIASGGAANYAN SIKGRFTASRDNAKNTVYLQMNGLKPEDTAVYYCNAFENWGQGTQVTV (SEQ ID NO: 51)

CDR1: GGIFNIRP (SEQ ID NO: 58)

CDR2: IASGGAA (SEQ ID NO: 69)

CDR3: NAFEN (SEQ ID NO: 70)

hz28A2v1

QVQLQESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKP (SEQ ID NO: 52)

CDR1: GGIFAIKP (SEQ ID NO: 61)

CDR2: TTSSGAT (SEQ ID NO: 62)

CDR3: NVFEY (SEQ ID NO: 63)

hz28A2v1-1

EVQLQESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKP (SEQ ID NO: 53)

CDR1: GGIFAIKP (SEQ ID NO: 61)

CDR2: TTSSGAT (SEQ ID NO: 62)

CDR3: NVFEY (SEQ ID NO: 63)

hz28A2v2

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKP (SEQ ID NO: 54)

CDR1: GGIFAIKP (SEQ ID NO: 61)

CDR2: TTSSGAT (SEQ ID NO: 62)

CDR3: NVFEY (SEQ ID NO: 63)

hz28A2v3

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKP (SEQ ID NO: 55)

CDR1: GGIFAIKP (SEQ ID NO: 61)

CDR2: TTSSGAT (SEQ ID NO: 62)

CDR3: NVFEY (SEQ ID NO: 63)

hz28A2v4:

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVTTTTSSGATNYAE SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKP (SEQ ID NO: 56)

CDR1: GGIFAIKP (SEQ ID NO: 61)

CDR2: TTSSGAT (SEQ ID NO: 62)

CDR3: NVFEY (SEQ ID NO: 63)

hz28A2v5:

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKP (SEQ ID NO: 57)

CDR1: GGIFAIKP (SEQ ID NO: 61)

CDR2: TTSSGAT (SEQ ID NO: 62)

CDR3: NVFEY (SEQ ID NO: 63)

[00162] In other embodiments, the PD-L1 binding portion is derived from the extracellular domain of PD-1 containing at least the IgV domain as shown below:

PTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRF RVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVT (SEQ ID NO: 71)

[00163] In some embodiments, the PDL1 binding domain comprises or is derived from a known anti-PDL1 antibody sequence or antigen-binding fragment thereof. In some

embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence disclosed in PCT Publication No. WO 2016/149201, the contents of which are hereby incorporated by reference in their entirety.

[00164] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a variable heavy chain (VH) sequence and a variable light chain (VL) sequence selected from the group consisting of:

VH Sequences:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYGFSWVRQAPGQGLEWMGWITAYNGNTNYA QKLQGRVTMTTDTSTSTVYMELRSLRSDDTAVYYCARDYFYGMDVWGQGTTVTVSS (SEQ ID NO: 72)

QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSTYAISWVRQAPGQGLEWMGGIIPIFGKAHYA QKFQGRVTITADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVTVS S (SEQ ID NO: 73)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDVHWVRQAPGQRLEWMGWLHADTGITKFS QKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCARERIQLWFDYWGQGT (SEQ ID NO: 74)

QVQLVQSGAEVKKPGSSVKVSCKVSGGIFSTYAINWVRQAPGQGLEWMGGIIPIFGTANHA QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDQGIAAALFDYWGQGTLVTVSS (SEQ ID NO: 75)

EVQLVESGGGLVQPGRSLRLSCAVSGFTFDDYVVHWVRQAPGKGLEWVSGNSGNIGYADSV KGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAVPFDYWGQGTLVTVSS (SEQ ID NO: 76)

QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSSYAISWVRQAPGQGLEWMGGIIPIFGRAHYA QKFQGRVTITADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVTVS S (SEQ ID NO: 77)

QVQLVQSGAEVKKPGSSVKVSCKTSGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGKAHYA
QKFQGRVTITADESTTTAYMELSSLRSEDTAVYYCARKYDYVSGSPFGMDVWGQGTTVTVS
S (SEQ ID NO: 78)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAINWVRQAPGQGLEWMGGIIPIFGSANYA
QKFQDRVTITADESTSAAYMELSSLRSEDTAVYYCARDSSGWSRYYMDVWGQGTTVTVSS
(SEQ ID NO: 79)

QVQLVQSGAEVKEPGSSVKVSCKASGGTFNSYAISWVRQAPGQGLEWMGGIIPLFGIAHYA
QKFQGRVTITADESTNTAYMDLSSLRSEDTAVYYCARKYSYVSGSPFGMDVWGQGTTVTVS
S (SEQ ID NO: 80)

EVQLVESGGGLVQPGRSLRLSCAASGITFDDYGMHWVRQAPGKGLEWVSGISWNRGRIEYA
DSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKGRFRYFDWFLDYWGQGTLVTVSS
(SEQ ID NO: 81)

QMQLVQSGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSEKYYV
DSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDYFWSGFSAFDIWGKGTLVTVS
(SEQ ID NO: 82)

VL Sequences:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLVWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIK (SEQ ID
NO: 83)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPTFGQGTKVEIK (SEQ ID NO: 84)

DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK (SEQ ID
NO: 85)

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPD
RFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK (SEQ ID
NO: 86)

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPD
RFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPFGGGTKVEIK (SEQ ID NO: 87)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPTFGQGTRLEIK (SEQ ID NO: 88)

AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPFTFGPGTKVDIK (SEQ ID
NO: 89)

DIVMTQSPSTLSASVGDRVTITCRASQGISSWLAWYQQKPGRAPKVLIYKASTLESGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPWTFGQGTKLEIK (SEQ ID
NO: 90)

[00165] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVANIKQDGSEKYYV
DSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAREGGWFGELAFDYWGQGTLVTVSS
(SEQ ID NO: 91)

VL Sequence:
EIVLTQSPGTLSLSPGERATLSCRASQRVSSSYLAWYQQKPGQAPRLLIYDASSRATGIPD
RFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSLPWTFGQGTKVEIK (SEQ ID
NO: 92)

[00166] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequences:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYA DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSA (SEQ ID NO: 93)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSGSWIHWVRQAPGKGLEWVAWILPYGGSSYYA DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSA (SEQ ID NO: 94)

VL Sequences:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO: 95)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYYNVPWTFGQGTKVEIKR (SEQ ID NO: 96)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYYAPPWTFGQGTKVEIKR (SEQ ID NO: 97)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYYTVPWTFGQGTKVEIKR (SEQ ID NO: 98)

DIQMTQSPSSLSASVGDRVTITCRASQVINTFLAWYQQKPGKAPKLLIYSASTLASGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYYTVPRTFGQGTKVEIKR (SEQ ID NO: 99)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQGYGVPRTFGQGTKVEIKR (SEQ ID NO: 100)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYLFTPPTFGQGTKVEIKR (SEQ ID NO: 101)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYFITPTTFGQGTKVEIKR (SEQ ID NO: 102)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYYYTPPTFGQGTKVEIKR (SEQ ID NO: 103)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQFFYTPPTFGQGTKVEIKR (SEQ ID NO: 104)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQSLFTPPTFGQGTKVEIKR (SEQ ID NO: 105)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQSLYTPPTFGQGTKVEIKR (SEQ ID NO: 106)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQSWYHPPTFGQGTKVEIKR (SEQ ID NO: 107)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYFYIPPTFGQGTKVEIKR (SEQ ID NO: 108)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYWYTPTTFGQGTKVEIKR (SEQ ID NO: 109)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQSYFIPPTFGQGTKVEIKR (SEQ ID NO: 110)

[00167] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequences:

METGLRWLLLVAVLKGVQCLSVEESGGRLVTPGTPLTLTCTASGFTITNYHMFWVRQAPGK GLEWIGVITSSGIGSSSTTYYATWAKGRFTISKTSTTVNLRITSPTTEDTATYFCARDYFT NTYYALDIWGPGTLVTVSS (SEQ ID NO: 111)

QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSTYAISWVRQAPGQGLEWMGGIIPIFGKAHYA QKFQGRVTITADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVTVS S (SEQ ID NO: 112)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDVHWVRQAPGQRLEWMGWLHADTGITKFS QKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCARERIQLWFDYWGQGTLVTVSS (SEQ ID NO: 113)

QVQLVQSGAEVKKPGSSVKVSCKVSGGIFSTYAINWVRQAPGQGLEWMGGIIPIFGTANHA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDQGIAAALFDYWGQGTLVTVSS
(SEQ ID NO: 114)

EVQLVESGGGLVQPGRSLRLSCAVSGFTFDDYVVHWVRQAPGKGLEWVSGISGNSGNIGYA
DSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAVPFDYWGQGTLVTVSS (SEQ ID
NO: 115)

QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSSYAISWVRQAPGQGLEWMGGIIPIFGRAHYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVTVS
S (SEQ ID NO: 116)

QVQLVQSGAEVKKPGSSVKVSCKTSGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGKAHYA
QKFQGRVTITADESTTTAYMELSSLRSEDTAVYYCARKYDYVSGSPFGMDVWGQGTTVTVS
S (SEQ ID NO: 117)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAINWVRQAPGQGLEWMGGIIPIFGSANYA
QKFQDRVTITADESTSAAYMELSSLRSEDTAVYYCARDSSGWSRYYMDVWGQGTTVTVSS
(SEQ ID NO: 118)

