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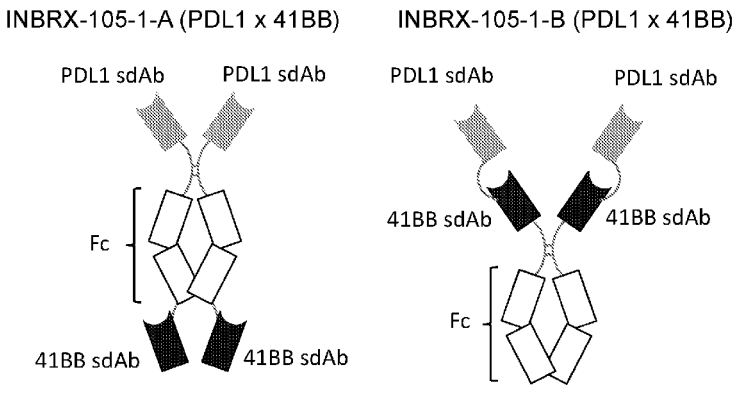
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[Continued on next page]

(54) Title: MULTIVALENT AND MULTISPECIFIC 41BB-BINDING FUSION PROTEINS

FIG. 13



(57) Abstract: This invention relates generally to molecules that specifically engage 41BB, a member of the TNF receptor superfamily (TNFRSF). More specifically, this invention relates to multivalent and multispecific molecules that bind at least 41BB.

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MULTIVALENT AND MULTISPECIFIC 41BB-BINDING FUSION PROTEINS

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/277,028, 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 41BB, a member of the TNF receptor superfamily (TNFRSF). More specifically, this invention relates to multivalent and/or multispecific molecules that bind at least 41BB.

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 purpose. 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 41BB (also known as tumor necrosis factor receptor superfamily, member 4 (TNFRSF9) and/or CD137)). The use of the term "41BB" is intended to cover any variation thereof, such as, by way of non-limiting example, 41-BB and/or 4-1BB, and all variations are used herein interchangeably. These molecules that bind at least 41BB are referred to herein as "41BB-targeting molecules" or "41BB-targeting fusions" or "41BB-targeting proteins" or "41BB-targeting fusion polypeptides" or "41BB-targeting fusion proteins." In some embodiments, the 41BB-

targeting molecule is a multivalent molecule, for example, a multivalent 41BB-targeting fusion protein. In some embodiments, the 41BB-targeting molecule is a multispecific molecule, for example, a multispecific 41BB-targeting fusion protein. In some embodiments, the 41BB-targeting molecule is a multivalent and multispecific molecule, for example, a multivalent and multispecific 41BB-targeting fusion protein. As used herein, the term "fusion protein" or "fusion polypeptide" or "41BB-targeting fusion protein" or "41BB-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 41BB. These molecules that bind at least PDL1 are referred to herein as "PDL1x41BB-targeting molecules" or "PDL1x41BB-targeting fusions" or "PDL1x41BB-targeting proteins" or "PDL1x41BB-targeting fusion polypeptides" or "PDL1x41BB-targeting fusion proteins." In some embodiments, the PDL1x41BB-targeting molecule is a multivalent molecule, for example, a multivalent PDL1x41BB-targeting fusion protein. In some embodiments, the PDL1x41BB-targeting

molecule is a multispecific molecule, for example, a multispecific PDL1x41BB-targeting fusion protein. In some embodiments, the PDL1x41BB-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 "PDL1x41BB-targeting fusion protein" or "PDL1x41BB-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] In some embodiments, the multivalent and/or multispecific fusion protein binds at least 41BB. 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 41BB. 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 41BB. In some embodiments, the fusion protein contains multiple copies of a TBD that binds 41BB. For example, in some embodiments, the fusion protein contains at least two copies of a TBD that binds 41BB. In some embodiments, the fusion protein contains at least three copies of a TBD that binds 41BB. In some embodiments, the fusion protein contains at least four copies of a TBD that binds 41BB. In some embodiments, the fusion protein contains at least five copies of a TBD that binds

41BB. In some embodiments, the fusion protein contains at least six copies of a TBD that binds 41BB. In some embodiments, the fusion protein contains six or more copies of a TBD that binds 41BB.

[0010] In other embodiments, the fusion proteins of the present disclosure bind 41BB and a second TNFRSF member for example GITR, OX40, 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] In these embodiments, binding to the TNFRSF member by the TBD induces minimal signaling unless the second antigen is co-engaged. For example, the multispecific fusion proteins of the present disclosure are capable binding 41BB and PD-L1 and 41BB-dependent signaling is greatly enhanced when the fusion protein is bound to a PD-L1 expressing cell. In another example, the multispecific fusion proteins of the present disclosure are capable binding 41BB and Folate Receptor Alpha (FRα) and 41BB-dependent signaling is greatly enhanced when the fusion protein is bound to a FRα expressing cell.

[0014] The present disclosure provides isolated polypeptides that specifically bind 41BB. 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_{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 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.

[0015] In some embodiments, the isolated polypeptide includes an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 20, 23, 25, 29, 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83. In some embodiments, the isolated polypeptide includes an amino acid sequence selected from the group consisting of SEQ ID NO: 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83.

[0016] 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, 20, 23, 25, 29, 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83. 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: 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83.

[0017] 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: 17, 21, 26, 30, 50, 65, and 69; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 27, 31, 42, 44, 48, 52, 61, 63, 71, 73, 75, 77, and 79; and a

complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 19, 22, 24, 28, 32, 55, and 57.

[0018] 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.

[0019] 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.

[0020] The present disclosure provides isolated polypeptides that specifically bind 41BB. 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_{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 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.

[0021] In some embodiments, the isolated polypeptide includes an amino acid sequence selected from the group consisting of SEQ ID NO: 100, 104, 108, 112, 114, 116, and 119-124. In some embodiments, the isolated polypeptide includes an amino acid sequence selected from the group consisting of SEQ ID NO: 119-124.

[0022] 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: 100, 104, 108, 112, 114, 116, and 119-124. 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: 119-124.

[0023] 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: 101, 105, and 109; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 102, 106, 110, and 117; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 103, 107, 111, 113, 115, and 118.

[0024] In some embodiments, the present disclosure provides isolated polypeptides that specifically bind at least 41BB 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.

[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, 20, 23, 25, 29, 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83, and a second amino acid sequence that binds PDL1 selected from the group consisting of SEQ ID NO: 100, 104, 108, 112, 114, 116, and 119-124.

[0026] In some embodiments, the isolated polypeptide includes a first amino acid sequence that binds 4B11 selected from the group consisting of SEQ ID NO: 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83, and a second amino acid sequence that binds PDL1 selected from the group consisting of SEQ ID NO: 119-124.

[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, 20, 23, 25, 29, 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83, 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: 100, 104, 108, 112, 114, 116, and 119-124.

[0028] 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: 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83, 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: 119-124.

[0029] 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: 17, 21, 26, 30, 50, 65, and 69; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 27, 31, 42, 44, 48, 52, 61, 63, 71, 73, 75, 77, and 79; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 19, 22, 24, 28, 32, 55, and 57; 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: 101, 105, and 109; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 102, 106, 110, and 117; and a CDR3 comprising

an amino acid sequence selected from the group consisting of SEQ ID NO: 103, 107, 111, 113, 115, and 118.

[0030] In some embodiments, the binding domains (BDs) of the present disclosure, e.g., the 41BB-binding domains and/or the PDL1-binding domains, 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 BDs are derived from non-antibody scaffold proteins for example but not limited to designed ankyrin repeat proteins (darpins), avimer, anticalin/lipocalins, centyrins and fynomers.

[0031] 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 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.

[0032] In some embodiments, all of the BDs of the fusion protein are TBDs that 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 41BB. 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 41BB. 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 41BB or OX40 and 41BB, or CD27 and 41BB.

[0033] In some embodiments, the multispecific fusion protein is a bispecific molecule that targets 41BB and PDL1. In some embodiments, the bispecific fusion protein includes a 41BB-targeting binding domain selected from the group consisting of SEQ ID NO: 16, 20, 23, 25, 29, 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83, operably linked to a second binding domain (BD2) that binds PDL1. In some embodiments, the BD2 comprises an amino acid sequence that specifically binds PDL1. In some embodiments, the BD2 comprises a PDL1-targeting domain selected from the group consisting of SEQ ID NO: 100, 104, 108, 112, 114, 116, and 119-124. some embodiments, the BD2 comprises a PDL1-targeting domain selected from the group consisting of SEQ ID NO: 119-124. In some embodiments, the BD2 comprises an amino acid sequence that specifically binds PDL1 and is selected from the group consisting of SEQ ID NO: 126-408.

[0034] In some embodiments, the multispecific fusion protein is a bispecific molecule that targets 41BB and PDL1. In some embodiments, the bispecific fusion protein includes a 41BB-targeting binding domain selected from the group consisting of SEQ ID NO: 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83, operably linked to a second binding domain (BD2) that binds PDL1. In some embodiments, the BD2 comprises an amino acid sequence that specifically binds PDL1. In some embodiments, the BD2 comprises a PDL1-targeting domain selected from the group consisting of SEQ ID NO: 100, 104, 108, 112, 114, 116, and 119-124. In some embodiments, the BD2 comprises a PDL1-targeting domain selected from the group consisting of SEQ ID NO: 119-124. In some embodiments, the BD2 comprises an amino acid sequence that specifically binds PDL1 and is selected from the group consisting of SEQ ID NO: 126-408.

[0035] In some embodiments, the multispecific fusion protein is a bispecific molecule that targets 41BB and PDL1. In some embodiments, the bispecific fusion protein includes a PDL1-targeting binding domain selected from the group consisting of SEQ ID NO: 100, 104, 108, 112, 114, 116, and 119-124, operably linked to a second TBD (TBD2) that binds 41BB. In some embodiments, the TBD2 comprises an amino acid sequence that specifically binds 41BB. In some embodiments, the TBD2 comprises a 41BB-targeting domain selected from the group consisting of SEQ ID NO: 16, 20, 23, 25, 29, 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83. In some

embodiments, the TBD2 comprises an amino acid sequence that specifically binds 41BB and is selected from the group consisting of SEQ ID NO: 84-99.

[0036] In some embodiments, the multispecific fusion protein is a bispecific molecule that targets 41BB and PDL1. In some embodiments, the bispecific fusion protein includes a PDL1-targeting binding domain selected from the group consisting of SEQ ID NO: 119-124, operably linked to a second TBD (TBD2) that binds 41BB. In some embodiments, the TBD2 comprises an amino acid sequence that specifically binds 41BB. In some embodiments, the TBD2 comprises a 41BB-targeting domain selected from the group consisting of SEQ ID NO: 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83. In some embodiments, the TBD2 comprises an amino acid sequence that specifically binds 41BB and is selected from the group consisting of SEQ ID NO: 84-99.

[0037] In some embodiments, the multispecific fusion protein is a bispecific molecule that targets 41BB and PDL1 and comprises an amino acid sequence that is selected from the group consisting of SEQ ID NO: 448-456.

[0038] In some embodiments, the multispecific fusion protein is a bispecific molecule that targets 41BB 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: 448-456.

[0039] 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 contain multiple BDs that target distinct regions of the PDL1. In some embodiments, the BDs may recognize different epitopes on PDL1.

[0040] 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.

[0041] In some embodiments, the fusion protein contains the BDs 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.

[0042] 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 subclass, IgG2 subclass, IgG3 subclass, and IgG4 subclass.

[0043] 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 subclass, having an amino acid sequence:

PAP**ELL**[G]GPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
DGVEVHNAKT KPREEQY[N]ST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV
EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
EALHNHYTQK SLSLSPGK (SEQ ID NO: 1)

[0044] 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.

[0045] 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.*, Leu234Ala (L234A). In some embodiments, the Fc region of the fusion protein is 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 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*).

[0046] 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).

[0047] 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)

[0048] 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.

[0049] In some embodiments, the immunoglobulin Fc region or immunologically active fragment of the fusion protein is of human IgG2 subclass, 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)

[0050] 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.

[0051] 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*).

[0052] In some embodiments, the immunoglobulin Fc region or immunologically active fragment of the fusion protein is of human IgG3 subclass, 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)

[0053] 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.

[0054] 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 218 of SEQ ID NO: 4 (EU index of *Kabat et al* 1991 *Sequences of Proteins of Immunological Interest*).

[0055] In some embodiments, the immunoglobulin Fc region or immunologically active fragment of the fusion protein is of human IgG4 subclass, having an amino acid sequence:

PAPEFLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV
DGVEVHNAKT KPREEQFNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP
SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV
EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH
EALHNHYTQK SLSLSLGK (SEQ ID NO: 5)

[0056] 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.