QVQLVQSGAEVKEPGSSVKVSCKASGGTFNSYAISWVRQAPGQGLEWMGGIIPLFGIAHYA
QKFQGRVTITADESTNTAYMDLSSLRSEDTAVYYCARKYSYVSGSPFGMDVWGQGTTVTVS
S (SEQ ID NO: 119)

EVQLVESGGGLVQPGRSLRLSCAASGITFDDYGMHWVRQAPGKGLEWVSGISWNRGRIEYA
DSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKGRFRYFDWFLDYWGQGTLVTVSS
(SEQ ID NO: 120)

VL Sequences:
MDTRAPTQLLGLLLLWLPGARCALVMTQTPSSTSTAVGGTVTIKCQASQSISVYLAWYQQK
PGQPPKLLIYSASTLASGVPSRFKGSRSGTEYTLTISGVQREDAATYYCLGSAGS (SEQ
ID NO: 121)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLVWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIK (SEQ ID
NO: 122)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPTFGQGTKVEIK (SEQ ID
NO: 123)

DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK (SEQ ID
NO: 124)

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPD
RFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK (SEQ ID
NO: 125)

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPD
RFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPFGGGTKVEIK (SEQ ID
NO: 126)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPTFGQGTRLEIK (SEQ ID
NO: 127)

AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPFTFGPGTKVDIK (SEQ ID
NO: 128)

[00168] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequences:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGLEWVSSIYPSGGITFYA
DTVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARIKLGTVTTVDYWGQGTLVTVSS
(SEQ ID NO: 129)

VL Sequences:

QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTRVFGTGTKVTVL (SEQ ID
NO: 130)

[00169] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequences:

EVKLQESGPSLVKPSQTLSLTCSVTGYSITSDYWNWIRKFPGNKLEYVGYISYTGSTYYNP
SLKSRISITRDTSKNQYYLQLNSVTSEDTATYYCARYGGWLSPFDYWGQGTTLTVSS
(SEQ ID NO: 131)

EVQLQESGPGLVAPSQSLSITCTVSGFSLTTYSINWIRQPPGKGLEWLGVMWAGGGTNSNS
VLKSRLIISKDNSKSQVFLKMNSLQTDDTARYYCARYYGNSPYYAIDYWGQGTSVTVSS
(SEQ ID NO: 132)

EVKLQESGPSLVKPSQTLSLTCSVTGYSIISDYWNWIRKFPGNKLEYLGYISYTGSTYYNP
SLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCARRGGWLLPFDYWGQGTTLTVSS
(SEQ ID NO: 133)

EVKLQESGPSLVKPGASVKLSCKASGYTFTSYDINWVKQRPGQGLEWIGWIFPRDNNTKYN
ENFKGKATLTVDTSSTTAYMELHSLTSEDSAVYFCTKENWVGDFDYWGQGTTLTLSS
(SEQ ID NO: 134)

EVQLQQSGPDLVTPGASVRISCQASGYTFPDYYMNWVKQSHGKSLEWIGDIDPNYGGTTYN
QKFKGKAILTVDRSSSTAYMELRSLTSEDSAVYYCARGALTDWGQGTSLTVSS (SEQ ID
NO: 135)

EIVLTQSPATLSLSPGERATLSCRASSSVSYIYWFQQKPGQSPRPLIYAAFNRATGIPARF
SGSGSGTDYTLTISSLEPEDFAVYYCQQWSNNPLTFGQGTKVEIK (SEQ ID
NO: 136)

QVQLVQSGAEVKKPGASVKVSCKASGYTFPDYYMNWVRQAPGQGLEWMGDIDPNYGGTNYA
QKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGALTDWGQGTMVTVSS (SEQ ID
NO: 137)

QVQLVQSGAEVKKPGASVKVSCKASGYTFPDYYMNWVRQAPGQSLEWMGDIDPNYGGTNYN
QKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGALTDWGQGTMVTVSS (SEQ ID
NO: 138)

EVQLVQSGAEVKKPGASVKVSCKASGYTFPDYYMNWVRQAPGQSLEWMGDIDPNYGGTNYN
QKFQGRVTMTVDRSSSTAYMELSRLRSDDTAVYYCARGALTDWGQGTMVTVSS (SEQ ID
NO: 139)

EVQLVESGGGLVQPGRSLRLSCTASGYTFPDYYMNWVRQAPGKGLEWVGDIDPNYGGTTYA
ASVKGRFTISVDRSKSIAYLQMSSLKTEDTAVYYCTRGALTDWGQGTMVTVSS (SEQ ID
NO: 140)

EVQLVESGGGLVQPGRSLRLSCTASGYTFPDYYMNWVRQAPGKGLEWVGDIDPNYGGTTYN
ASVKGRFTISVDRSKSIAYLQMSSLKTEDTAVYYCARGALTDWGQGTMVTVSS (SEQ ID
NO: 141)

VL Sequences:
DIVMTQSHKLMSTSVGDRVSITCKASQDVGTAVAWYQQKPGQSPKLLIYWASTRHTGVPDR
FTGSGSGTDFTLTISNVQSEDLADYFCQQDSSYPLTFGAGTKVELK (SEQ ID
NO: 142)

DIVTTQSHKLMSTSVGDRVSITCKASQDVGTAVAWYQQKPGQSPKLLIYWASTRHTGVPDR FTGSGSGTDFTLTISNVQSEDLADYFCQQDSSYPLTFGAGTKVELK (SEQ ID NO: 143)

DIVMTQSPSSLAVSVGEKVSMGCKSSQSLLYSSNQKNSLAWYQQKPGQSPKLLIDWASTRE SGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYGYPLTFGAGTKLELK (SEQ ID NO: 144)

DIVMTQSPAIMSASPGEKVTMTCSASSSIRYMHWYQQKPGTSPKRWISDTSKLTSGVPARF SGSGSGTSYALTISSMEAEDAATYYCHQRSSYPWTFGGGTKLEIK (SEQ ID NO: 145)

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIYWFQQKPGSSPKPWIYATFNLASGVPARF SGSGSGTSYSLTISRVETEDAATYYCQQWSNNPLTFGAGTKLELK (SEQ ID NO: 146)

EIVLTQSPATLSLSPGERATLSCRASSSVSYIYWFQQKPGQAPRLLIYAAFNRATGIPARF SGSGSGTDYTLTISSLEPEDFAVYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 147)

QIVLTQSPATLSLSPGERATLSCRASSSVSYIYWFQQKPGQSPRPLIYATFNLASGIPARF SGSGSGTSYTLTISRLEPEDFAVYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 148)

DIQLTQSPSSLSASVGDRVTITCRASSGVSYIYWFQQKPGKAPKLLIYAAFNLASGVPSRF SGSGSGTEYTLTISSLQPEDFATYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 149)

DIQLTQSPSSLSASVGDRVTITCRASSGVSYIYWFQQKPGKAPKPLIYAAFNLASGVPSRF SGSGSGTEYTLTISSLQPEDFATYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 150)

DIQLTQSPSILSASVGDRVTITCRASSSVSYIYWFQQKPGKAPKPLIYATFNLASGVPSRF SGSGSGTSYTLTISSLQPEDFATYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 151)

[00170] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequences:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYA QKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARALPSGTILVGGWFDPWGQGTLVTV SS (SEQ ID NO: 152)

EVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYALSWVRQAPGKGLEWVSAISGGGGSTYYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDVFPETFSMNYGMDVWGQGTLVTV SS (SEQ ID NO: 153)

QVQLVQSGGGVVQPGGSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSLISGDGGSTYYA DSVKGRFTISRDNSKNSLYLQMNSLRTEDTALYYCAKVLLPCSSTSCYGSVGAFDIWGQGT TVTVSS (SEQ ID NO: 154)

QVQLVQSGGSVVRPGESLRLSCVASGFIFDNYDMSWVRQVPGKGLEWVSRVNWNGGSTTYA DAVKGRFTISRDNTKNSLYLQMNNLRAEDTAVYYCVREFVGAYDLWGQGTTVTVSS (SEQ ID NO: 155)

QVQLVQSGAEVKKPGATVKVSCKVFGDTFRGLYIHWVRQAPGQGLEWMGGIIPIFGTANYA QKFQGRVTITTDESTSTAYMELSSLRSEDTAVYYCASGLRWGIWGWFDPWGQGTLVTVSS (SEQ ID NO: 156)

EVQLVQSGAELKKPGSSVKVSCKAFGGTFSDNAISWVRQAPGQGPEWMGGIIPIFGKPNYA QKFQGRVTITADESTSTAYMVLSSLRSEDTAVYYCARTMVRGFLGVMDVWGQGTTVTVSS (SEQ ID NO: 157)

QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDQFVTIFGVPRYGMDVWGQGTTVT
VSS (SEQ ID NO: 158)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYA
QKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGRQMFGAGIDFWGPGTLVTVSS
(SEQ ID NO: 159)

EVQLVESGAEVKKPGSSVKVSCKVSGGTFGTYALNWVRQAPGQGLEWMGRIVPLIGLVNYA
HNFEGRISITADKSTGTAYMELSNLRSDDTAVYYCAREVYGGNSDYWGQGTLVTVSS
(SEQ ID NO: 160)

QVQLVQSGGEVKKPGASVKVSCKASGYTLSSHGITWVRQAPGQGLEWMGWISAHNGHASNA
QKVEDRVTMTTDTSTNTAYMELRSLTADDTAVYYCARVHAALYYGMDVWGQGTLVTVSS
(SEQ ID NO: 161)

QVQLQESGGGVVQPGRSLRLSCSASGFTFSRHGMHWVRQAPGKGLEWVAVISHDGSVKYYA
DSMKGRFSISRDNSNNTLYLQMDSLRADDTAVYYCARGLSYQVSGWFDPWGQGTLVTVSS
(SEQ ID NO: 162)

NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSSPTTVIYEDNQRPSGVPD
RFSGSIDTSSNSASLTISGLKTKDEADYYCQSYDGITVIFGGGTKLTVL (SEQ ID
NO: 163)

NFMLTQPHSVSGSPGKTVTLPCTRSSGSIASHYVQWYQQRPGSAPTTVIYEDNKRPSGVPD
RFSGSIDSSSNSASLSISGLKTEDEADYYCQSYDSSNRWVFGGGTKLTVL (SEQ ID
NO: 164)

LPVLTQPASLSASPGASASLTCTLRSGLNVGSYRIYWYQQKPGSRPQYLLNYKSDSNKQQA
SGVPSRFSGSKDASANAGILLISGLQSEDEADYYCMIWYSSAVVFGGGTKLTVL (SEQ
ID NO: 165)

VL Sequences:

NFMLTQPHSVSESPGKTVTISCTRSSGNIASNYVQWYQQRPGSAPTTVIYEDNQRPSGVPD
RFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSSNLWVFGGGTKLTVL (SEQ ID
NO: 166)

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRF
SGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHYVFGTGTKVTVL (SEQ ID
NO: 167)

LPVLTQAPSVSVAPGKTARITCGGSDIGRKSVHWYQQKPGQAPALVIYSDRDRPSGISERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDNNSDHYVFGAGTELIVL (SEQ ID
NO: 168)

QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSTLPFGGGTKLTVL (SEQ ID
NO: 169)

EIVLTQSPATLSLSPGERATLSCRASQSIGNSLAWYQQKPGQAPRLLMYGASSRATGIPDR
FSGSGAGTDFTLTISSLEPEDFATYYCQQHTIPTFSFGPGTKVEVK (SEQ ID
NO: 170)

DIVMTQTPSFLSASIGDRVTITCRASQGIGSYLAWYQQRPGEAPKLLIYAASTLQSGVPSR
FSGSGSGTDFTLTISNLQPEDFATYYCQQLNNYPITFGQGTRLEIK (SEQ ID
NO: 171)

QSALTQPPSVSVSPGQTANIPCSGDKLGNKYAYWYQQKPGQSPVLLIYQDIKRPSRIPERF
SGSNSADTATLTISGTQAMDEADYYCQTWDNSVVFGGGTKLTVL (SEQ ID NO: 172)

NFMLTQPHSVSESPGKTVTISCTRSSGSIDSNYVQWYQQRPGSAPTTVIYEDNQRPSGVPD
RFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSNNRHVIFGGGTKLTVL (SEQ ID
NO: 173)

NFMLTQPHSVSESPGKTVTISCTRSSGNIGTNYVQWYQQRPGSAPVALIYEDYRRPSGVPD
RFSGSIDSSSNSASLIISGLKPEDEADYYCQSYHSSGWEFGGGTKLTVL (SEQ ID
NO: 174)

QSVLTQPPSVSVAPGQTARITCGGNNIGSKGVHWYQQKPGQAPVLVVYDDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHWVFGGGTKLTVL (SEQ ID
NO: 175)

NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTTVIYEDNQRPSGVPD
RFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSTTPSVFGGGTKLTVL (SEQ ID
NO: 176)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWTSPHNGLTAFA
QILEGRVTMTTDTSTNTAYMELRNLTFDDTAVYFCAKVHPVFSYALDVWGQGTLVTVSS
(SEQ ID NO: 177)

EVQLVESGAEVMNPGSSVRVSCRGSGGDFSTYAFSWVRQAPGQGLEWMGRIIPILGIANYA
QKFQGRVTITADKSTSTAYMELSSLRSDDTAVYYCARDGYGSDPVLWGQGTLVTVSS
(SEQ ID NO: 178)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGISWVRQAPGQGLEWMGWISAYNGNTNYA
QKVQGRVTMTTDTSTSTGYMELRSLRSDDTAVYYCARGDFRKPFDYWGQGTLVTVSS
(SEQ ID NO: 179)

[00171] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequences:

EVQLVQSGPELKKPGASVKMSCKASGYTFTSYVMHWVKQAPGQRLEWIGYVNPFNDGTKYN
EMFKGRATLTSDKSTSTAYMELSSLRSEDSAVYYCARQAWGYPWGQGTLVTVSS (SEQ
ID NO: 180)

EVQLVQSGAEVKKPGASVKMSCKASGYTFTSYVMHWVKQAPGQRLEWIGYVNPFNDGTKYN EMFKGRATLTSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS (SEQ ID NO: 181)

EVQLVQSGAEVKKPGASVKMSCKASGYTFTSYVMHWVRQAPGQRLEWIGYVNPFNDGTKYN EMFKGRATLTSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS (SEQ ID NO: 182)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVMHWVRQAPGQRLEWIGYVNPFNDGTKYN EMFKGRATLTSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS (SEQ ID NO: 183)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVMHWVRQAPGQRLEWIGYVNPFNDGTKYN EMFKGRATITSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS (SEQ ID NO: 354)

VL Sequences:

DIVLTQSPASLALSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDSG VPSRFSGSGSGTDFTLTINSLEEEDAAMYFCQQSRRVPYTFGQGTKLEIK (SEQ ID NO: 184)

DIVLTQSPATLSLSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDSG VPSRFSGSGSGTDFTLTINSLEAEDAAMYFCQQSRRVPYTFGQGTKLEIK (SEQ ID NO: 185)

EIVLTQSPATLSLSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDSG VPSRFSGSGSGTDFTLTINSLEAEDAAMYFCQQSRRVPYTFGQGTKLEIK (SEQ ID NO: 186)

DIVLTQSPATLSLSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDSG VPSRFSGSGSGTDFTLTINSLEAEDAATYFCQQSRRVPYTFGQGTKLEIK (SEQ ID NO: 187)

[00172] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequences:

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYA QKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCAREGTIYDSSGYSFDYWGQGTLVTVS S (SEQ ID NO: 188)

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGIINPSGGSTSYA QKFQGRVSMTRDTSTSTVYMELSSLTSEDTAVYYCARDLFPHIYGNYYGMDIWGQGTTVTV SS (SEQ ID NO: 189)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYA QKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARLAVPGAFDIWGQGTMVTVSS (SEQ ID NO: 190)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLAVISYDGSNKYYADSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGQWLVTELDYWGQGTLVTVSS (SEQ ID NO: 191)

EVQLVESGSEVEKPGSSVKVSCKASGGTFSDSGISWVRQAPGQGLEWMGGIIPMFATPYYA QKFQDRVTITADESTSTVYMELSGLRSDDTAVFYCARDRGRGHLPWYFDLWGRGTLVTVSS (SEQ ID NO: 192)

EVQLVESGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYA QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARAPYYYYYMDVWGQGTTVTVSS (SEQ ID NO: 193)

EVQLLESGAEVKKPGSSVKVSCKASGGTLSRYALSWVRQAPGQGPEWVGAIIPIFGTPHYS
KKFQDRVIITVDTSTNTAFMELSSLRFEDTALYFCARGHDEYDISGYHRLDYWGQGTLVTV
SS (SEQ ID NO: 194)

QVQLVQSGSELKKPGSSVKVSCKASGYSFSGYYIHWVRQAPGQGLEWMGWIDPNSGVTNYV
RRFQGRVTMTRDTSLSTAYMELSGLTADDTAVYYCARDENLWQFGYLDYWGQGTLVTVSS
(SEQ ID NO: 195)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSRYGVHWVRQAPGQGLEWMGRLIPIVSMTNYA
QKFQDRVSITTDKSTGTAYMELRSLTSEDTALYYCASVGQQLPWVFFAWGQGTLVTVSS
(SEQ ID NO: 196)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 197)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 198)

EVQLVQSGGGLVQPGGSLRLSCAASGFTFSDYGMHWVRQPPGKGLEWLAVISYDGSYKIHA
DSVQGRFTISRDNAKNSVFLQMNSLKTEDTAVYYCTTDRKWLAWHGMDVWGQGTTVTVSS
(SEQ ID NO: 199)

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDGIVADFQHWGQGTLVTVSS
(SEQ ID NO: 200)

EVQLVESGAEVKKPGASVKVSCKASGDTFSRYGITWVRQAPGRGLEWMGNIVPFFGATNYA
QKFQGRLTITADKSSYTSYMDLSSLRSDDTAVYYCARDHFYGSGGYFDYWGQGTLVTVSS
(SEQ ID NO: 201)