[0057] 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: 5 (EU index of *Kabat et al* 1991 *Sequences of Proteins of Immunological Interest*).

[0058] 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 KPREEQFNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP

SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH EALHNHYTQK SLSLSLGK (SEQ ID NO: 6)

[0059] 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.

[0060] 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.

[0061] 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: 2, 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).

[0062] 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: 2, 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.

[0063] 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.

[0064] 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).

[0065] 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 subclasses. 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).

[0066] 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 linker and hinge polypeptides.

[0067] 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.

[0068] 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.

[0069] In some embodiments, the BDs 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.

[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 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.

[0072] 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.

[0073] 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.

[0074] 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 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.

[0075] 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).

[0076] 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.

[0077] 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.

[0078] 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.

[0079] In some embodiments, the TBD of the hexavalent TNFRSF binding fusion protein is a single domain antibody or VHH. In some embodiments, each TBD of the hexavalent TNFRSF binding fusion protein is a single domain antibody or VHH. 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.

[0080] 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.

[0081] 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.

[0082] 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.

[0083] 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 some of these embodiments, the TNFRSF binding fusion protein is tetravalent and has the following structure VHH-Linker-VHH-Linker-VHH-Linker-VHH-Linker. In some of these embodiments, the TNFRSF binding fusion protein is pentavalent and has the following structure VHH-Linker-VHH-Linker-VHH-Linker-VHH-Linker-VHH. In some of 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 any of these embodiments, the VHH is a humanized or fully human VHH sequence.

[0084] 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).

[0085] 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.

[0086] In some embodiments, the fusion proteins are multispecific containing an anti-41BB 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-41BB binding domain. In some embodiments, the second antigen binding domain is located N-terminal an anti-41BB binding domain. In other embodiments, the second antigen binding domain is located to C-terminal to the an anti-41BB 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-41BB binding domain.

[0087] 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.

[0088] 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.

[0089] In some embodiments, the TBD within the multispecific TNFRSF binding fusion protein is a single domain antibody or VHH that binds 41BB. In some embodiments, the anti-41BB 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-41BB 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-41BB 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-41BB binding domain. In some embodiments, the anti-41BB 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.

[0090] 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.

[0091] In some embodiments, the anti-41BB binding domain of the multispecific TNFRSF binding fusion protein is a bispecific antibody or antigen-binding fragment thereof.

[0092] In some embodiments, the anti-PDL1 binding domain of the multispecific fusion protein is a bispecific antibody or antigen-binding fragment thereof.

[0093] 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.

[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, 20, 23, 25, 29, 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83.

[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: 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83.

[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: 17, 21, 26, 30, 50, 65, and 69; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 27, 31, 42, 44, 48, 52, 61, 63, 71, 73, 75, 77, and 79; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 19, 22, 24, 28, 32, 55, and 57.

[0097] In any of the above embodiments, at least one BD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 100, 104, 108, 112, 114, 116, and 119-124.

[0098] In any of the above embodiments, at least one BD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 119-124.

[0099] 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: 101, 105, and 109; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 102, 106, 110, and 117; and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 103, 107, 111, 113, 115, and 118.

[00100] 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, 20, 23, 25, 29, 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83, and at least one BD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 100, 104, 108, 112, 114, 116, and 119-124.

[00101] In any of the above embodiments, at least one TBD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83, and at least one BD

comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 119-124.

[00102] 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: 17, 21, 26, 30, 50, 65, and 69; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 27, 31, 42, 44, 48, 52, 61, 63, 71, 73, 75, 77, and 79; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 19, 22, 24, 28, 32, 55, and 57, and at least one BD comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 101, 105, and 109; a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 102, 106, 110, and 117; and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 103, 107, 111, 113, 115, and 118.

BRIEF DESCRIPTION OF THE DRAWINGS

[00103] Figure 1 is schematic of exemplary multivalent and multispecific fusion proteins of the present disclosure.

[00104] Figures 2A and 2B are a pair of graphs demonstrating the ability of 41BB single domain antibodies (sdAbs) to bind recombinant human 41BB (Figure 2A) or cyno 41BB (Figure 2B). Binding was assessed by ELISA wherein recombinant 41BB-mFc protein was immobilized on a Medisorp 96 well plate.

[00105] Figure 3 is a graph demonstrating the ability of 41BB single domain antibodies (sdAbs) to bind cell surface 41BB. Binding was assessed by flow cytometry using 41BB expressing CHO cells and data is presented as median fluorescence intensity.

[00106] Figure 4 is a graph demonstrating the ability of 41BB single domain antibodies, RH3 and 4H04 to bind cynomolgus monkey 41BB. Binding was assessed by ELISA wherein recombinant 41BB-mFc protein was immobilized on a Medisorp 96 well plate.

[00107] Figure 5 is a graph demonstrating the capacity of 41BB single domain antibodies (VHHs) to block the interaction between 41BB and 41BBL. All single domain antibodies tested, with the exception of RH3 blocks the interaction between 41BB and

41BBL. Blocking was assessed by flow cytometry using a recombinant 41BB fusion protein and 41BB expressing CHO cells, data is presented as median fluorescence intensity.

[00108] Figure 6 is a graph demonstrating the inability of a conventional bivalent anti-41BB antibody PF-05082566 to induce 41BB signaling unless further clustered with an exogenous crosslinking anti-human IgG antibody. 41BB signaling was monitored using a NF-kB reporter 293 cell line expressing 41BB.

[00109] Figures 7A and 7B are a pair of graphs demonstrating the capacity of an exemplary PDL1 single domain antibody (28A10) to bind cell surface PDL1 and to block the interaction with PD1. Binding (Figure 7A) was assessed by flow cytometry on PDL1 expressing CHO cells. Blocking (Figure 7B) was assessed by flow cytometry using a recombinant PD1 fusion protein and PDL1 expressing CHO cells, data is presented as median fluorescence intensity.

[00110] Figures 8A, 8B, and 8C are a series of illustrations and a graph depicting PDL1-dependent 41BB agonism mediated by bispecific PDL1-41BB targeting fusion proteins of the present disclosure. Figures 8A and 8B are conceptual schematics, wherein the bispecific fusion proteins have minimal 41BB agonistic properties (Figure 8A) unless bound by a PD-L1 expressing cell (Figure 8B). Figure 8C is a graph demonstrating the ability of a PDL1-positive cell (here PDL1 transfected CHO cells) to mediate 41BB signaling and the inability of PDL1-negative cell (here untransfected CHO cells) to mediate 41BB signaling. 41BB signaling was monitored using a NF-kB reporter 293 cell line expressing 41BB.

[00111] Figures 9A, 9B, 9C, 9D, and 9E are a series of graphs demonstrating the binding to human (Figure 9A and Figure 9C) or cynomolgus monkey (Figure 9B and Figure 9D) 41BB of humanized RH3 variants. Binding was assessed by flow cytometry on 41BB expressing 293freestyle cells. Figure 9E is a graph demonstrating that the humanized variants hzRH3v5-1 and hzRH3v9 do not block binding of 41BBL to cell surface 41BB. Herein a recombinant fusion protein 41BBL-mFc, containing a mouse Fc region was used and bound 41BBL was detected using an anti-mouse IgG-Fc specific secondary antibody.

[00112] Figure 10 is a graph demonstrating the specific binding of hzRH3v5-1 (40nM) to 41BB compared to other TNFRSF members OX40 and GITR. Binding was assessed by flow cytometry using CHO cells expressing the given TNFRSF member.

[00113] Figures 11A, 11B, 11C, and 11D are a series of graphs demonstrating the binding to human (Figure 11A and Figure 11C) or cynomolgus monkey (Figure 11B) 41BB

of humanized 4E01 variants. Binding was assessed by flow cytometry on 41BB expressing 293freestyle cells. Figure 11D is a graph demonstrating that the humanized variants hz4E01v16, hz4E01v18, hz4E01v21, hz4E01v22 and hz4E01v23 block binding of 41BBL to cell surface 41BB. In these studies, a recombinant fusion protein 41BBL-mFc, containing a mouse Fc region was used and bound 41BBL was detected using an anti-mouse IgG-Fc specific secondary antibody.

[00114] Figure 12 is a graph demonstrating binding of humanized single domain antibodies targeting PDL1. Binding was assessed by flow cytometry on PDL1-expressing CHO cells.

[00115] Figure 13 is a schematic of two exemplary formats of a PDL1x41BB bispecific, INBRX-105-1. INBRX-105-1-A (left) has the PDL1 and 41BB binding domains, located at opposing terminal positions with a central Fc region, whereas INBRX-105-1-B (right) has the PDL1 and 41BB binding domains positioned in tandem, N-terminal to an Fc region.

[00116] Figures 14A, 14B, and 14C are a series of graphs demonstrating the equivalent binding to human (Figure 14A) or cynomolgus monkey (Figure 14B) 41BB by the two distinct formats of a bispecific fusion protein targeting PDL1 and 41BB referred to herein as INBRX-105-1-A and INBRX-105-1-B. Binding was assessed by flow cytometry on 41BB expressing 293freestyle cells. Figure 14C is a graph that demonstrates that the bispecific fusion protein containing hzRh3v5-1 does not block 41BBL binding to cell surface 41BB. Herein a recombinant fusion protein of 41BBL and mouse Fc region was used and bound 41BBL was detected using an anti-mouse IgG-Fc specific secondary antibody.

[00117] Figures 15A, 15B, 15C, and 15D are a series of graphs demonstrating the equivalent binding (Figure 15A and Figure 15C). and PD1 blocking (Figure 15B and Figure 15D) by the two distinct formats of a bispecific fusion protein targeting PDL1 and 41BB referred to herein as INBRX-105-1-A and INBRX-105-1-B. Binding was assessed by flow cytometry on human (Figure 15A) or cynomolgus monkey (Figure 15C) PDL1 expressing 293freestyle cells. Blocking was assessed by flow cytometry using on human (Figure 15B) or cynomolgus monkey (Figure 15D) PDL1 expressing 293freestyle cells with either recombinant human (Figure 15B) or cynomolgus monkey (Figure 15D) PD1-mFc fusion protein. Bound PD1 was detected using an anti-mouse IgG-Fc specific secondary antibody.

[00118] Figure 16 is a graph demonstrating the ability of humanized versions of a PDL1x41BB bispecific fusion protein (INBRX-105-1) to induce PDL1-dependent 41BB agonism. A 41BB-expressing HEK293 NF-kB reporter cell line was used to assess 41BB signaling and a PDL1-expressing CHO cell line was used as the source of PDL1.

[00119] Figures 17A and 17B are a pair of graphs demonstrating the 41BB-specific binding by the 41BB-binding portion of a PDL1x41BB bispecific fusion protein (INBRX-105-1) of the present disclosure. Binding was assessed on 41BB (Figure 17A) or the closest homolog, TNFRSF21/DR6 (Figure 17B), expressing 293freestyle cells by flow cytometry. An anti-DR6 antibody (Invitrogen) was used to as positive control for DR6 expression.

[00120] Figures 18A, 18B, and 18C are a series of graphs demonstrating the PDL1-specific binding by the PDL1-binding portion of a PDL1x41BB bispecific fusion protein (INBRX-105-1) of the present disclosure. Binding was assessed on PDL1 (Figure 18A), and its closest homologs PDL2 (Figure 18B) or VISTA/PDL3 (Figure 18C), expressing 293freestyle cells by flow cytometry. Anti-PDL2 and anti-VISTA antibodies were used to as positive controls for PDL2 and PDL3 expression respectively.

[00121] Figures 19A and 19B are a pair of graphs demonstrating the ability of a PDL1x41BB bispecific fusion protein to simultaneously bind PDL1 and 41BB. Bound 41BB was detected using an anti-mouse IgG-Fc specific secondary antibody. Figure 19A. is a graph showing the binding of INBRX-105-1 to the PDL1 expressing K562 cells. Figure 19B is a graph showing the binding of recombinant 41BB to INBRX-105-1 on the PDL1 expressing cells.

[00122] Figure 20 is a graph demonstrating the ability of a PDL1x41BB bispecific fusion protein to simultaneously bind recombinant PDL1 and recombinant 41BB in an ELISA. Bound recombinant 41BB was detected via streptavidin-HRP.

[00123] Figures 21A, 21B, and 21C are a series of graphs demonstrating the effect of a PDL1x41BB bispecific fusion protein (INBRX-105-1) of the present disclosure on T-cell activation and proliferation. INFγ production in the cell supernatant was monitored using an ELISA and normalized to the standard curve. T-cell proliferation was monitored by flow cytometry using CTV labeling of T-cells. T-cell activation was assessed by the presence of the activation marker CD25 monitored by flow cytometry. Antibodies were used at 10nM.