EVQLLESGAEVKKPGASVKVSCKASGYTFNSYDINWVRQAPGQGLEWMGGIIPVFGTANYA
ESFQGRVTMTADHSTSTAYMELNNLRSEDTAVYYCARDRWHYESRPMDVWGQGTTVTVSS
(SEQ ID NO: 202)

EVQLVESGGGLVRPGGSLRLACAASGFSFSDYYMTWIRQAPGRGLEWIAYISDSGQTVHYA
DSVKGRFTISRDNTKNSLFLQVNTLRAEDTAVYYCAREDLLGYYLQSWGQGTLVTVSS
(SEQ ID NO: 203)

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYN
DYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCARDEPRAVAGSQAYYYYGMDVWG
QGTTVTVSS (SEQ ID NO: 204)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSDGSTSYA
QKFQGRVTMTRDTSTSTVHMELSSLRSEDTAVYYCARDLFPHIYGNYYGMDIWGQGTTVTV
SS (SEQ ID NO: 205)

QMQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 206)

QVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 207)

VL Sequences:

QSVLTQPPSVSAAPGQKVTISCSGNNSNIANNYVSWYQQLPGTAPKLLIYDNNYRPSGIPD
RFSGSKSGTSATLDITGLQTGDEADYYCGVWDGSLTTGVFGGGTKLTVL (SEQ ID
NO: 208)

AIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPKLLIYAASTLESGVPSR
FSGSGSGTDFTLTISSLQPEDLATYYCQQLHTFPLTFGGGTKVEIK (SEQ ID
NO: 209)

QPVLTQPPSASGSPGQSVTISCTGTSSDVGAYNFVSWYRQHPGKAPKLMIYEVNKRPSGVP
DRFSGSKSGNTASLTVSGLQAEDEADYYCSSYAGTNSLGIFGTGTKLTVL (SEQ ID
NO: 210)

QSVVTQPPSVSAAPGQKVTISCSGSSSDIGNHYVSWYQQLPGTAPKLLIYDNNQRPSGIPD
RFSGSKSGTSATLAITGLQTGDEADYYCGTWDNSLSPHLLFGGGTKLTVL (SEQ ID
NO: 211)

QSVLTQPPSVSAAPGQKVTISCSGSSSNMGNNYVSWYKQVPGTAPKLLIYENDKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDNSLSGFVFASGTKVTVL (SEQ ID
NO: 212)

QSALTQPASVSGSLGQSVTISCTGSSSDVGSYNLVSWYQQHPGKAPNLMIYDVSKRSGVSN
RFSGSKSGNTASLTISGLQAEDEADYYCSSYTGISTVVFGGGTKLTVL (SEQ ID
NO: 213)

QSVLTQPASVSGSPGQSITISCTGTSSDVGSYNLVSWYQQHPGKAPKLMIYEVSKRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYGGFNNLLFGGGTKLTVL (SEQ ID
NO: 214)

DIVMTQSPSSLSASIGDRVTITCRASQRISAYVNWYQQKPGKAPKVLIYAASSLRSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQTYSSPWTFGQGTKVEIK (SEQ ID
NO: 215)

QSVLTQPPSASGSPGQSVTISCTGTSSDIGGYDSVSWYQQHPGKAPKLMIYDVSKRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSIFFYVFGTGTKVTVL (SEQ ID
NO: 216)

LPVLTQPASVSGSPGQSITISCTGTTSDIGGYDYVSWYQQHPGKAPKLMIYDVSKRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTHVFGTGTKLTVL (SEQ ID
NO: 217)

QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYRSSTLGPVFGGGTKLTVL (SEQ ID
NO: 218)

QAGLTQPPSVSEAPRQRVTISCSGSSSNIGNNAVNWYQQLPGKAPKLLIYYDDLLPSGVSD
RFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGYVFGTGTKLTVL (SEQ ID
NO: 219)

QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGVP
DRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSTTHVFGTGTKVTVL (SEQ ID
NO: 220)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSVWVFGGGTQLTVL (SEQ ID
NO: 221)

QSVLTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGRAPRLMIYDVSNRPSGVS
NRFSGSKSGNTASLTISGLQAEDEGDYYCSSYTSGGTLGPVFGGGTKLTVL (SEQ ID
NO: 222)

QAGLTQPPSASGTPGQRVTISCSGSSSNIGSNTVNWYQQLPGTAPKLLIYSNNQRPSGVPD
RFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGWVFGGGTKLTVL (SEQ ID
NO: 223)

AIRMTQSPSSLSASVGDRVTITCRASQSISNYLNWYQQRPGKAPNLLIYAASSLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQTYSTPYTFGQGTKLEIK (SEQ ID
NO: 224)

QSVLTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYRQHPGKAPKLMIYDVSYRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYTDSSTRYVFGTGTKLTVL (SEQ ID
NO: 225

QPVLTQPPSASGTPGQRVAISCSGSRSNIEINSVNWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGSWDSSLSADVFGTGTKLTVL (SEQ ID
NO: 226)

QSVLTQPPSVSAAPGKKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYRNNQRPSGVPD
RFSGSKSGTSASLAISGLQSEDEADYYCATWDDSLNGWVFGGGTKLTVL (SEQ ID
NO: 227)

QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNNNRHSGVP
DRFSGSKSGTSASLAITGLQAEDEAEFFCGTWDSRLTTYVFGSGTKLTVL (SEQ ID
NO: 228)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAVVFGGGTKLTVL (SEQ ID
NO: 229)

VIWMTQSPSSLSASVGDRVTITCAASSLQSWYQQKPGKAPKLLIYEASTLESGVPSRFSGS
GSGTEFTLTISSLQPEDFATYYCQQSYSTPYTFGQGTKLEIK (SEQ ID NO: 230)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQVPGTAPKLLIYDNNKRPSGIPD
RFSGSNSDTSATLGITGLQTGDEADYYCGTWDSSLSAWVFGGGTKLTVL (SEQ ID
NO: 231)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAGSVVFGGGTKLTVL (SEQ ID
NO: 232)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCLVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 233)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 234)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 235)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 236)

[00173] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a heavy chain (HC) and a light chain sequence (LC) selected from the group consisting of:

HC Sequences:

QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFN
EKFKNRVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTTVTVSSAS
TKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLSLGK (SEQ ID NO: 237)

QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDGSKRYYA
DSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSSASTKGPSVF
PLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD

WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN
HYTQKSLSLSLGK (SEQ ID NO: 238)

LC Sequences:

EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESG
VPARFSGSGSGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 239)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK
ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 240)

[00174] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequences:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYA
DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSSASTK
(SEQ ID NO: 241)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYA
DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSS
(SEQ ID NO: 242)

HC Sequences:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYA
DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSSASTK

GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPG (SEQ ID NO: 243)

VL Sequences:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID
NO: 244)

LC Sequences:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK
ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 245)

[00175] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequences:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRFWMSWVRQAPGKGLEWVANINQDGTEKYYV
DSVKGRFTISRDNAKNSLYLQMNSLRAGDTAVYYCANTYYDFWSGHFDYWGQGTLVTVSS
(SEQ ID NO: 246)

QEHLVESGGGVVQPGRSLRLSCEASGFTFSNFGMHWVRQAPGKGLEWVAALWSDGSNKYYA
DSVKGRVTISRDNSKNTLYLQMNSLRAEDTAVYYCARGRGAPGIPIFGYWGQGTLVTVSS
(SEQ ID NO: 247)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEWVGRIKRKTDGGTTD
YAAPVKGRFTISRDDSKNTLHLQMNSLKTEDTAVYYCTTDDIVVVPAVMREYYFGMDVWGQ
GTTVTVSS (SEQ ID NO: 248)

QVQLVQSGAEVKKPGASVQVSCKASGYSFTGYYIHWVRQAPGQGLEWMGWINPNSGTKKYA
HKFQGRVTMTRDTSIDTAYMILSSLISDDTAVYYCARDEDWNFGSWFDSWGQGTLVTVSS
(SEQ ID NO: 249)

QVHLVQSGAEVKKPGASVKVSCKASGYTFTGYYIHWVRQAPGHGLEWMGWLNPNTGTTKYI
QNFQGRVTMTRDTSSSTAYMELTRLRSDDTAVYYCARDEDWNYGSWFDTWGQGTLVTVSS
(SEQ ID NO: 250)

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMTWVRQAPGRGLEWVSGIHWHGKRTGYA
DSVKGRFTISRDNAKKSLYLQMNSLKGEDTALYHCVRGGMSTGDWFDPWGQGTLVIVSS
(SEQ ID NO: 251)

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMTWVRQVPGKGLEWVSGIHWSGRSTGYA
DSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCARGGMSTGDWFDPWGQGTLVTVSS
(SEQ ID NO: 252)

EVQLVESGGGLVQPGGSLRLSCAASGFTVGSNYMNWVRQAPGKGLEWVSVIYSGGSTYYAD
SVKGRFTISRLTSKNTLYLQMSSLRPEDTAVYYCARGIRGLDVWGQGTTVTVSS (SEQ
ID NO: 253)

EERLVESGGDLVQPGGSLRLSCAASGITVGTNYMNWVRQAPGKGLEWVSVISSGGNTHYAD
SVKGRFIMSRQTSKNTLYLQMNSLETEDTAVYYCARGIRGLDVWGQGTMVTVSS (SEQ
ID NO: 254)

QVQLVQSGAEVKMPGSSVRVSCKASGGIFSSSTISWVRQAPGQGLEWMGEIIPVFGTVNYA
QKFQDRVIFTADESTTTAYMELSSLKSGDTAVYFCARNWGLGSFYIWGQGTMVTVSS
(SEQ ID NO: 255)

EVQLVESGGDLVHPGRSLRLSCAASGFPFDEYAMHWVRQVPGKGLEWVSGISWSNNNIGYA
DSVKGRFTISRDNAKNSLYLQMNSLRPEDTAFYYCAKSGIFDSWGQGTLVTVSS (SEQ
ID NO: 256)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVTLISYEGRNKYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDRTLYGMDVWGQGTTVTVSS
(SEQ ID NO: 257)

QVTLRESGPALVKTTQTLTLTCTFSGFSLSTNRMCVTWIRQPPGKALEWLARIDWDGVKYY
NTSLKTRLTISKDTSKNQVVLTMTNMDPVDTATFYCARSTSLTFYYFDYWGQGTLVTVSS
(SEQ ID NO: 258)

EVQLVESGGGLVQPGGSLRLSCAASEFTVGTNHMNWVRQAPGKGLEWVSVIYSGGNTFYAD
SVKGRFTISRHTSKNTLYLQMNSLTAEDTAVYYCARGLGGMDVWGQGTTVTVSS (SEQ
ID NO: 259)

EVQLVESGGGLVQRGESLRLYCAASGFTFSKYWMNWVRQAPGKGLEWVANIKGDGSEKYYV
DSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDYWGSGYYFDFWGQGTLVTVSS
(SEQ ID NO: 260)

EVQLVESGGGLVQSGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSEKYYV
DSVKGRFTISRDNAKNSLYLQMNSLRADDTAVYYCARDDIVVVPAPMGYYYYYFGMDVWGQ
GTTVTVSS (SEQ ID NO: 261)

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDFAMHWVRQAPGKGLEWVSGISWTGGNMDYA
NSVKGRFTISREDAKNSLYLQMNSLRAADTALYYCVKDIRGIVATGGAFDIWGRGTMVTVS
S (SEQ ID NO: 262)

EVQLVESGGGLVQPGGSLRLSCAASGFTVGTNYMNWVRQAPGKGLEWISVIYSGGSTFYAD
SVKGRFTISRQTSQNTLYLQMNSLRPEDTAVYYCARGIRGFDIWGQGTMVTVSS (SEQ
ID NO: 263)

EVQLVESGGGLVQPGGSLRLSCAASGFTISTNYMNWVRQAPGKGLEWVAVIYSSGSTYYID SVKGRFTISRLTSKNTVYLQMSSLNSEDTAVYYCARGIRGFDIWGQGTMVTVSS (SEQ ID NO: 264)

EVQLVESGGGLVQPGRSLRLSCAASGFTIDDSAMHWVRQTPGKGLEWVSGISWKSGSIGYA DSVRGRFTISRDNAKNSLYLQMNSLRVEDTALYYCVKDIRGNWNYGGNWFDPWGQGTLVTV SS (SEQ ID NO: 265)

EVQLVESGGGLVQPGGSLRLSCEASGFTVGVNHMNWVRQAPGKGLEWVSVIFSSGRTFYGD YVKGRLTIFRQTSQNTVYLQMNSLRSEDTAIYYCARGIGGLDIWGRGTMVTVSS (SEQ ID NO: 266)

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYALHWVRQAPGKGLEWVSGISWTGGTIDYA DSVKGRFTISRDNAKNSLYLQMSSLRTEDTAIYYCTRDIRGNWKYGGWFDPWGQGTLVTVS S (SEQ ID NO: 267)

QVQLVQSGTEVKKPGASVKVSCKASGYTFTAYYMHWVRQAPGQGLDWMGWISPNSGFTNYA QKFQGRVTMTRDTSINTFYMELSGLRSDDTAVYYCAREGSTHHNSFDPWGQGTLVTVSS (SEQ ID NO: 268)

EVQLVESGGGLVQPGGSLRLSCAASGFTVGTNFMNWVRQAPGKGLEWVSAIYSGGTANYAD SVKGRFTISRDTSRNTLYLQMNSLRTEDTAVYYCARGGGMDVWGQGTTVTVSS (SEQ ID NO: 269)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFNTYVLSWVRQAPGQGLEWMGEIIPILGAANYA QNFQGRVTFTTDESTNTAYMDLSSLRSEDTAVYYCARDRTSGGFDPWGQGTLVTVSS (SEQ ID NO: 270)

QVQLVQSGAEVEKPGASVKVSCKASGYIFTHYGISWVRQAPGQGLEWVGWISPYNGYTDYA QKLQGRVTLTTDTSTTTAYMELRNLRSDDTAMYYCSRGRGPYWSFDLWGRGTLVTVSS (SEQ ID NO: 271)

VL Sequences:

DIQMTQSPSTLSASVGDRVTITCRASQSISNWLAWYQQKPGKAPKLLIYKASSLESGVPSR FSGSGSGTEFTLTISSLQPDDFATYYCQQYHSYSYTFGQGTKEIK (SEQ ID NO: 272)

DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYTASSLQSGVPSR FSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPLTFGGGTKVAIK (SEQ ID NO: 273)

DIQMTQSPSSLSASVGDRVTITCRTSQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSR FSGSGSGTEFTLTISSLQPEDFATYYCLQHNNYPYTFGQGTKLEIK (SEQ ID NO: 274)

DIVMTQTPLSSPVTLGQPASISCRSSQTLVHGDGNTYLSWIQQRPGQPPRLLIYKVSNQFS GVPDRFSGSGAGTDFTLKISRVEAEDVGLYFCMQATHFPITFGQGTRLEIK (SEQ ID NO: 275)

DIVMTQTPLSSPVTLGQPASISCRSSPSLVHSDGNTYLSWLQQRPGQPPRLLIYKISNRFS GVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCMQATHFPITFGQGTRLEIR (SEQ ID NO: 276)

DIQMTQSPSSLSASLGDRVTITCRASQSINSYLNWYQQKPGKAPKLLIYVASSLQSGVPSR FSGSGSGTEFTLTISNLQPEDFATYYCQQSYSTPPITFGQGTRLEIK (SEQ ID NO: 277)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYVASSLQSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPITFGQGTRLEIK (SEQ ID NO: 278)

DIQMTQSPSSLSASVGDRVTITCRASQTINIYLNWYQQKPGRAPRLLIYAASSLQSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCHQSYSTPPITFGQGTRLEIK (SEQ ID NO: 279)

DIQMTQSPSSLSASVGDRVTITCRASQSMSSYLNWYQQKPGRAPKLLIFAASSLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPITFGQGTRLEIK (SEQ ID
NO: 280)

EIVLTQSPGTLSLSPGERATLSCRASQSFNFNYLAWYQQKPGQAPRLLIYGASSRATGIPD
RFSGSGSGTDFTLTINRLEPEDFGVFYCQQYESAPWTFGQGTKVEIK (SEQ ID
NO: 2812)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKLLIYAASSLQSGVPSRFSG
GGSGTDFTLTISSLRPEDFATYYCQQSYCTPPITFGQGTRLEIK (SEQ ID NO: 282)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPITFGQGTRLEIK (SEQ ID
NO: 283)

DRVTITCRASQVISNYLAWYQQKPGKVPRLLIYAASTLQSGVPSRFSGSGSGTDFTLTISS
LQPEDVATYYCQKYNSAPRTFGQGTKVEIK (SEQ ID NO: 284)

DIQMTQSPSSLSASVGDRVTITCRASQNINNYLNWYQQKPGKAPKLLIYAASSFQNAVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQSYNTPLTFGGGTKVEIK (SEQ ID
NO: 285)

DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSR
FSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPYTFGQGTKLEIK (SEQ ID
NO: 286)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPITFGQGTRLEIK (SEQ ID
NO: 287)

[00176] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequences:

QSLEESGGRLVKPDETLTITCTVSGIDLSSNGLTWVRQAPGEGLEWIGTINKDASAYYASW AKGRLTISKPSSTKVDLKITSPTTEDTATYFCGRIAFKTGTSIWGPGTLVTVSS (SEQ ID NO: 288)

VL Sequences:

AIVMTQTPSPVSAAVGGTVTINCQASESVYSNNYLSWFQQKPGQPPKLLIYLASTLASGVP SRFKGSGSGTQFTLTISGVQCDDAATYYCIGGKSSSTDGNAFGGGTEVVVR (SEQ ID NO: 289)

[00177] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequences:

QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYA QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGNIVATITPLDYWGQGTLVTVSS (SEQ ID NO: 290)

QPVLTQPPSVSAAPGQKVTISCSGSSSNIANNYVSWYQQLPGTAPKLLIFANNKRPSGIPD RFSGSKSGTSAALDITGLQTGDEADYYCGTWDSDLRAGVFGGGTKLTVL (SEQ ID NO: 291)