[00124] Figures 22A and 22B are a pair of graphs demonstrating PDL1-dependent 41BB agonism mediated by a PDL1x41BB bispecific fusion protein (INBRX-105-1) of the present disclosure. $CD8^+$ T-cell proliferation (Figure 22A) was monitored using CTV

labeling and INFγ production (Figure 22B) in the cell supernatant was monitored using an ELISA and normalized to the standard curve.

[00125] Figure 23 is a graph demonstrating the capacity of a PDL1x41BB bispecific fusion protein (INBRX-105-1) of the present disclosure to enhance the Th1 lineage defining transcription factor, T-bet, expression in T-cell populations. T-bet expression was assessed on $CD4^+$ and $CD8^+$ T-cell population by flow cytometry via intracellular staining following fixation and permeabilization.

[00126] Figures 24A and 24B are a pair graphs contrasting the capacity of a PDL1x41BB bispecific fusion protein (INBRX-105-1) of the present disclosure and the combination of monospecific antibodies Atezolizumab (anti-PDL1) and Utomilumab (anti-41BB) to induce INFγ (Figure 24A) or TNFα (Figure 24B) production from $CD4^+$ or $CD8^+$ T-cells. Cytokine expression was assessed on $CD4^+$ and $CD8^+$ T-cell population by flow cytometry via intracellular staining following fixation and permeabilization.

[00127] Figures 25A and 25B are a pair of graphs demonstrating the agonistic capacity of a tetravalent 41BB-binding fusion protein and PDL1x41BB bispecific fusion proteins of the present disclosure in the presence of an additional PDL1 positive (Figure 25A) or negative (Figure 25B) cell line. Herein a 41BB-expressing HEK293 NF-kB reporter cell was used and co-incubated with either the PDL1-negative K562 cell line (Figure 25B) or a stably transfected, PDL1-expressing K562 cell line (Figure 25A).

DETAILED DESCRIPTION OF THE INVENTION

[00128] 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

[00129] 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.

[00130] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[00131] 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, Fv, scFvs, a Fab expression library, and single domain antibody (sdAb) fragments, for example V_HH, V_{NAR}, engineered V_H or V_K.

[00132] 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.

[00133] 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.

[00134] 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).

[00135] The single domain antibody (sdAb) fragments portions of the fusion proteins of the present disclosure are referred to interchangeably herein as targeting polypeptides herein.

[00136] 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 ≤ 1 mM, for example, ≤ 1 μM; *e.g.*, ≤ 100 nM, for example, ≤ 10 nM and for example, ≤ 1 nM.

[00137] 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 ≤1 μM, for example, ≤ 100 nM, for example, ≤ 10 nM, and for example, ≤ 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.

[00138] 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.

[00139] 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.

[00140] 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.

[00141] 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.

[00142] 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.

[00143] 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.

[00144] 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 for example, 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.

[00145] 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.

[00146] 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".

[00147] 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.

[00148] 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, preferably 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.

[00149] 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.

[00150] 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".

[00151] 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, for example, at least 95 percent sequence identity, and for example, at least 99 percent sequence identity.

[00152] In some embodiments, residue positions which are not identical differ by conservative amino acid substitutions.

[00153] 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.

[00154] 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%, and for example, 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 disclosure.

[00155] 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).

[00156] 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, for example, at least 20 amino acids long, usually at least 50 amino acids long, and for example, 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.

[00157] 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.

[00158] 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.

[00159] 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.

[00160] 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.

[00161] 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.

[00162] 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)).

[00163] 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 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.

[00164] 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.

[00165] 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.

[00166] 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 disclosure 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.

[00167] 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.

[00168] 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.

[00169] 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.

[00170] 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.

[00171] 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.

[00172] 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."

41BB (CD137, TNFRSF9) Targeting

[00173] 41BB is a member of the TNF receptor superfamily that is predominately expressed on activated T-cells and NK cells and serves as a co-stimulatory molecule. Agonizing 41BB enhances T cell proliferation and survival, cytolytic activity and cytokine secretion (e.g., IL-2, TNFα and INFγ). In mice, 41BB engagement has been shown to enhance anti-tumor immunity. (Croft, 2009, Nat Rev Immunol 9:271-285; Lynch, 2008, Immunol Rev. 22: 277-286). Importantly, tumor infiltrating cytotoxic T-cells (CTLs), have been shown to be express 41BB and it is these 41BB positive CTLs that have the highest anti-tumor cytotoxic activity (Ye *et al* Clin Cancer Res; 20(1): 44-55). The ligand for 41BB, 41BBL, naturally forms a homotrimer any thereby suggests that signaling is mediated by higher order clustering of 41BB. This is activation mechanism is shared with many members of the TNFRSF. Interest in exploiting 41BB signaling for anti-tumor immunotherapy has prompted the development of therapeutic 41BB antibodies. However, the capacity of bivalent 41BB antibodies to induce signaling is weak in absence of an exogenous clustering event. This can be achieved to some degree through the interaction with Fcγ-receptors (FcγRs), yet this can also lead to depletion of the 41BB-expressing cell through effector mechanisms (e.g. ADCC and ADCP). Furthermore, competition with the high concentration of IgG in serum attenuates efficient FcγR interactions. Therefore, current bivalent antibodies targeting 41BB are either ineffective agonists or have the liability of depleting the vary cells wherein 41BB signaling is desired. It has previously been shown that the therapeutic 41BB antibody, PF-05082566 is only capable of mediated 41BB signaling with cross-linked with anti-human secondary antibody (Fisher et al Cancer Immunol Immunother (2012) 61:1721–1733). Therefore, there exists a need for optimized 41BB agonist capable of mediating signaling in the absence of an exogenous crosslinking agent or FcγR interaction. The fusion proteins of the present disclosure are capable of mediating potent 41BB signaling 1) without any additional interactions when formatted as a multivalent fusion protein or 2) conditionally when engaged with at least a second antigen interaction when formatted as a multispecific fusion protein. The fusion proteins of the

present disclosure are capable of standalone (multivalent) or conditional (multispecific) co-stimulatory activity on T-cell and NK cells.

[00174] Exemplary amino acid sequences of 41BB binding single domain antibodies are shown below:

4H04:

QVQLQESGGGLVQAGDSLRLSCAASGWAFDNYGMAWFRQAPGKEREFIGRLAWNGGSTDYA
DSVKGRFTISRDNPKNTLYLQMNNLKPEDTAVYYCARQRSYSGYGIRTPQTYDYWGQGTQV
T (SEQ ID NO: 16)

CDR1: GWAFDNYG (SEQ ID NO: 17)

CDR2: LAWNGGST (SEQ ID NO: 18)

CDR3: ARQRSYSGYGIRTPQTYDY (SEQ ID NO: 19)

4E1:

QVQLQQSGGGLVQAGDSLRLSCAASGWAFGNYGMAWFRRAPGKEREFIGRLAWNGGSTDYV
DSVKGRFTISRDNPKNTLYLQMNNLKPDDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTQV
T (SEQ ID NO: 20)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWNGGST (SEQ ID NO: 18)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

4F5:

QVQLVQSGGGLVQPGGSLRLSCAASGWAFDNYGMAWFRQAPGKEREFIGRLAWNGGSTDYA
DSVKGRFTISRDNPKNTLYLQMNSLKPEDTAVYYCARQRSYSRYGIRAPQTYDYWGQGTQV
T (SEQ ID NO: 23)

CDR1: GWAFDNYG (SEQ ID NO: 17)

CDR2: LAWNGGST (SEQ ID NO: 18)

CDR3: ARQRSYSRYGIRAPQTYDY (SEQ ID NO: 24)

RH3:

QVQLQESGGGLVQPGGSLRLSCAVSGFSFSINAMGWYRQAPGKRREFLAAIDSGRNTVYAV SVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCGLLKGNRVVSPSVAYWGQGTQVT (SEQ ID NO: 25)

CDR1: GFSFSINA (SEQ ID NO: 26)

CDR2: IDSGRNT (SEQ ID NO: 27)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

D1:

EVQPVQSGGGLVQAGESLRLSCAASATIFSNNAMGWYRQAPGKQRELVATITTGGFTNYRD SVKGRFDISRDNAKNTVYLQMNNLKPEDTAVYYCNVVLRYSRDYSYTTVKEYWGQGTQV (SEQ ID NO: 29)

CDR1: ATIFSNNA (SEQ ID NO: 30)

CDR2: ITTGGFT (SEQ ID NO: 31)

CDR3: NVVLRYSRDYSYTTVKEY (SEQ ID NO: 32)

1G3:

QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIPAGDGSTKYA DSVKGRFTISRDNAKNTVYLQMDSLKPEDTAVYFCAKSRGWSTVDDMDYWGKGTQV (SEQ ID NO: 432)

CDR1: GFTFSSYA (SEQ ID NO: 433)

CDR2: IPAGDGST (SEQ ID NO: 434)

CDR3: AKSRGWSTVDDMDY (SEQ ID NO: 435)

1H4:

QVQLVQSGGGLVQPGGSLRLSCVVSGFTFRSYAMSWVRQAPGKGLEWVSTINSGESSTKYA DSVKGRFTISRDDAKNTLYLQMSDLKPEDTAVYFCAKHRGWSTVDDINYWGKGTQV (SEQ ID NO: 436

CDR1: GFTFRSYA (SEQ ID NO: 437)

CDR2: INSGESST (SEQ ID NO: 438)

CDR3: AKHRGWSTVDDINY (SEQ ID NO: 439)

1H1:

QVQLVQSGGGLVQPGGSLRLSCAASGFTFDDHAMSWVRQAPGKGLEWVSAISWNGHYTYYA
ESMKGRFAISRDNAKNTLYLQMNSLKSEDTAVYYCVKGWRGSYTRDRPFASWGQGTQV
(SEQ ID NO: 440)

CDR1: GFTFDDHA (SEQ ID NO: 441)

CDR2: ISWNGHYT (SEQ ID NO: 442)

CDR3: VKGWRGSYTRDRPFAS (SEQ ID NO: 443)

1H8:

EVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYYMSWVRQAPGKGLEWVSTISTNTGGGSTY
YAYADSVKGRFTISRDNAKNTLYLEMNSLKPEDTAQYYCVRTRWEGVYDYWGLGTQV
(SEQ ID NO: 444)

CDR1: GFTFSSYY (SEQ ID NO: 445)

CDR2: ISTNTGGGST (SEQ ID NO: 446)

CDR3: VRTRWEGVYDY (SEQ ID NO: 447)

Hz4E1-v1:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKGLEWVARLAWNGGSTDYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 33)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWNGGST (SEQ ID NO: 18)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

Hz4E1-v3:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKGREFVARLAWNGGSTDYA
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 34)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWNGGST (SEQ ID NO: 18)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v7-1:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFVSRLAWNGGSTDYV
AESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTL
VTVK (SEQ ID NO: 35)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWNGGST (SEQ ID NO: 18)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v8:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFIGRLAWNGGSTDYV
ESVKGRFTISRDNPKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 36)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWNGGST (SEQ ID NO: 18)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v9:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFVSRLAWNGGSTDYV
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 37)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWNGGST (SEQ ID NO: 18)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v10:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFVSRLAWNGGSTDYV
ESVKGRFTISRDNPKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 38)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWNGGST (SEQ ID NO: 18)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v11:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFIGRLAWNGGSTDYV
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 39)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWNGGST (SEQ ID NO: 18)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v12:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFIGRLAWNGGSTDYV
ESVKGRFTISRDNPKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 40)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWNGGST (SEQ ID NO: 18)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v13:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFIGRLAWQGGSTDYV
ESVKGRFTISRDNPKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 41)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWQGGST (SEQ ID NO: 42)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v14:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFIGRLAWNAGSTDYV
ESVKGRFTISRDNPKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 43)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWNAGST (SEQ ID NO: 44)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v16:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFVSRLAWQGGSTDYV
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 45)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWQGGST (SEQ ID NO: 42)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v17:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFVSRLAWNAGSTDYV
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 46)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWNAGST (SEQ ID NO: 44)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v18:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFVSRLAWGGGSTDYV
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 47)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWGGGST (SEQ ID NO: 48)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v21:

EVQLLESGGGEVQPGGSLRLSCAASGWAFSNYGMAWFRQAPGKEREFVSRLAWGGGSTDYV
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 49)

CDR1: GWAFSNYG (SEQ ID NO: 50)

CDR2: LAWGGGST (SEQ ID NO: 48)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v22:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFVSRLAWSGGSTDYV
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 51)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWSGGST (SEQ ID NO: 52)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v23:

EVQLLESGGGEVQPGGSLRLSCAASGWAFSNYGMAWFRQAPGKEREFVSRLAWSGGSTDYV
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 53)

CDR1: GWAFSNYG (SEQ ID NO: 50)

CDR2: LAWSGGST (SEQ ID NO: 52)

CDR3: ARQRSYSRYDIRTPQTYDY (SEQ ID NO: 22)

hz4E01v24:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFVSRLAWGGSTDYV
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSGYDIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 54)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWGGGST (SEQ ID NO: 48)

CDR3: ARQRSYSGYDIRTPQTYDY (SEQ ID NO: 55)

hz4E01v25:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFVSRLAWGGSTDYV
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYGIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 56)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWGGGST (SEQ ID NO: 48)

CDR3: ARQRSYSRYGIRTPQTYDY (SEQ ID NO: 57)

hz4E01v26:

EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFVSRLAWGGGSTDYV
ESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSGYGIRTPQTYDYWGQGTLV
TVKP (SEQ ID NO: 58)

CDR1: GWAFGNYG (SEQ ID NO: 21)

CDR2: LAWGGGST (SEQ ID NO: 48)

CDR3: ARQRSYSGYGIRTPQTYDY (SEQ ID NO: 19)

hzRH3-v1:

EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKGLEWVAAIDSGRNTVYAE
SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 59)

CDR1: GFSFSINA (SEQ ID NO: 26)

CDR2: IDSGRNT (SEQ ID NO: 27)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v5-1:

EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIESGRNTVYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 60)

CDR1: GFSFSINA (SEQ ID NO: 26)

CDR2: IESGRNT (SEQ ID NO: 61)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v5-2:

EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIYSGRNTVYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 62)

CDR1: GFSFSINA (SEQ ID NO: 26)

CDR2: IYSGRNT (SEQ ID NO: 63)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v5-3

EVQLLESGGGEVQPGGSLRLSCAASGFTFSINAMGWYRQAPGKRREFVAAIESGRNTVYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 64)

CDR1: GFTFSINA (SEQ ID NO: 65)

CDR2: IESGRNT (SEQ ID NO: 61)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v5-6

EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMSWYRQAPGKRREFVAAIESGRNTVYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 66)

CDR1: GFSFSINA (SEQ ID NO: 67)

CDR2: IESGRNT (SEQ ID NO: 61)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v5-8

EVQLLESGGGEVQPGGSLRLSCAASGFTFSSNAMGWYRQAPGKRREFVAAIESGRNTVYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 68)

CDR1: GFTFSSNA (SEQ ID NO: 69)

CDR2: IESGRNT (SEQ ID NO: 61)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v5-10

EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIESSRNTVYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 70)

CDR1: GFSFSINA (SEQ ID NO: 26)

CDR2: IESSRNT (SEQ ID NO: 71)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v5-12

EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIESGSNTVYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 72)

CDR1: GFSFSINA (SEQ ID NO: 26)

CDR2: IESGSNT (SEQ ID NO: 73)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v5-14

EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIESGRSTVYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 74)

CDR1: GFSFSINA (SEQ ID NO: 26)

CDR2: IESGRST (SEQ ID NO: 75)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v5-15

EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIESGRNTYYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 76)

CDR1: GFSFSINA (SEQ ID NO: 26)

CDR2: IESGRNT (SEQ ID NO: 77)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v5-16

EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIYSGSSTVYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 78)

CDR1: GFSFSINA (SEQ ID NO: 26)

CDR2: IYSGSST (SEQ ID NO: 79)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v7

EVQLLESGGGEVQPGGSLRLSCAVSGFSFSINAMGWYRQAPGKRREFVAAIESGRNTVYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 80)

CDR1: GFSFSINA (SEQ ID NO: 26)

CDR2: IESGRNT (SEQ ID NO: 61)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v8

EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIESGRNTVYAV
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 81)

CDR1: GFSFSINA (SEQ ID NO: 26)

CDR2: IESGRNT (SEQ ID NO: 61)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v9

EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKGREFVAAIESGRNTVYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 82)

CDR1: GFSFSINA (SEQ ID NO: 26)

CDR2: IESGRNT (SEQ ID NO: 61)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

hzRH3v13

EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFLAAIESGRNTVYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKP
(SEQ ID NO: 83)

CDR1: GFSFSINA (SEQ ID NO: 26)

CDR2: IESGRNT (SEQ ID NO: 61)

CDR3: GLLKGNRVVSPSVAY (SEQ ID NO: 28)

[00175] In some embodiments, the 41BB 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:
QVQLVQSGAEVKKPGSSVKVSCKASGGTFNSYAISWVRQAPGQGLEWMGGIIPGFGTANYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARKNEEDGGFDHWGQGTLVTVSS
(SEQ ID NO: 84)

QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYYMHWVRQAPGKGLEWVSVISGSGSNTYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLYAQFEGDFWGQGTLVTVSS
(SEQ ID NO: 85)

QVQLVQSGAEVKKPGESLKISCKGSGYSFSTYWISWVRQMPGKGLEWMGKIYPGDSYTNYS
PSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARGYGIFDYWGQGTLVTVSS (SEQ
ID NO: 86)

EVQLVQSGAEVKKPGESLRISCKGSGYSFSTYWISWVRQMPGKGLEWMGKIYPGDSYTNYS
PSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARGYGIFDYWGQGTLVTVSS (SEQ
ID NO: 87)

VL Sequences:

DIELTQPPSVSVAPGQTARISCSGDNLGDYYASWYQQKPGQAPVLVIYDDSNRPSGIPERF
SGSNSGNTATLTISGTQAEDEADYYCQTWDGTLHFVFGGGTKLTVL (SEQ ID
NO: 88)

DIELTQPPSVSVAPGQTARISCSGDNIGSKYVSWYQQKPGQAPVLVIYSDSERPSGIPERF
SGSNSGNTATLTISGTQAEDEADYYCQSWDGSISRVFGGGTKLTVL (SEQ ID
NO: 89)

DIELTQPPSVSVAPGQTARISCSGDNIGDQYAHWYQQKPGQAPVVVIYQDKNRPSGIPERF
SGSNSGNTATLTISGTQAEDEADYYCATYTGFGSLAVFGGGTKLTVL (SEQ ID
NO: 90)

SYELTQPPSVSVSPGQTASITCSGDNIGDQYAHWYQQKPGQSPVLVIYQDKNRPSGIPERF
SGSNSGNTATLTISGTQAMDEADYYCATYTGFGSLAVFGGGTKLTVL (SEQ ID
NO: 91)

SYELTQPPSVSVSPGQTASITCSGDNIGDQYAHWYQQKPGQSPVVVIYQDKNRPSGIPERF
SGSNSGNTATLTISGTQAMDEADYYCATYTGFGSLAVFGGGTKLTVL (SEQ ID
NO: 92)

DIELTQPPSVSVAPGQTARISCSGDNIGDQYAHWYQQKPGQAPVVVIYQDKNRPSGIPERF
SGSNSGNTATLTISGTQAEDEADYYCSTYTFVGFTTVFGGGTKLTVL (SEQ ID
NO: 93)

SYELTQPPSVSVSPGQTASITCSGDNIGDQYAHWYQQKPGQSPVLVIYQDKNRPSGIPERF
SGSNSGNTATLTISGTQAMDEADYYCSTYTFVGFTTVFGGGTKLTVL (SEQ ID
NO: 94)

SYELTQPPSVSVSPGQTASITCSGDNIGDQYAHWYQQKPGQSPVVVIYQDKNRPSGIPERF
SGSNSGNTATLTISGTQAMDEADYYCSTYTFVGFTTVFGGGTKLTVL (SEQ ID
NO: 95)

[00176] In some embodiments, the 41BB binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof that includes a combination of a heavy chain (HC) sequence and a light chain (LC) sequence selected from the group consisting of:

HC Sequences:

QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQSPEKGLEWIGEINHGGYVTYNP
SLESRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDYGPGNYDWYFDLWGRGTLVTVSSA

STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS
VMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 96)

QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQSPEKGLEWIGEINHGGYVTYNP
SLESRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDYGPGNYDWYFDLWGRGTLVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 97)

LC Sequences:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPALTFGGGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 98)

[00177] In some embodiments, the 41BB binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof selected from the antibody sequences described in US Patent Application Publication No. 20160244528, the contents of which are hereby incorporated by reference in their entirety.

[00178] In some embodiments, the 41BB binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof selected from the antibody sequences described in US Patent No. 8,337,850, the contents of which are hereby incorporated by reference in their entirety.

[00179] In some embodiments, the 41BB binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof selected from the antibody

sequences described in PCT Publication No. WO 2005/035584, the contents of which are hereby incorporated by reference in their entirety.

[00180] In some embodiments, the 41BB binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof selected from the antibody sequences described in EP Patent No. EP 1670828 B1, the contents of which are hereby incorporated by reference in their entirety.

[00181] In some embodiments, the 41BB binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof selected from the antibody sequences described in PCT Publication No. WO 2006/088447, the contents of which are hereby incorporated by reference in their entirety.

[00182] In some embodiments, the 41BB binding domain comprises or is derived from an antibody sequence or antigen-binding fragment thereof selected from the antibody sequences described in US Patent Application Publication No. 20080166336, the contents of which are hereby incorporated by reference in their entirety.

[00183] In some embodiments, the 41BB binding domain comprises or is derived from an anti-cancer fusion protein sequence or antigen-binding fragment thereof selected from the sequences described in PCT Publication No. WO 2016/177802, the contents of which are hereby incorporated by reference in their entirety. In some embodiments, the 41BB binding domain comprises or is derived from an amino acid sequence comprising:

QDSTSDLIPAPPLSKVPLQQNFQDNQFHGKWYVVGQAGNIRLREDKDPIKMMATIYELKED KSYDVTMVKFDDKKCMYDIWTFVPGSQPGEFTLGKIKSFPGHTSSLVRVVSTNYNQHAMVF FKFVFQNREEFYITLYGRTKELTSELKENFIRFSKSLGLPENHIVFPVPIDQCIDG (SEQ ID NO: 99)

[00184] In some embodiments, the 41BB binding domain comprises or is derived from an 41BB-targeting polypeptide sequence or antigen-binding fragment thereof selected from the sequences described in PCT Publication No. WO 2016/177762, the contents of which are hereby incorporated by reference in their entirety. In some embodiments, the 41BB binding domain comprises or is derived from an amino acid sequence comprising:

PDL1 Targeting

[00185] 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.

[00186] 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.

[00187] In some embodiments, the PD-L1 binding portion is single domain antibody. In some embodiments, the PDL1 binding portion of the fusion blocks or dampens the interaction of PDL1 and PD-1. Exemplary PDL1-targeting single domain sequences are shown below:

28A10:

QVQLQESGGGLVQAGGSLRLACTTSGGIFNIRPISWYRQPPGMQREWVATIAFGGATNYAN SIKGRFTASRDNAKNTVYLQMNGLKPEDTAVYYCNAFEIWGQGTQVTV (SEQ ID NO: 100)

CDR1: GGIFNIRP (SEQ ID NO: 101)

CDR2: IAFGGAT (SEQ ID NO: 102)

CDR3: NAFEI (SEQ ID NO: 103)

28A2:

QLQLQESGGGLVRAGGSLRLACTTSGGIFAIKPISWYRQPPGQEREWVTTTTSSGATNYAN SIKGRFTVARDNAKNTVYLQMNDLKLEDTAVYYCNVFEYWGQGTQVTV (SEQ ID NO: 104)

CDR1: GGIFAIKP (SEQ ID NO: 105)

CDR2: TTSSGAT (SEQ ID NO: 106)

CDR3: NVFEY (SEQ ID NO: 107)

B03:

QVQLQESGGDLVQAGSSLRLACATSGGVFNIRPISWYRQPPGKQREWVATIASGGATNYAN SIKGRFTASRDNAKNTVYLQMNGLKPEDTAVYYCNAFEVWGQGTQVTV (SEQ ID NO: 108)

CDR1: GGVFNIRP (SEQ ID NO: 109)

CDR2: IASGGAT (SEQ ID NO: 110)

CDR3: NAFEV (SEQ ID NO: 111)

B10:

QVQLQQSGGGLVQAGGSLRLACTTSGGIFNIRPISWYRQPPGMQREWVATIASGGATNYAN SIKGRFTASRDNAKNTVYLQMNGLKPEDTAVYYCNTLNFWGRGTQVTV (SEQ ID NO: 112)

CDR1: GGIFNIRP (SEQ ID NO: 101)

CDR2: IASGGAT (SEQ ID NO: 110)

CDR3: NTLNF (SEQ ID NO: 113)

D02:

QVQLQESGGGLVQAGGSLRLACTTSGGIFNIRPISWYRQPPGMQREWVATIASGGATNYAN SIKGRFTASRDNAKNTVYLQMNGLKPEDTAVYYCNVFEIWGQGTQVTV (SEQ ID NO: 114)

CDR1: GGIFNIRP (SEQ ID NO: 101)

CDR2: IASGGAT (SEQ ID NO: 110)

CDR3: NVFEI (SEQ ID NO: 115)

A03:

QVQLQQSGGGLVQAGGSLRLACITSGGIFNIRPISWYRQPPGKQREWVATIASGGAANYAN SIKGRFTASRDNAKNTVYLQMNGLKPEDTAVYYCNAFENWGQGTQVTV (SEQ ID NO: 116)

CDR1: GGIFNIRP (SEQ ID NO: 101)

CDR2: IASGGAA (SEQ ID NO: 117)

CDR3: NAFEN (SEQ ID NO: 118)

hz28A2v1

QVQLQESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKP (SEQ ID NO: 119)

CDR1: GGIFAIKP (SEQ ID NO: 105)

CDR2: TTSSGAT (SEQ ID NO: 106)

CDR3: NVFEY (SEQ ID NO: 107)

hz28A2v1-1

EVQLQESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKP (SEQ ID NO: 120)

CDR1: GGIFAIKP (SEQ ID NO: 105)

CDR2: TTSSGAT (SEQ ID NO: 106)

CDR3: NVFEY (SEQ ID NO: 107)

hz28A2v2

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKP (SEQ ID NO: 121)

CDR1: GGIFAIKP (SEQ ID NO: 105)

CDR2: TTSSGAT (SEQ ID NO: 106)

CDR3: NVFEY (SEQ ID NO: 107)

hz28A2v3

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKP (SEQ ID NO: 122)

CDR1: GGIFAIKP (SEQ ID NO: 105)

CDR2: TTSSGAT (SEQ ID NO: 106)

CDR3: NVFEY (SEQ ID NO: 107)

hz28A2v4:

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVTTTTSSGATNYAE SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKP (SEQ ID NO: 123)

CDR1: GGIFAIKP (SEQ ID NO: 105)

CDR2: TTSSGAT (SEQ ID NO: 106)

CDR3: NVFEY (SEQ ID NO: 107)

hz28A2v5:

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKP (SEQ ID NO: 124)

CDR1: GGIFAIKP (SEQ ID NO: 105)

CDR2: TTSSGAT (SEQ ID NO: 106)

CDR3: NVFEY (SEQ ID NO: 107)

[00188] 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: 125)

[00189] 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.

[00190] 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: 126)

QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSTYAISWVRQAPGQGLEWMGGIIPIFGKAHYA QKFQGRVTITADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVTVS S (SEQ ID NO: 127)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDVHWVRQAPGQRLEWMGWLHADTGITKFS QKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCARERIQLWFDYWGQGT (SEQ ID NO: 128)

QVQLVQSGAEVKKPGSSVKVSCKVSGGIFSTYAINWVRQAPGQGLEWMGGIIPIFGTANHA QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDQGIAAALFDYWGQGTLVTVSS (SEQ ID NO: 129)

EVQLVESGGGLVQPGRSLRLSCAVSGFTFDDYVVHWVRQAPGKGLEWVSGNSGNIGYADSV KGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAVPFDYWGQGTLVTVSS (SEQ ID NO: 130)

QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSSYAISWVRQAPGQGLEWMGGIIPIFGRAHYA QKFQGRVTITADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVTVS S (SEQ ID NO: 131)

QVQLVQSGAEVKKPGSSVKVSCKTSGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGKAHYA QKFQGRVTITADESTTTAYMELSSLRSEDTAVYYCARKYDYVSGSPFGMDVWGQGTTVTVS S (SEQ ID NO: 132)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAINWVRQAPGQGLEWMGGIIPIFGSANYA QKFQDRVTITADESTSAAYMELSSLRSEDTAVYYCARDSSGWSRYYMDVWGQGTTVTVSS (SEQ ID NO: 133)

QVQLVQSGAEVKEPGSSVKVSCKASGGTFNSYAISWVRQAPGQGLEWMGGIIPLFGIAHYA
QKFQGRVTITADESTNTAYMDLSSLRSEDTAVYYCARKYSYVSGSPFGMDVWGQGTTVTVS
S (SEQ ID NO: 134)

EVQLVESGGGLVQPGRSLRLSCAASGITFDDYGMHWVRQAPGKGLEWVSGISWNRGRIEYA
DSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKGRFRYFDWFLDYWGQGTLVTVSS
(SEQ ID NO: 135)

QMQLVQSGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSEKYYV
DSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDYFWSGFSAFDIWGKGTLVTVS
(SEQ ID NO: 136)

VL Sequences:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLVWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIK (SEQ ID
NO: 137)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPTFGQGTKVEIK (SEQ ID
NO: 138)

DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK (SEQ ID
NO: 139)

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPD
RFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK (SEQ ID
NO: 140)

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPD
RFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPFGGGTKVEIK (SEQ ID
NO: 141)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPTFGQGTRLEIK (SEQ ID
NO: 142)

AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPFTFGPGTKVDIK (SEQ ID
NO: 143)

DIVMTQSPSTLSASVGDRVTITCRASQGISSWLAWYQQKPGRAPKVLIYKASTLESGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPWTFGQGTKLEIK (SEQ ID
NO: 144)

[00191] 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: 145)

VL Sequence:

EIVLTQSPGTLSLSPGERATLSCRASQRVSSSYLAWYQQKPGQAPRLLIYDASSRATGIPD
RFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSLPWTFGQGTKVEIK (SEQ ID
NO: 146)

[00192] 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: 147)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSGSWIHWVRQAPGKGLEWVAWILPYGGSSYYA
DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSA
(SEQ ID NO: 148)

VL Sequences:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID
NO: 149)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYYNVPWTFGQGTKVEIKR (SEQ ID
NO: 150)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYYAPPWTFGQGTKVEIKR (SEQ ID
NO: 151)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYYTVPWTFGQGTKVEIKR (SEQ ID
NO: 152)

DIQMTQSPSSLSASVGDRVTITCRASQVINTFLAWYQQKPGKAPKLLIYSASTLASGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYYTVPRTFGQGTKVEIKR (SEQ ID NO: 153)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQGYGVPRTFGQGTKVEIKR (SEQ ID NO: 154)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYLFTPPTFGQGTKVEIKR (SEQ ID NO: 155)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYFITPTTFGQGTKVEIKR (SEQ ID NO: 156)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQYYYTPPTFGQGTKVEIKR (SEQ ID NO: 157)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQFFYTPPTFGQGTKVEIKR (SEQ ID NO: 158)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQSLFTPPTFGQGTKVEIKR (SEQ ID NO: 159)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQSLYTPPTFGQGTKVEIKR (SEQ ID NO: 160)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQSWYHPPTFGQGTKVEIKR (SEQ ID
NO: 161)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYFYIPPTFGQGTKVEIKR (SEQ ID
NO: 162)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYWYTPTTFGQGTKVEIKR (SEQ ID
NO: 163)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQSYFIPPTFGQGTKVEIKR (SEQ ID
NO: 164)

[00193] 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: 165)

QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSTYAISWVRQAPGQGLEWMGGIIPIFGKAHYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVTVS
S (SEQ ID NO: 166)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDVHWVRQAPGQRLEWMGWLHADTGITKFS
QKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCARERIQLWFDYWGQGTLVTVSS
(SEQ ID NO: 167)

QVQLVQSGAEVKKPGSSVKVSCKVSGGIFSTYAINWVRQAPGQGLEWMGGIIPIFGTANHA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDQGIAAALFDYWGQGTLVTVSS
(SEQ ID NO: 168)

EVQLVESGGGLVQPGRSLRLSCAVSGFTFDDYVVHWVRQAPGKGLEWVSGISGNSGNIGYA
DSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAVPFDYWGQGTLVTVSS (SEQ ID
NO: 169)

QVQLVQSGAEVKKPGSSVKVSCKTSGDTFSSYAISWVRQAPGQGLEWMGGIIPIFGRAHYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYFCARKFHFVSGSPFGMDVWGQGTTVTVS
S (SEQ ID NO: 170)

QVQLVQSGAEVKKPGSSVKVSCKTSGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGKAHYA
QKFQGRVTITADESTTTAYMELSSLRSEDTAVYYCARKYDYVSGSPFGMDVWGQGTTVTVS
S (SEQ ID NO: 171)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAINWVRQAPGQGLEWMGGIIPIFGSANYA
QKFQDRVTITADESTSAAYMELSSLRSEDTAVYYCARDSSGWSRYYMDVWGQGTTVTVSS
(SEQ ID NO: 172)

QVQLVQSGAEVKEPGSSVKVSCKASGGTFNSYAISWVRQAPGQGLEWMGGIIPLFGIAHYA
QKFQGRVTITADESTNTAYMDLSSLRSEDTAVYYCARKYSYVSGSPFGMDVWGQGTTVTVS
S (SEQ ID NO: 173)

EVQLVESGGGLVQPGRSLRLSCAASGITFDDYGMHWVRQAPGKGLEWVSGISWNRGRIEYA
DSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKGRFRYFDWFLDYWGQGTLVTVSS
(SEQ ID NO: 174)

VL Sequences:

MDTRAPTQLLGLLLLWLPGARCALVMTQTPSSTSTAVGGTVTIKCQASQSISVYLAWYQQK
PGQPPKLLIYSASTLASGVPSRFKGSRSGTEYTLTISGVQREDAATYYCLGSAGS (SEQ
ID NO: 175)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLVWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIK (SEQ ID
NO: 176)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPTFGQGTKVEIK (SEQ ID
NO: 177)

DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK (SEQ ID
NO: 178)

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPD
RFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK (SEQ ID
NO: 179)

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPD
RFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPFGGGTKVEIK (SEQ ID
NO: 180)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPTFGQGTRLEIK (SEQ ID
NO: 181)

AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPFTFGPGTKVDIK (SEQ ID
NO: 182)

[00194] 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: 183)

VL Sequences:

QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTRVFGTGTKVTVL (SEQ ID
NO: 184)

[00195] 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: 185)

EVQLQESGPGLVAPSQSLSITCTVSGFSLTTYSINWIRQPPGKGLEWLGVMWAGGGTNSNS
VLKSRLIISKDNSKSQVFLKMNSLQTDDTARYYCARYYGNSPYYAIDYWGQGTSVTVSS
(SEQ ID NO: 186)

EVKLQESGPSLVKPSQTLSLTCSVTGYSIISDYWNWIRKFPGNKLEYLGYISYTGSTYYNP
SLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCARRGGWLLPFDYWGQGTTLTVSS
(SEQ ID NO: 187)

EVKLQESGPSLVKPGASVKLSCKASGYTFTSYDINWVKQRPGQGLEWIGWIFPRDNNTKYN
ENFKGKATLTVDTSSTTAYMELHSLTSEDSAVYFCTKENWVGDFDYWGQGTTLTLSS
(SEQ ID NO: 188)

EVQLQQSGPDLVTPGASVRISCQASGYTFPDYYMNWVKQSHGKSLEWIGDIDPNYGGTTYN QKFKGKAILTVDRSSSTAYMELRSLTSEDSAVYYCARGALTDWGQGTSLTVSS (SEQ ID NO: 189)

EIVLTQSPATLSLSPGERATLSCRASSSVSYIYWFQQKPGQSPRPLIYAAFNRATGIPARF SGSGSGTDYTLTISSLEPEDFAVYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 190)

QVQLVQSGAEVKKPGASVKVSCKASGYTFPDYYMNWVRQAPGQGLEWMGDIDPNYGGTNYA QKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGALTDWGQGTMVTVSS (SEQ ID NO: 191

QVQLVQSGAEVKKPGASVKVSCKASGYTFPDYYMNWVRQAPGQSLEWMGDIDPNYGGTNYN QKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGALTDWGQGTMVTVSS (SEQ ID NO: 192)

EVQLVQSGAEVKKPGASVKVSCKASGYTFPDYYMNWVRQAPGQSLEWMGDIDPNYGGTNYN QKFQGRVTMTVDRSSSTAYMELSRLRSDDTAVYYCARGALTDWGQGTMVTVSS (SEQ ID NO: 193)

EVQLVESGGGLVQPGRSLRLSCTASGYTFPDYYMNWVRQAPGKGLEWVGDIDPNYGGTTYA ASVKGRFTISVDRSKSIAYLQMSSLKTEDTAVYYCTRGALTDWGQGTMVTVSS (SEQ ID NO: 194)