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYA QKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCAREGTIYDSSGYSFDYWGQGTLVTVS S (SEQ ID NO: 292)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 293)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 294)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 295)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 296)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 297)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 298)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSRYGVHWVRQAPGQGLEWMGRLIPIVSMTNYA
QKFQDRVSITTDKSTGTAYMELRSLTSEDTALYYCASVGQQLPWVFFAWGQGTLVTVSS
(SEQ ID NO: 299)

QMQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 300)

QVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 301)

QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAYSWVRQAPGQGLEWMGGIIPSFGTANYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGPIVATITPLDYWGQGTLVTVSS
(SEQ ID NO: 302)

QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAYSWVRQAPGQGLEWMGGIIPIFGTANYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGPIVATITPLDYWGQGTLVTVSS
(SEQ ID NO: 303)

QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAYSWVRQAPGQGLEWMGGIIPSFGTANYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGPIVATITPLDYWGQGTLVTVSS
(SEQ ID NO: 304)

QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPAFGTANYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGPIVATITPLDYWGQGTLVTVSS
(SEQ ID NO: 305)

VL Sequences:

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 306)

AIRMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYTTSSLKSGVPSR
FSGSGSGTDFTLTISRLQPEDFATYYCQQSYSSTWTFGRGTKVEIK (SEQ ID
NO: 307)

QSVLTQPPSVSAAPGQKVTISCSGNNSNIANNYVSWYQQLPGTAPKLLIYDNNYRPSGIPD
RFSGSKSGTSATLDITGLQTGDEADYYCGVWDGSLTTGVFGGGTKLTVL (SEQ ID
NO: 308)

LPVLTQPASVSGSPGQSITISCTGTTSDIGGYDYVSWYQQHPGKAPKLMIYDVSKRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTHVFGTGTKLTVL (SEQ ID
NO: 309)

QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYRSSTLGPVFGGGTKLTVL (SEQ ID
NO: 310)

QAGLTQPPSVSEAPRQRVTISCSGSSSNIGNNAVNWYQQLPGKAPKLLIYYDDLLPSGVSD
RFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGYVFGTGTKLTVL (SEQ ID
NO: 311)

QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGVP
DRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSTTHVFGTGTKVTVL (SEQ ID
NO: 312)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSVWVFGGGTQLTVL (SEQ ID
NO: 313)

QSVLTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGRAPRLMIYDVSNRPSGVS
NRFSGSKSGNTASLTISGLQAEDEGDYYCSSYTSGGTLGPVFGGGTKLTVL (SEQ ID
NO: 314)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAVVFGGGTKLTVL (SEQ ID
NO: 315)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQVPGTAPKLLIYDNNKRPSGIPD
RFSGSNSDTSATLGITGLQTGDEADYYCGTWDSSLSAWVFGGGTKLTVL (SEQ ID
NO: 316)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAGSVVFGGGTKLTVL (SEQ ID
NO: 317)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCLVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 318)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 319)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 320)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 321)

[00178] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequences:
QVQLVQSGSEVKKSGSSVKVSCKTSGGTFSITNYAINWVRQAPGQGLEWMGGILPIFGAAK
YAQKFQDRVTITADESTNTAYLELSSLTSEDTAMYYCARGKRWLQSDLQYWGQGTLVTVSS
(SEQ ID NO: 322)

VL Sequences:
QPVLTQPASVSGSPGQSITISCTGSSSDVGSYDLVSWYQQSPGKVPKLLIYEGVKRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYAGTRNFVFGGGTQLTVL (SEQ ID
NO: 323)

[00179] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a VH sequence and a VL sequence selected from the group consisting of:

VH Sequences:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGQSRPGFDYWGQGTLVTVSS (SEQ ID NO: 324)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGQSWPGFDYWGQGTLVTVSS (SEQ ID NO: 325)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGQSFPGFDYWGQGTLVTVSS (SEQ ID NO: 326)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAAFDYWGQGTLVTVSS (SEQ ID NO: 327)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAGYDYWGQGTLVTVSS (SEQ ID NO: 328)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSKGFDYWGQGTLVTVSS (SEQ ID NO: 329)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWKQGIVTVYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTV (SEQ ID NO: 330)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRNGIVTVYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 331)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSDIWKQGMVTVYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 332)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRQGLATAYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 333)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSEIVATGILTSYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 334)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIGRQGLITVYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 335)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWYQGLVTVYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 336)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSDIWKQGFATADS VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 337)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWKQGIVTVYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 338)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRQGLATAYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 339)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRNGIVTVYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAAFDYWGQGTLVTVSS (SEQ ID NO: 340)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRNGIVTVYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAGYDYWGQGTLVTVSS (SEQ ID NO: 341)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRNGIVTVYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSKGFDYWGQGTLVTVSS (SEQ ID NO: 342)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMetSWVRQAPGKGLEWVSSIWYQGLVTV YADSVKGRFTISRDNSKNTLYLQMetNSLRAEDTAVYYCAKWSAAFDYWGQGTLVTVSS (SEQ ID NO: 343)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWYQGLVTVYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAGYDYWGQGTLVTVSS (SEQ ID NO: 344)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWYQGLVTVYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSKGFDYWGQGTLVTVSS (SEQ ID NO: 345)

VL Sequences:

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYYASTLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQDNGYPSTFGQGTKVEIKR (SEQ ID
NO: 346)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYYASTLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQDNGYPSTFGQGTKVEIKR (SEQ ID
NO: 347)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQDNGYPSTFGGGTKVEIKR (SEQ ID
NO: 348)

[00180] In some embodiments, the PDL1 binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a single chain Fv (scFv) sequence selected from the group consisting of:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSDITASGQRTTYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSKIAFDYWGQGTLVTVSSGGGGSG
GGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYKA
SRLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQRALKPVTFGQGTKVEIKR
(SEQ ID NO: 349)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSINKDGHYTSYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKNLDEFDYWGQGTLVTVSSGGGGSG
GGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAA
SSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPNTFGQGTKVEIKR
(SEQ ID NO: 350)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIMATGAGTLYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDGAGFDYWGQGTLVTVSSGGGGSG
GGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYSA

SQLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSRPSTFGQGTKVEIKR
(SEQ ID NO: 351)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLQWVSTITSSGAATYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKNYTGFDYWGQGTLVTVSSGGGGSG
GGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYNA
SSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYTYGPGTFGQGTKVEIKR
(SEQ ID NO: 352)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSSGGGGSG
GGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYYA
STLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQDNGYPSTFGQGTKVEIKR
(SEQ ID NO: 353)

PDL1 x OX40 Dual Targeting

[00181] In some embodiments, the fusion proteins are bispecific molecules that include a TBD that binds OX40 and a binding domain directed toward PDL1. In these, embodiments, the binding to PDL1 is capable of providing the additional crosslinking function and TNFRSF activation can be achieved with only one or two anti- OX40 TBDs. In these embodiments, the TNFRSF signaling is enhanced and focused by the presence of a PDL1 expressing cell.

Tetravalent hz1D10v1 IgG1-wt:
EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGLEWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCSRDVDGDFRGQGTLVTVKPGGSGGSE
VQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGLEWVSSISNRGLKTAYAE
SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCSRDVDGDFRGQGTLVTVKPGGGGDKTH
TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 387)

Tetravalent hz1D10v1 IgG1-xELL:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGLEWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCSRDVDGDFRGQGTLVTVKPGGSGGSE
VQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGLEWVSSISNRGLKTAYAE
SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCSRDVDGDFRGQGTLVTVKPGGGGDKTH
TCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 388)

Tetravalent hzG3v9 IgG1-wt:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKEREWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKPGGSGGS
EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKEREWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKPGGGGDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 389)

Tetravalent hzG3v9 IgG1-xELL:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKEREWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKPGGSGGS
EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKEREWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKPGGGGDK
THTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 390)

Hexavalent hz1D10v1 IgG1-wt:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGLEWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCSRDVDGDFRGQGTLVTVKPGGSGGSE

VQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGLEWVSSISNRGLKTAYAE
SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCSRDVDGDFRGQGTLVTVKPGGSGGSEV
QLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGLEWVSSISNRGLKTAYAES
VKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCSRDVDGDFRGQGTLVTVKPGGGGDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 391)

Hexavalent hz1D10v1 IgG1-xELL:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGLEWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCSRDVDGDFRGQGTLVTVKPGGSGGSE
VQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGLEWVSSISNRGLKTAYAE
SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCSRDVDGDFRGQGTLVTVKPGGSGGSEV
QLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKGLEWVSSISNRGLKTAYAES
VKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCSRDVDGDFRGQGTLVTVKPGGGGDKTHT
CPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 392)

Hexavalent hzG3v9 IgG1-wt:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKEREWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKPGGSGGS
EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKEREWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKPGGSGGS
EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKEREWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKPGGGGDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 393)

Hexavalent hzG3v9 IgG1-xELL:

EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKEREWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKPGGSGGS
EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKEREWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKPGGSGGS
EVQLLESGGGEVQPGGSLRLSCAASGFTFSDAFMYWVRQAPGKEREWVSSISNRGLKTAYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCVEGDWNLGPRGQGTLVTVKPGGGGDK
THTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 394)

[00182] In some embodiments, the fusion proteins are multispecific containing a TBD and a binding domain directed toward Folate Receptor Alpha (FRα). In these, embodiments, the binding to FRα is capable of providing the additional crosslinking function and TNFRSF activation can be achieved with only one or two TBDs. In these embodiments, the TNFRSF signaling is enhanced and focused by the presence of a FRα expressing cell.