EVQLVESGGGLVQPGRSLRLSCTASGYTFPDYYMNWVRQAPGKGLEWVGDIDPNYGGTTYN ASVKGRFTISVDRSKSIAYLQMSSLKTEDTAVYYCARGALTDWGQGTMVTVSS (SEQ ID NO: 195)

VL Sequences:

DIVMTQSHKLMSTSVGDRVSITCKASQDVGTAVAWYQQKPGQSPKLLIYWASTRHTGVPDR FTGSGSGTDFTLTISNVQSEDLADYFCQQDSSYPLTFGAGTKVELK (SEQ ID NO: 196)

DIVTTQSHKLMSTSVGDRVSITCKASQDVGTAVAWYQQKPGQSPKLLIYWASTRHTGVPDR FTGSGSGTDFTLTISNVQSEDLADYFCQQDSSYPLTFGAGTKVELK (SEQ ID NO: 197)

DIVMTQSPSSLAVSVGEKVSMGCKSSQSLLYSSNQKNSLAWYQQKPGQSPKLLIDWASTRE SGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYGYPLTFGAGTKLELK (SEQ ID NO: 198)

DIVMTQSPAIMSASPGEKVTMTCSASSSIRYMHWYQQKPGTSPKRWISDTSKLTSGVPARF SGSGSGTSYALTISSMEAEDAATYYCHQRSSYPWTFGGGTKLEIK (SEQ ID NO: 199)

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIYWFQQKPGSSPKPWIYATFNLASGVPARF SGSGSGTSYSLTISRVETEDAATYYCQQWSNNPLTFGAGTKLELK (SEQ ID NO: 200)

EIVLTQSPATLSLSPGERATLSCRASSSVSYIYWFQQKPGQAPRLLIYAAFNRATGIPARF SGSGSGTDYTLTISSLEPEDFAVYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 201)

QIVLTQSPATLSLSPGERATLSCRASSSVSYIYWFQQKPGQSPRPLIYATFNLASGIPARF SGSGSGTSYTLTISRLEPEDFAVYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 202)

DIQLTQSPSSLSASVGDRVTITCRASSGVSYIYWFQQKPGKAPKLLIYAAFNLASGVPSRF SGSGSGTEYTLTISSLQPEDFATYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 203)

DIQLTQSPSSLSASVGDRVTITCRASSGVSYIYWFQQKPGKAPKPLIYAAFNLASGVPSRF SGSGSGTEYTLTISSLQPEDFATYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 204)

DIQLTQSPSILSASVGDRVTITCRASSSVSYIYWFQQKPGKAPKPLIYATFNLASGVPSRF SGSGSGTSYTLTISSLQPEDFATYYCQQWSNNPLTFGQGTKVEIK (SEQ ID NO: 205)

[00196] 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: 206)

EVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYALSWVRQAPGKGLEWVSAISGGGGSTYYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDVFPETFSMNYGMDVWGQGTLVTV SS (SEQ ID NO: 207)

QVQLVQSGGGVVQPGGSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSLISGDGGSTYYA DSVKGRFTISRDNSKNSLYLQMNSLRTEDTALYYCAKVLLPCSSTSCYGSVGAFDIWGQGT TVTVSS (SEQ ID NO: 208)

QVQLVQSGGSVVRPGESLRLSCVASGFIFDNYDMSWVRQVPGKGLEWVSRVNWNGGSTTYA DAVKGRFTISRDNTKNSLYLQMNNLRAEDTAVYYCVREFVGAYDLWGQGTTVTVSS (SEQ ID NO: 209)

QVQLVQSGAEVKKPGATVKVSCKVFGDTFRGLYIHWVRQAPGQGLEWMGGIIPIFGTANYA QKFQGRVTITTDESTSTAYMELSSLRSEDTAVYYCASGLRWGIWGWFDPWGQGTLVTVSS (SEQ ID NO: 210)

EVQLVQSGAELKKPGSSVKVSCKAFGGTFSDNAISWVRQAPGQGPEWMGGIIPIFGKPNYA QKFQGRVTITADESTSTAYMVLSSLRSEDTAVYYCARTMVRGFLGVMDVWGQGTTVTVSS (SEQ ID NO: 211)

QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDQFVTIFGVPRYGMDVWGQGTTVT
VSS (SEQ ID NO: 212)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYA
QKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGRQMFGAGIDFWGPGTLVTVSS
(SEQ ID NO: 213)

EVQLVESGAEVKKPGSSVKVSCKVSGGTFGTYALNWVRQAPGQGLEWMGRIVPLIGLVNYA
HNFEGRISITADKSTGTAYMELSNLRSDDTAVYYCAREVYGGNSDYWGQGTLVTVSS
(SEQ ID NO: 214)

QVQLVQSGGEVKKPGASVKVSCKASGYTLSSHGITWVRQAPGQGLEWMGWISAHNGHASNA
QKVEDRVTMTTDTSTNTAYMELRSLTADDTAVYYCARVHAALYYGMDVWGQGTLVTVSS
(SEQ ID NO: 215)

QVQLQESGGGVVQPGRSLRLSCSASGFTFSRHGMHWVRQAPGKGLEWVAVISHDGSVKYYA
DSMKGRFSISRDNSNNTLYLQMDSLRADDTAVYYCARGLSYQVSGWFDPWGQGTLVTVSS
(SEQ ID NO: 216)

NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSSPTTVIYEDNQRPSGVPD
RFSGSIDTSSNSASLTISGLKTKDEADYYCQSYDGITVIFGGGTKLTVL (SEQ ID
NO: 217)

NFMLTQPHSVSGSPGKTVTLPCTRSSGSIASHYVQWYQQRPGSAPTTVIYEDNKRPSGVPD
RFSGSIDSSSNSASLSISGLKTEDEADYYCQSYDSSNRWVFGGGTKLTVL (SEQ ID
NO: 218)

LPVLTQPASLSASPGASASLTCTLRSGLNVGSYRIYWYQQKPGSRPQYLLNYKSDSNKQQA
SGVPSRFSGSKDASANAGILLISGLQSEDEADYYCMIWYSSAVVFGGGTKLTVL (SEQ
ID NO: 219)

VL Sequences:

NFMLTQPHSVSESPGKTVTISCTRSSGNIASNYVQWYQQRPGSAPTTVIYEDNQRPSGVPD RFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSSNLWVFGGGTKLTVL (SEQ ID NO: 220)

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRF SGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHYVFGTGTKVTVL (SEQ ID NO: 221)

LPVLTQAPSVSVAPGKTARITCGGSDIGRKSVHWYQQKPGQAPALVIYSDRDRPSGISERF SGSNSGNTATLTISRVEAGDEADYYCQVWDNNSDHYVFGAGTELIVL (SEQ ID NO: 222)

QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVS NRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSTLPFGGGTKLTVL (SEQ ID NO: 223)

EIVLTQSPATLSLSPGERATLSCRASQSIGNSLAWYQQKPGQAPRLLMYGASSRATGIPDR FSGSGAGTDFTLTISSLEPEDFATYYCQQHTIPTFSFGPGTKVEVK (SEQ ID NO: 224)

DIVMTQTPSFLSASIGDRVTITCRASQGIGSYLAWYQQRPGEAPKLLIYAASTLQSGVPSR FSGSGSGTDFTLTISNLQPEDFATYYCQQLNNYPITFGQGTRLEIK (SEQ ID NO: 225)

QSALTQPPSVSVSPGQTANIPCSGDKLGNKYAYWYQQKPGQSPVLLIYQDIKRPSRIPERF SGSNSADTATLTISGTQAMDEADYYCQTWDNSVVFGGGTKLTVL (SEQ ID NO: 226)

NFMLTQPHSVSESPGKTVTISCTRSSGSIDSNYVQWYQQRPGSAPTTVIYEDNQRPSGVPD RFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSNNRHVIFGGGTKLTVL (SEQ ID NO: 227)

NFMLTQPHSVSESPGKTVTISCTRSSGNIGTNYVQWYQQRPGSAPVALIYEDYRRPSGVPD
RFSGSIDSSSNSASLIISGLKPEDEADYYCQSYHSSGWEFGGGTKLTVL (SEQ ID
NO: 228)

QSVLTQPPSVSVAPGQTARITCGGNNIGSKGVHWYQQKPGQAPVLVVYDDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHWVFGGGTKLTVL (SEQ ID
NO: 229)

NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTTVIYEDNQRPSGVPD
RFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSTTPSVFGGGTKLTVL (SEQ ID
NO: 230)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWTSPHNGLTAFA
QILEGRVTMTTDTSTNTAYMELRNLTFDDTAVYFCAKVHPVFSYALDVWGQGTLVTVSS
(SEQ ID NO: 231)

EVQLVESGAEVMNPGSSVRVSCRGSGGDFSTYAFSWVRQAPGQGLEWMGRIIPILGIANYA
QKFQGRVTITADKSTSTAYMELSSLRSDDTAVYYCARDGYGSDPVLWGQGTLVTVSS
(SEQ ID NO: 232)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGISWVRQAPGQGLEWMGWISAYNGNTNYA
QKVQGRVTMTTDTSTSTGYMELRSLRSDDTAVYYCARGDFRKPFDYWGQGTLVTVSS
(SEQ ID NO: 233)

[00197] 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: 234)

EVQLVQSGAEVKKPGASVKMSCKASGYTFTSYVMHWVKQAPGQRLEWIGYVNPFNDGTKYN EMFKGRATLTSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS (SEQ ID NO: 235)

EVQLVQSGAEVKKPGASVKMSCKASGYTFTSYVMHWVRQAPGQRLEWIGYVNPFNDGTKYN EMFKGRATLTSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS (SEQ ID NO: 236)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVMHWVRQAPGQRLEWIGYVNPFNDGTKYN EMFKGRATLTSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS (SEQ ID NO: 237)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYVMHWVRQAPGQRLEWIGYVNPFNDGTKYN EMFKGRATITSDKSTSTAYMELSSLRSEDTAVYYCARQAWGYPWGQGTLVTVSS (SEQ ID NO: 238)

VL Sequences:

DIVLTQSPASLALSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDSG VPSRFSGSGSGTDFTLTINSLEEEDAAMYFCQQSRRVPYTFGQGTKLEIK (SEQ ID NO: 239)

DIVLTQSPATLSLSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDSG VPSRFSGSGSGTDFTLTINSLEAEDAAMYFCQQSRRVPYTFGQGTKLEIK (SEQ ID NO: 240)

EIVLTQSPATLSLSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDSG VPSRFSGSGSGTDFTLTINSLEAEDAAMYFCQQSRRVPYTFGQGTKLEIK (SEQ ID NO: 241)

DIVLTQSPATLSLSPGERATLSCRATESVEYYGTSLVQWYQQKPGQPPKLLIYAASSVDSG VPSRFSGSGSGTDFTLTINSLEAEDAATYFCQQSRRVPYTFGQGTKLEIK (SEQ ID NO: 242)

[00198] 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: 243)

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGIINPSGGSTSYA QKFQGRVSMTRDTSTSTVYMELSSLTSEDTAVYYCARDLFPHIYGNYYGMDIWGQGTTVTV SS (SEQ ID NO: 244)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYA QKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARLAVPGAFDIWGQGTMVTVSS (SEQ ID NO: 245)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLAVISYDGSNKYYADSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGQWLVTELDYWGQGTLVTVSS (SEQ ID NO: 246)

EVQLVESGSEVEKPGSSVKVSCKASGGTFSDSGISWVRQAPGQGLEWMGGIIPMFATPYYA QKFQDRVTITADESTSTVYMELSGLRSDDTAVFYCARDRGRGHLPWYFDLWGRGTLVTVSS (SEQ ID NO: 247)

EVQLVESGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYA QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARAPYYYYYMDVWGQGTTVTVSS (SEQ ID NO: 248)

EVQLLESGAEVKKPGSSVKVSCKASGGTLSRYALSWVRQAPGQGPEWVGAIIPIFGTPHYS
KKFQDRVIITVDTSTNTAFMELSSLRFEDTALYFCARGHDEYDISGYHRLDYWGQGTLVTV
SS (SEQ ID NO: 249)

QVQLVQSGSELKKPGSSVKVSCKASGYSFSGYYIHWVRQAPGQGLEWMGWIDPNSGVTNYV
RRFQGRVTMTRDTSLSTAYMELSGLTADDTAVYYCARDENLWQFGYLDYWGQGTLVTVSS
(SEQ ID NO: 250)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSRYGVHWVRQAPGQGLEWMGRLIPIVSMTNYA
QKFQDRVSITTDKSTGTAYMELRSLTSEDTALYYCASVGQQLPWVFFAWGQGTLVTVSS
(SEQ ID NO: 251)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 252)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 253)