[00183] Exemplary FRα -targeting single domain sequences are shown below:

Fra-5:

QLQLQESGGGLVQAGGSLRLSCAASGIMFYISDMGWYRQAPGKQREFVATITSGGTTNYAD
SVEGRFSISRDNAKNTVYLQMNSLEPEDTAVYYCTAHGPTYGSTWDDLWGQGTQVTVKPGG
(SEQ ID NO: 354)

CDR1: GIMFYISD (SEQ ID NO: 360)

CDR2: ITSGGTT (SEQ ID NO: 361)

CDR3: TAHGPTYGSTWDDL (SEQ ID NO: 362)

Fra-6:

QVQLQESGGGLVQAGGSLRLSCAASETFGVVFTLGWYRQTPGKQREFVARVTGTDTVDYAD
SVKGRFTISSDFARNTVYLQMNNLKPEDTAVYYCNTGAYWGQGTQVTVKPGG (SEQ ID
NO: 355)

CDR1: ETFGVVFT (SEQ ID NO: 363)

CDR2: VTGTDTV (SEQ ID NO: 364)

CDR3: NTGAY (SEQ ID NO: 365)

Fra-57:

QVQLVQSGGGLVQTGGSLRLSCAASGRTASTYSMGWFRQAPGKERQFVARIIWSTGSTYYT
NSVEGRFTISRDIAKNTLYLQMNSLEPEDTAVYYCTAREPTGYDYWGQGTQVTVKPGG
(SEQ ID NO: 356)

CDR1: GRTASTYS (SEQ ID NO: 366)

CDR2: IWSTGST (SEQ ID NO: 367)

CDR3: TAREPTGYDY (SEQ ID NO: 368)

1A3:

QLQLQESGGGLVQAGGSLGLSCAASGSIFRFGARGWYRQAPGKQRELVAIITSGGSTNYAD
SVQGRFTISRDNAKNMVYLQMNGLKSGDTAVYYCAADRSDAVGVGWDYWGQGTQVTVKPGG
(SEQ ID NO: 357)

CDR1: GSIFRFGA (SEQ ID NO: 369)

CDR2: ITSGGST (SEQ ID NO: 370)

CDR3: AADRSDAVGVGWDY (SEQ ID NO: 371)

1F3:

QVQLQQSGGGLVQTGGSLRLSCAASGRTASTYSMGWFRQAPGKERQFVARIIWSTGSTYYT
NSVEGRFTISRDIAKNTLYLQMNSLEPEDTAVYYCTARDPTGYDYWGQGTQVTVKPGG
(SEQ ID NO: 358)

CDR1: GRTASTYS (SEQ ID NO: 366)

CDR2: IIWSTGST (SEQ ID NO: 372)

CDR3: TARDPTGYDY (SEQ ID NO: 373)

1G10:

QLQLQESGGGLVQAGGSLRLSCAASGSIFSIDATAWYRQAPGKQRELVAIITSSGSTNYPD
SVKGRFTISRDNAKNTVYLQMNSLNPEDTALYSCNAITRMGGSTYDFWGQGTQVTVKPGG
(SEQ ID NO: 359)

CDR1: GSIFSIDA (SEQ ID NO: 374)

CDR2: ITSSGST (SEQ ID NO: 375)

CDR3: NAITRMGGSTYDF (SEQ ID NO: 376)

[00184] In some embodiments, the fusion proteins contain a TBD fused to a tumor, bacterial or viral antigen (vaccine sequence). In these, embodiments, the binding to the TBD facilitates enhanced immunogenicity of the vaccine sequence thereby promoting acquired immunity to a tumor, bacteria or virus that expresses the vaccine sequence. In some embodiments, the TBD and the vaccine may be non-fused and introduced separately. In these, embodiments, the vaccine may be nucleic acid sequence, protein sequence or whole cells such as tumor cell, bacterial cells or virus. Vaccines may be monovalent (also called univalent) or multivalent (also called polyvalent). A monovalent vaccine is designed to immunize against a single antigen or single microorganism or cancer type. A multivalent or polyvalent vaccine is designed to immunize against two or more strains of the same microorganism or cancer type, or against two or more distinct microorganisms or cancer types.

[00185] The disclosure will be further described in the following examples, which do not limit the scope of the disclosure described in the claims.

EXAMPLES

Example 1. OX40-Targeting Single Domain Antibodies Bind OX40

[00186] The OX40-targeting single domain antibodies (sdAbs) referred to herein as 1A06 (SEQ ID NO: 16), 2B07, 2C09 (SEQ ID NO: 19), 1D10 (SEQ ID NO: 22), 2E4 (SEQ ID NO: 18), 2H06 (SEQ ID NO: 17), 3E11 (SEQ ID NO: 23), 3G9 (SEQ ID NO: 24), and G3 (SEQ ID NO: 25) bind cell surface OX40 expressed on CHO cells (Figures 2A-2B). Binding was assessed by flow cytometry using OX40 expressing CHO cells and data is presented as median fluorescence intensity.

[00187] The OX40-targeting sdAbs referred to herein as 1D10, G3, and 3E11 were also evaluated for their ability to bind cynomolgus OX40 expressed on CHO cells (Figure 4). Binding was assessed by flow cytometry using cynoOX40 expressing CHO cells and data is presented as median fluorescence intensity.

[00188] Various humanized versions of OX40-targeting sdAbs coupled to an Fc region were also evaluated for their ability to bind human OX40 and cynomolgus OX40

(Figures 12A and 12B). Binding was assessed by flow cytometry using OX40 expressing CHO cells and data is presented as median fluorescence intensity.

[00189]

Example 2. OX40-Targeting Single Domain Antibodies Block OX40

[00190] The OX40-targeting single domain antibodies (sdAbs) referred to herein as 1D10 (SEQ ID NO: 22), 2E4 (SEQ ID NO: 18), G3 (SEQ ID NO: 25), 3E11 (SEQ ID NO: 23), and H11 block the interaction between OX40 and OX40L. As shown in Figure 3, 2E4 blocks the interaction between OX40 and OX40L, while the other single domain antibodies do not. A single domain antibody that binds GITR was included as a negative control. Blocking was assessed by flow cytometry using a OX40L-citrine fusion protein and data is presented as median fluorescence intensity.

Example 3. Multivalent OX40-Targeting Molecules

[00191] Multiple copies of binding domains, such as, for example, single domain antibodies (sdAbs), can be operably linked together to produce multivalent OX-40 targeting molecules. In some embodiments, multiple OX40-targeting VHHs are operably linked to an Fc region polypeptide to produce multivalent OX40-targeting molecules.

[00192] Figure 5A is schematic depicting the format of various embodiments of multivalent OX40 binding fusion proteins and the estimated molecular weights for each. Figure 5B is a photograph of a Coomassie blue stain SDS-PAGE gel of multivalent OX40 binding fusion proteins under reducing and non-reducing conditions.

[00193] The OX40 multivalent molecules exhibit that enhanced OX40 signaling is mediated by higher valency binding. Figure 6 depicts the comparison between tetravalent and bivalent binding of OX40 using the fusion proteins of the present disclosure. OX40 signaling was monitored using an NF-kB reporter 293 cell line expressing OX40. Fusion proteins incorporating 1D10 binding domain (SEQ ID NO: 22) as the TBD were used in these assays.

[00194] Figures 7A depicts the comparison between tetravalent and hexavalent binding of OX40 using the fusion proteins of the present disclosure. Figure 7B depicts the comparison between hexavalent binding of OX40 using a fusion protein of the present

disclosure and a known hexameric OX40L-fusion protein, which is described in U.S. Patent No. 7,959,925). The multivalent OX40 binding fusion proteins of the present disclosure displayed equivalent or enhanced OX40 agonistic activity compared to the hexameric OX40L fusion protein. OX40 signaling was monitored using a NF-kB reporter 293 cell line expressing OX40. Fusion proteins incorporating 1D10 as the TBD were used in these assays.

[00195] Figures 8A and 8B are a series of graphs depicting the elution profile from a size exclusion chromatography (SEC) column (Superdex 200) for tetravalent (Fig. 8A) and hexavalent (Fig. 8B) 1D10. Calculated molecular weights based on the retention volume (standard curve) are also shown.

[00196] Figure 9 is a graph demonstrating OX40 signaling is mediated by various multivalent formats of the fusion proteins of the present disclosure, including hexavalent with three tandem OX40 VHHs linked to an Fc region, tetravalent with two tandem OX40 VHHs linked to an Fc region and tetravalent with an N-terminal and C-terminal OX40 VHH separated by an Fc region. OX40 signaling was monitored using an NF-kB reporter 293 cell line expressing OX40. Fusion proteins incorporating 1D10 as the TBD were used in for these assays.

[00197] Figures 14A and 14B are a pair of graphs demonstrating OX40 signaling is mediated by various multivalent formats of the fusion proteins of the present disclosure, including hexavalent with three tandem OX40 VHHs linked to an Fc region, tetravalent with two tandem OX40 VHHs linked to an Fc region and tetravalent with an N-terminal and C-terminal OX40 VHH separated by an Fc region. OX40 signaling was monitored using an NF-kB reporter 293 cell line expressing OX40. Fusion proteins incorporating hzG3v9 as the TBD were used in the tetravalent and hexavalent molecules for these assays.