EVQLVQSGGGLVQPGGSLRLSCAASGFTFSDYGMHWVRQPPGKGLEWLAVISYDGSYKIHA
DSVQGRFTISRDNAKNSVFLQMNSLKTEDTAVYYCTTDRKWLAWHGMDVWGQGTTVTVSS
(SEQ ID NO: 254)

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDGIVADFQHWGQGTLVTVSS
(SEQ ID NO: 255)

EVQLVESGAEVKKPGASVKVSCKASGDTFSRYGITWVRQAPGRGLEWMGNIVPFFGATNYA
QKFQGRLTITADKSSYTSYMDLSSLRSDDTAVYYCARDHFYGSGGYFDYWGQGTLVTVSS
(SEQ ID NO: 256)

EVQLLESGAEVKKPGASVKVSCKASGYTFNSYDINWVRQAPGQGLEWMGGIIPVFGTANYA
ESFQGRVTMTADHSTSTAYMELNNLRSEDTAVYYCARDRWHYESRPMDVWGQGTTVTVSS
(SEQ ID NO: 257)

EVQLVESGGGLVRPGGSLRLACAASGFSFSDYYMTWIRQAPGRGLEWIAYISDSGQTVHYA
DSVKGRFTISRDNTKNSLFLQVNTLRAEDTAVYYCAREDLLGYYLQSWGQGTLVTVSS
(SEQ ID NO: 258)

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYN
DYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCARDEPRAVAGSQAYYYYGMDVWG
QGTTVTVSS (SEQ ID NO: 259)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSDGSTSYA
QKFQGRVTMTRDTSTSTVHMELSSLRSEDTAVYYCARDLFPHIYGNYYGMDIWGQGTTVTV
SS (SEQ ID NO: 260)

QMQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 261)

QVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 262)

VL Sequences:

QSVLTQPPSVSAAPGQKVTISCSGNNSNIANNYVSWYQQLPGTAPKLLIYDNNYRPSGIPD
RFSGSKSGTSATLDITGLQTGDEADYYCGVWDGSLTTGVFGGGTKLTVL (SEQ ID
NO: 263)

AIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPKLLIYAASTLESGVPSR
FSGSGSGTDFTLTISSLQPEDLATYYCQQLHTFPLTFGGGTKVEIK (SEQ ID
NO: 264)

QPVLTQPPSASGSPGQSVTISCTGTSSDVGAYNFVSWYRQHPGKAPKLMIYEVNKRPSGVP
DRFSGSKSGNTASLTVSGLQAEDEADYYCSSYAGTNSLGIFGTGTKLTVL (SEQ ID
NO: 265)

QSVVTQPPSVSAAPGQKVTISCSGSSSDIGNHYVSWYQQLPGTAPKLLIYDNNQRPSGIPD
RFSGSKSGTSATLAITGLQTGDEADYYCGTWDNSLSPHLLFGGGTKLTVL (SEQ ID
NO: 266)

QSVLTQPPSVSAAPGQKVTISCSGSSSNMGNNYVSWYKQVPGTAPKLLIYENDKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDNSLSGFVFASGTKVTVL (SEQ ID
NO: 267)

QSALTQPASVSGSLGQSVTISCTGSSSDVGSYNLVSWYQQHPGKAPNLMIYDVSKRSGVSN
RFSGSKSGNTASLTISGLQAEDEADYYCSSYTGISTVVFGGGTKLTVL (SEQ ID
NO: 268)

QSVLTQPASVSGSPGQSITISCTGTSSDVGSYNLVSWYQQHPGKAPKLMIYEVSKRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYGGFNNLLFGGGTKLTVL (SEQ ID
NO: 269)

DIVMTQSPSSLSASIGDRVTITCRASQRISAYVNWYQQKPGKAPKVLIYAASSLRSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQTYSSPWTFGQGTKVEIK (SEQ ID
NO: 270)

QSVLTQPPSASGSPGQSVTISCTGTSSDIGGYDSVSWYQQHPGKAPKLMIYDVSKRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSIFFYVFGTGTKVTVL (SEQ ID
NO: 271)

LPVLTQPASVSGSPGQSITISCTGTTSDIGGYDYVSWYQQHPGKAPKLMIYDVSKRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTHVFGTGTKLTVL (SEQ ID
NO: 272)

QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYRSSTLGPVFGGGTKLTVL (SEQ ID
NO: 273)

QAGLTQPPSVSEAPRQRVTISCSGSSSNIGNNAVNWYQQLPGKAPKLLIYYDDLLPSGVSD
RFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGYVFGTGTKLTVL (SEQ ID
NO: 274)

QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGVP
DRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSTTHVFGTGTKVTVL (SEQ ID
NO: 275)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSVWVFGGGTQLTVL (SEQ ID
NO: 276)

QSVLTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGRAPRLMIYDVSNRPSGVS
NRFSGSKSGNTASLTISGLQAEDEGDYYCSSYTSGGTLGPVFGGGTKLTVL (SEQ ID
NO: 277)

QAGLTQPPSASGTPGQRVTISCSGSSSNIGSNTVNWYQQLPGTAPKLLIYSNNQRPSGVPD
RFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGWVFGGGTKLTVL (SEQ ID
NO: 278)

AIRMTQSPSSLSASVGDRVTITCRASQSISNYLNWYQQRPGKAPNLLIYAASSLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQTYSTPYTFGQGTKLEIK (SEQ ID
NO: 279)

QSVLTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYRQHPGKAPKLMIYDVSYRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYTDSSTRYVFGTGTKLTVL (SEQ ID
NO: 280)

QPVLTQPPSASGTPGQRVAISCSGSRSNIEINSVNWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGSWDSSLSADVFGTGTKLTVL (SEQ ID
NO: 281)

QSVLTQPPSVSAAPGKKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYRNNQRPSGVPD
RFSGSKSGTSASLAISGLQSEDEADYYCATWDDSLNGWVFGGGTKLTVL (SEQ ID
NO: 282)

QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNNNRHSGVP
DRFSGSKSGTSASLAITGLQAEDEAEFFCGTWDSRLTTYVFGSGTKLTVL (SEQ ID
NO: 283)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAVVFGGGTKLTVL (SEQ ID
NO: 284)

VIWMTQSPSSLSASVGDRVTITCAASSLQSWYQQKPGKAPKLLIYEASTLESGVPSRFSGS
GSGTEFTLTISSLQPEDFATYYCQQSYSTPYTFGQGTKLEIK (SEQ ID NO: 285)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQVPGTAPKLLIYDNNKRPSGIPD
RFSGSNSDTSATLGITGLQTGDEADYYCGTWDSSLSAWVFGGGTKLTVL (SEQ ID
NO: 286)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAGSVVFGGGTKLTVL (SEQ ID
NO: 287)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCLVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 288)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 289)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 290)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 291)

[00199] 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: 292)

QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDGSKRYYA
DSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSSASTKGPSVF
PLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD

WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN
HYTQKSLSLSLGK (SEQ ID NO: 293)

LC Sequences:

EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESG
VPARFSGSGSGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 294)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK
ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 295)

[00200] 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: 296)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYA
DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSS
(SEQ ID NO: 297)

HC Sequences:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYA
DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSSASTK

GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPG (SEQ ID NO: 298)

VL Sequences:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID
NO: 299)

LC Sequences:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK
ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 300)

[00201] 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: 301)

QEHLVESGGGVVQPGRSLRLSCEASGFTFSNFGMHWVRQAPGKGLEWVAALWSDGSNKYYA
DSVKGRVTISRDNSKNTLYLQMNSLRAEDTAVYYCARGRGAPGIPIFGYWGQGTLVTVSS
(SEQ ID NO: 302)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEWVGRIKRKTDGGTTD YAAPVKGRFTISRDDSKNTLHLQMNSLKTEDTAVYYCTTDDIVVVPAVMREYYFGMDVWGQ GTTVTVSS (SEQ ID NO: 303)

QVQLVQSGAEVKKPGASVQVSCKASGYSFTGYYIHWVRQAPGQGLEWMGWINPNSGTKKYA HKFQGRVTMTRDTSIDTAYMILSSLISDDTAVYYCARDEDWNFGSWFDSWGQGTLVTVSS (SEQ ID NO: 304)

QVHLVQSGAEVKKPGASVKVSCKASGYTFTGYYIHWVRQAPGHGLEWMGWLNPNTGTTKYI QNFQGRVTMTRDTSSSTAYMELTRLRSDDTAVYYCARDEDWNYGSWFDTWGQGTLVTVSS (SEQ ID NO: 305)

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMTWVRQAPGRGLEWVSGIHWHGKRTGYA DSVKGRFTISRDNAKKSLYLQMNSLKGEDTALYHCVRGGMSTGDWFDPWGQGTLVIVSS (SEQ ID NO: 306)

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMTWVRQVPGKGLEWVSGIHWSGRSTGYA DSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCARGGMSTGDWFDPWGQGTLVTVSS (SEQ ID NO: 307)

EVQLVESGGGLVQPGGSLRLSCAASGFTVGSNYMNWVRQAPGKGLEWVSVIYSGGSTYYAD SVKGRFTISRLTSKNTLYLQMSSLRPEDTAVYYCARGIRGLDVWGQGTTVTVSS (SEQ ID NO: 308)

EERLVESGGDLVQPGGSLRLSCAASGITVGTNYMNWVRQAPGKGLEWVSVISSGGNTHYAD SVKGRFIMSRQTSKNTLYLQMNSLETEDTAVYYCARGIRGLDVWGQGTMVTVSS (SEQ ID NO: 309)

QVQLVQSGAEVKMPGSSVRVSCKASGGIFSSSTISWVRQAPGQGLEWMGEIIPVFGTVNYA QKFQDRVIFTADESTTTAYMELSSLKSGDTAVYFCARNWGLGSFYIWGQGTMVTVSS (SEQ ID NO: 310)

EVQLVESGGDLVHPGRSLRLSCAASGFPFDEYAMHWVRQVPGKGLEWVSGISWSNNNIGYA DSVKGRFTISRDNAKNSLYLQMNSLRPEDTAFYYCAKSGIFDSWGQGTLVTVSS (SEQ ID NO: 311)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVTLISYEGRNKYYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDRTLYGMDVWGQGTTVTVSS (SEQ ID NO: 312)

QVTLRESGPALVKTTQTLTLTCTFSGFSLSTNRMCVTWIRQPPGKALEWLARIDWDGVKYY NTSLKTRLTISKDTSKNQVVLTMTNMDPVDTATFYCARSTSLTFYYFDYWGQGTLVTVSS (SEQ ID NO: 313)

EVQLVESGGGLVQPGGSLRLSCAASEFTVGTNHMNWVRQAPGKGLEWVSVIYSGGNTFYAD SVKGRFTISRHTSKNTLYLQMNSLTAEDTAVYYCARGLGGMDVWGQGTTVTVSS (SEQ ID NO: 314)

EVQLVESGGGLVQRGESLRLYCAASGFTFSKYWMNWVRQAPGKGLEWVANIKGDGSEKYYV DSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDYWGSGYYFDFWGQGTLVTVSS (SEQ ID NO: 315)

EVQLVESGGGLVQSGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSEKYYV DSVKGRFTISRDNAKNSLYLQMNSLRADDTAVYYCARDDIVVVPAPMGYYYYYFGMDVWGQ GTTVTVSS (SEQ ID NO: 316)

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDFAMHWVRQAPGKGLEWVSGISWTGGNMDYA NSVKGRFTISREDAKNSLYLQMNSLRAADTALYYCVKDIRGIVATGGAFDIWGRGTMVTVS S (SEQ ID NO: 317)

EVQLVESGGGLVQPGGSLRLSCAASGFTVGTNYMNWVRQAPGKGLEWISVIYSGGSTFYAD SVKGRFTISRQTSQNTLYLQMNSLRPEDTAVYYCARGIRGFDIWGQGTMVTVSS (SEQ ID NO: 318)

EVQLVESGGGLVQPGGSLRLSCAASGFTISTNYMNWVRQAPGKGLEWVAVIYSSGSTYYID SVKGRFTISRLTSKNTVYLQMSSLNSEDTAVYYCARGIRGFDIWGQGTMVTVSS (SEQ ID NO: 319)

EVQLVESGGGLVQPGRSLRLSCAASGFTIDDSAMHWVRQTPGKGLEWVSGISWKSGSIGYA DSVRGRFTISRDNAKNSLYLQMNSLRVEDTALYYCVKDIRGNWNYGGNWFDPWGQGTLVTV SS (SEQ ID NO: 320)