[00198] Figures 10A, 10B, and 10C depict PDL1-dependent OX40 agonism mediated by bispecific PDL1-OX40 targeting fusion proteins of the present disclosure. Figures 10 A and B are conceptual schematics wherein the bispecific fusion protein have minimal OX40 agonistic properties (Figure 10A) unless bound by a PDL1 expressing cell (Figure 10B). Figure 10C is graph demonstrating the ability of a PDL1-positive cell (here PDL1 transfected CHO cells) to mediate OX40 signaling and the inability of PDL1-negative cell (here untransfected CHO cells) to mediate OX40 signaling. OX40 signaling was monitored using a NF-kB reporter 293 cell line expressing OX40. Numerous distinct

bispecific fusion proteins are shown in this figure, each containing a distinct OX40 binding VHH (e.g., G3, 2E4, 3G9, 1D10) and the same PDL1 VHH, 28A10.

[00199] Figure 11 depicts FRα-dependent OX40 agonists signaling mediated by bispecific FRa-OX40 targeting fusion proteins of the present disclosure. An FRα expressing ovarian cancer cell line, SKOV3, was used in these assays. OX40 signaling was monitored using a NF-kB reporter 293 cell line expressing OX40. Numerous distinct bispecific fusion proteins are shown in this figure, each containing a distinct FRα binding VHH (e.g., 1G10, 1A3, 57, 5) and the same OX40 VHH, 1D10.

[00200] Figures 13A and 13B are a pair of graphs depicting whether various humanized OX40 single domain antibodies bind to various TNFRSF members. Various humanized sdAbs of the disclosure as the TBD were used in the tetravalent and hexavalent molecules for these assays.

What is claimed is:

1. An isolated polypeptide that binds OX40 and comprises a plurality of Tumor Necrosis Factor receptor superfamily (TNFRSF) binding domain (TBDs), wherein at least a first TBD (TBD1) binds OX40.

2. An isolated polypeptide that binds PDL1 and comprises a plurality of polypeptide binding domains (BDs), wherein at least a first BD (BD1) binds PDL1.

3. An isolated polypeptide that binds PDL1 and OX40 and comprises a plurality of polypeptide binding domains (BDs) and a plurality of Tumor Necrosis Factor receptor superfamily (TNFRSF) binding domain (TBDs), wherein at least a first binding domain (BD1) binds PLD1 and at least a first TNFRSF binding domain (TBD1) binds OX40.

4. The isolated polypeptide of any one of claims 1 to 3, wherein the isolated polypeptide is monospecific.

5. The isolated polypeptide of any one of claims 1 to 3, wherein the isolated polypeptide is multispecific.

6. The isolated polypeptide of any one of claims 1 to 3, wherein the isolated polypeptide is bispecific.

7. The isolated polypeptide of any one of claims 1 to 3, wherein the polypeptide comprises at least a second TBD (TBD2) that binds a second TNFRSF member.

8. The isolated polypeptide of claim 1, wherein each of TBD in the plurality of TBDs binds OX40.

9. The isolated polypeptide of claim 8, wherein the plurality of TBDs binds the same epitope on OX40.

10. The isolated polypeptide of claim 8, wherein at least two TBDs in the plurality of TBDs bind a different epitope on OX40.

11. The isolated polypeptide of any one of claims 8 to 10, wherein the plurality of TBDs comprises at least four TBDs.

12. The isolated polypeptide of any one of claims 8 to 10, wherein the plurality of TBDs comprises at least six TBDs.

13. The isolated polypeptide of claim 2, wherein the polypeptide comprises at least a second BD (BD2) that binds a second antigen.

14. The isolated polypeptide of claim 2, wherein each BD in the plurality of BDs binds PDL1.

15. The isolated polypeptide of claim 14, wherein the plurality of BDs binds the same epitope on PDL1.

16. The isolated polypeptide of claim 14, wherein at least two BDs in the plurality of BDs bind a different epitope on PDL1.

17. The isolated polypeptide of any one of claims 13 to 16, wherein the plurality of BDs comprises at least four BDs.

18. The isolated polypeptide of any one of claims 13 to 16, wherein the plurality of BDs comprises at least six BDs.

19. The isolated polypeptide of any one of claims 1 to 3, wherein each of the TBD in the plurality of TBDs or each of the BDs in the plurality of BDs are operably linked via a linker polypeptide.

20. The isolated polypeptide of any one of claims 1 to 3, wherein the isolated polypeptide comprises at least one binding domain that binds a target, provided that the target is not a TNFRSF member.

21. The isolated polypeptide of claim 1, wherein at least one of the TBD in the plurality of TBDs binds a second TNFRSF member selected from the group consisting of DR5, GITR, and CD137.

22. The isolated polypeptide of any one of claims 1 to 3, wherein the isolated polypeptide comprises a heterodimerization domain.

23. The isolated polypeptide of any one of claims 1 to 3, wherein the isolated polypeptide comprises an immunoglobulin Fc region polypeptide.

24. The isolated polypeptide of claim 23, wherein the immunoglobulin Fc region polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6.

25. The isolated polypeptide of any one of claims 1 to 24, wherein at least one TBD in the plurality of TBDs or at least one BD in the plurality of BDs comprises an antibody or antigen-binding fragment thereof.

26. The isolated polypeptide of any one of claims 1 to 24, wherein each TBD in the plurality of TBDs or each BD in the plurality of BDs comprises an antibody or antigen-binding fragment thereof.

27. The isolated polypeptide of claim 25 or claim 26, wherein the antibody or antigen-binding fragment thereof is a scFv, a Fab, a single domain antibody (sdAb), a V_{NAR}, or a VHH.

28. The isolated polypeptide of claim 25 or claim 26, wherein the antibody or antigen-binding fragment is a sdAb.

29. The isolated polypeptide of claim 28, wherein the sdAb is a human or humanized sdAb.

30. The isolated polypeptide of claim 28, wherein the sdAb is VHH, V_{NAR}, an engineered VH domain or an engineered VK domain.

31. The isolated polypeptide of claim 30, wherein the sdAb is generated from a cartilaginous fish heavy chain only antibody.

32. The isolated polypeptide of any one of the preceding claims, wherein at least one of the binding domains comprises a non-antibody scaffold protein.

33. The isolated polypeptide of claim 32, wherein the non-antibody scaffold protein is an ankyrin repeat protein, a darpin, an avimer, an anticalin/lipocalin, a centyrin, or a fynomer.

34. The isolated polypeptide of claim 1 or claim 3, wherein the polypeptide comprises an amino acid sequence that binds OX40 selected from the group consisting of SEQ ID NO: 16-29 and 377-386.

34. The isolated polypeptide of claim 1 or claim 3, wherein the polypeptide comprises an amino acid sequence that binds OX40 and comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 30, 36, and 44; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 31, 35, and 37; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 32-34, 48-43, and 45.

36. The isolated polypeptide of claim 2 or claim 3, wherein the polypeptide comprises an amino acid sequence that binds PDL1 selected from the group consisting of SEQ ID NO: 52-57.

37. The isolated polypeptide of claim 2 or claim 3, wherein the polypeptide comprises an amino acid sequence that binds PDL1 and comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 58, 61, and 64; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 59, 62, 65, and 69; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 60, 63, 66-68, and 70.

38. The isolated polypeptide of claim 3, wherein the polypeptide comprises a first amino acid sequence that binds OX40 selected from the group consisting of SEQ ID NO: 52-57, and a second amino acid sequence that binds PDL1 selected from the group consisting of SEQ ID NO: 52-57.

39. The isolated polypeptide of claim 3, wherein the polypeptide comprises an amino acid sequence that binds OX40 and comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 30, 36, and 44; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 31, 35, and 37; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 32-34, 48-43, and 45, and wherein the polypeptide comprises an amino acid sequence that binds PDL1 and comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 58, 61, and 64; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 59, 62, 65, and 69; and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 60, 63, 66-68, and 70.

40. The isolated polypeptide of any one of claims 1 to 3, wherein the polypeptide is tetravalent.

41. The isolated polypeptide of claim 40, wherein the polypeptide comprises the structure: VHH-Linker-VHH-Linker-Hinge-Fc, where the VHH is a humanized or fully human VHH sequence.

42. The isolated polypeptide of any one of claims 1 to 3, wherein the polypeptide is hexavalent.

43. The isolated polypeptide of claim 42, wherein the polypeptide comprises the structure: VHH-Linker-VHH-Linker-VHH-Linker-Hinge-Fc, where the VHH is a humanized or fully human VHH sequence.

44. An isolated polypeptide that binds OX40 and PDL1, wherein the polypeptide comprises an amino acid sequence that is selected from the group consisting of SEQ ID NO: 387-394.

45. Use of the polypeptide of any one of claims 1 to 44 for treating neoplasms.

46. Use of the polypeptide of any one of claims 1 to 44 for modulating immune cells to enhance tumor destruction.

47. Use of the polypeptide of any one of claims 1 to 44 for treating an inflammatory disorder.