EVQLVESGGGLVQPGGSLRLSCEASGFTVGVNHMNWVRQAPGKGLEWVSVIFSSGRTFYGD YVKGRLTIFRQTSQNTVYLQMNSLRSEDTAIYYCARGIGGLDIWGRGTMVTVSS (SEQ ID NO: 321)

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYALHWVRQAPGKGLEWVSGISWTGGTIDYA DSVKGRFTISRDNAKNSLYLQMSSLRTEDTAIYYCTRDIRGNWKYGGWFDPWGQGTLVTVS S (SEQ ID NO: 322)

QVQLVQSGTEVKKPGASVKVSCKASGYTFTAYYMHWVRQAPGQGLDWMGWISPNSGFTNYA QKFQGRVTMTRDTSINTFYMELSGLRSDDTAVYYCAREGSTHHNSFDPWGQGTLVTVSS (SEQ ID NO: 323)

EVQLVESGGGLVQPGGSLRLSCAASGFTVGTNFMNWVRQAPGKGLEWVSAIYSGGTANYAD SVKGRFTISRDTSRNTLYLQMNSLRTEDTAVYYCARGGGMDVWGQGTTVTVSS (SEQ ID NO: 324)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFNTYVLSWVRQAPGQGLEWMGEIIPILGAANYA QNFQGRVTFTTDESTNTAYMDLSSLRSEDTAVYYCARDRTSGGFDPWGQGTLVTVSS (SEQ ID NO: 325)

QVQLVQSGAEVEKPGASVKVSCKASGYIFTHYGISWVRQAPGQGLEWVGWISPYNGYTDYA QKLQGRVTLTTDTSTTTAYMELRNLRSDDTAMYYCSRGRGPYWSFDLWGRGTLVTVSS (SEQ ID NO: 326)

VL Sequences:

DIQMTQSPSTLSASVGDRVTITCRASQSISNWLAWYQQKPGKAPKLLIYKASSLESGVPSR FSGSGSGTEFTLTISSLQPDDFATYYCQQYHSYSYTFGQGTKEIK (SEQ ID NO: 327)

DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYTASSLQSGVPSR FSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPLTFGGGTKVAIK (SEQ ID NO: 328)

DIQMTQSPSSLSASVGDRVTITCRTSQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSR FSGSGSGTEFTLTISSLQPEDFATYYCLQHNNYPYTFGQGTKLEIK (SEQ ID NO: 329)

DIVMTQTPLSSPVTLGQPASISCRSSQTLVHGDGNTYLSWIQQRPGQPPRLLIYKVSNQFS GVPDRFSGSGAGTDFTLKISRVEAEDVGLYFCMQATHFPITFGQGTRLEIK (SEQ ID NO: 330)

DIVMTQTPLSSPVTLGQPASISCRSSPSLVHSDGNTYLSWLQQRPGQPPRLLIYKISNRFS GVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCMQATHFPITFGQGTRLEIR (SEQ ID NO: 331)

DIQMTQSPSSLSASLGDRVTITCRASQSINSYLNWYQQKPGKAPKLLIYVASSLQSGVPSR FSGSGSGTEFTLTISNLQPEDFATYYCQQSYSTPPITFGQGTRLEIK (SEQ ID NO: 332)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYVASSLQSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPITFGQGTRLEIK (SEQ ID NO: 333)

DIQMTQSPSSLSASVGDRVTITCRASQTINIYLNWYQQKPGRAPRLLIYAASSLQSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCHQSYSTPPITFGQGTRLEIK (SEQ ID NO: 334)

DIQMTQSPSSLSASVGDRVTITCRASQSMSSYLNWYQQKPGRAPKLLIFAASSLQSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPITFGQGTRLEIK (SEQ ID NO: 335)

EIVLTQSPGTLSLSPGERATLSCRASQSFNFNYLAWYQQKPGQAPRLLIYGASSRATGIPD RFSGSGSGTDFTLTINRLEPEDFGVFYCQQYESAPWTFGQGTKVEIK (SEQ ID NO: 336)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKLLIYAASSLQSGVPSRFSG GGSGTDFTLTISSLRPEDFATYYCQQSYCTPPITFGQGTRLEIK (SEQ ID NO: 337)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPITFGQGTRLEIK (SEQ ID NO: 338)

DRVTITCRASQVISNYLAWYQQKPGKVPRLLIYAASTLQSGVPSRFSGSGSGTDFTLTISS LQPEDVATYYCQKYNSAPRTFGQGTKVEIK (SEQ ID NO: 339)

DIQMTQSPSSLSASVGDRVTITCRASQNINNYLNWYQQKPGKAPKLLIYAASSFQNAVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQSYNTPLTFGGGTKVEIK (SEQ ID NO: 340)

DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSR FSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPYTFGQGTKLEIK (SEQ ID NO: 341)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPITFGQGTRLEIK (SEQ ID NO: 342)

[00202] 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: 343)

VL Sequences:

AIVMTQTPSPVSAAVGGTVTINCQASESVYSNNYLSWFQQKPGQPPKLLIYLASTLASGVP SRFKGSGSGTQFTLTISGVQCDDAATYYCIGGKSSSTDGNAFGGGTEVVVR (SEQ ID NO: 344)

[00203] 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: 345)

QPVLTQPPSVSAAPGQKVTISCSGSSSNIANNYVSWYQQLPGTAPKLLIFANNKRPSGIPD RFSGSKSGTSAALDITGLQTGDEADYYCGTWDSDLRAGVFGGGTKLTVL (SEQ ID NO: 346)

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYA QKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCAREGTIYDSSGYSFDYWGQGTLVTVS S (SEQ ID NO: 347)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 348)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 349)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 350)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 351)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 352)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 353)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSRYGVHWVRQAPGQGLEWMGRLIPIVSMTNYA
QKFQDRVSITTDKSTGTAYMELRSLTSEDTALYYCASVGQQLPWVFFAWGQGTLVTVSS
(SEQ ID NO: 354)

QMQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 355)

QVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISFDGSNKYYA
DSVRGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARGWLDRDIDYWGQGTLVTVSS
(SEQ ID NO: 356)

QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAYSWVRQAPGQGLEWMGGIIPSFGTANYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGPIVATITPLDYWGQGTLVTVSS
(SEQ ID NO: 357)

QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAYSWVRQAPGQGLEWMGGIIPIFGTANYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGPIVATITPLDYWGQGTLVTVSS
(SEQ ID NO: 358)

QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAYSWVRQAPGQGLEWMGGIIPSFGTANYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGPIVATITPLDYWGQGTLVTVSS
(SEQ ID NO: 359)

QMQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPAFGTANYA
QKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGPIVATITPLDYWGQGTLVTVSS
(SEQ ID NO: 360)

VL Sequences:

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 361)

AIRMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYTTSSLKSGVPSR
FSGSGSGTDFTLTISRLQPEDFATYYCQQSYSSTWTFGRGTKVEIK (SEQ ID
NO: 362)

QSVLTQPPSVSAAPGQKVTISCSGNNSNIANNYVSWYQQLPGTAPKLLIYDNNYRPSGIPD
RFSGSKSGTSATLDITGLQTGDEADYYCGVWDGSLTTGVFGGGTKLTVL (SEQ ID
NO: 363)

LPVLTQPASVSGSPGQSITISCTGTTSDIGGYDYVSWYQQHPGKAPKLMIYDVSKRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTHVFGTGTKLTVL (SEQ ID
NO: 364)

QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYRSSTLGPVFGGGTKLTVL (SEQ ID
NO: 365)

QAGLTQPPSVSEAPRQRVTISCSGSSSNIGNNAVNWYQQLPGKAPKLLIYYDDLLPSGVSD
RFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGYVFGTGTKLTVL (SEQ ID
NO: 366)

QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGVP
DRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSTTHVFGTGTKVTVL (SEQ ID
NO: 367)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSVWVFGGGTQLTVL (SEQ ID
NO: 368)

QSVLTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGRAPRLMIYDVSNRPSGVS
NRFSGSKSGNTASLTISGLQAEDEGDYYCSSYTSGGTLGPVFGGGTKLTVL (SEQ ID
NO: 369)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAVVFGGGTKLTVL (SEQ ID
NO: 370)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQVPGTAPKLLIYDNNKRPSGIPD
RFSGSNSDTSATLGITGLQTGDEADYYCGTWDSSLSAWVFGGGTKLTVL (SEQ ID
NO: 371)

QSVVTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSGIPD
RFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAGSVVFGGGTKLTVL (SEQ ID
NO: 372)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCLVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 373)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 374)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 375)

SYELMQPPSVSVAPGKTATIACGGENIGRKTVHWYQQKPGQAPVLVIYYDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHRIFGGGTKLTVL (SEQ ID
NO: 376)

[00204] 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: 377)

VL Sequences:

QPVLTQPASVSGSPGQSITISCTGSSSDVGSYDLVSWYQQSPGKVPKLLIYEGVKRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSYAGTRNFVFGGGTQLTVL (SEQ ID
NO: 378)

[00205] 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: 379)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGQSWPGFDYWGQGTLVTVSS (SEQ ID NO: 380)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGQSFPGFDYWGQGTLVTVSS (SEQ ID NO: 381)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAAFDYWGQGTLVTVSS (SEQ ID NO: 382)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAGYDYWGQGTLVTVSS (SEQ ID NO: 383)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSKGFDYWGQGTLVTVSS (SEQ ID NO: 384)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWKQGIVTVYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTV (SEQ ID NO: 385)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRNGIVTVYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 386)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSDIWKQGMVTVYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 387)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRQGLATAYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 388)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSEIVATGILTSYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 389)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIGRQGLITVYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 390)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWYQGLVTVYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 391)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSDIWKQGFATADS VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 392)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWKQGIVTVYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 393)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRQGLATAYD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSS (SEQ ID NO: 394)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRNGIVTVYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAAFDYWGQGTLVTVSS (SEQ ID NO: 395)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRNGIVTVYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAGYDYWGQGTLVTVSS (SEQ ID NO: 396)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWRNGIVTVYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSKGFDYWGQGTLVTVSS (SEQ ID NO: 397)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMetSWVRQAPGKGLEWVSSIWYQGLVTV YADSVKGRFTISRDNSKNTLYLQMetNSLRAEDTAVYYCAKWSAAFDYWGQGTLVTVSS (SEQ ID NO: 398)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWYQGLVTVYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSAGYDYWGQGTLVTVSS (SEQ ID NO: 399)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIWYQGLVTVYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKWSKGFDYWGQGTLVTVSS (SEQ ID NO: 400)

VL Sequences:

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYYASTLQSGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQQDNGYPSTFGQGTKVEIKR (SEQ ID NO: 401)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYYASTLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQDNGYPSTFGQGTKVEIKR (SEQ ID
NO: 402)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQDNGYPSTFGGGTKVEIKR (SEQ ID
NO: 403)

[00206] 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: 404)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSINKDGHYTSYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKNLDEFDYWGQGTLVTVSSGGGGSG
GGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAA
SSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPNTFGQGTKVEIKR
(SEQ ID NO: 405)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIMATGAGTLYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDGAGFDYWGQGTLVTVSSGGGGSG
GGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYSA
SQLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSRPSTFGQGTKVEIKR
(SEQ ID NO: 406)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLQWVSTITSSGAATYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKNYTGFDYWGQGTLVTVSSGGGGSG
GGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYNA

SSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYTYGPGTFGQGTKVEIKR
(SEQ ID NO: 407)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIYSTGGATAYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSSAGFDYWGQGTLVTVSSGGGGSG
GGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYYA
STLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQDNGYPSTFGQGTKVEIKR
(SEQ ID NO: 408)

PDL1 x 41BB Dual Targeting

[00207] In some embodiments, the fusion proteins are bispecific molecules that include a TBD that binds 41BB 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-41BB TBDs. In these embodiments, the TNFRSF signaling is enhanced and focused by the presence of a PDL1 expressing cell.

Tetravalent 41BB agonist: hzRH3v5-1
EVQLLESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIESGRNTVYAE
SVKGRFTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKPG
GGGDKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGGSGGGGS**EVQLLES**
GGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIESGRNTVYAESVKGRFT
ISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKPGG (SEQ
ID NO: 448)

Bispecific PDL1 x 41BB: hz28A2v5 x hzRH3v5-1
EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE
SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKPGGSGGS**EVQLL**
ESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIESGRNTVYAESVKGR
FTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKPGGGGDK

THTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 449)

Bispecific PDL1 x 41BB: hz28A2v5 x hzRH3v5-2

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE
SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKPGGSGGS**EVQLL**
ESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIYSGRNTVYAESVKGR
FTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKPGGGGDK
THTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 450)

Bispecific PDL1 x 41BB: hz28A2v5 x hzRH3v5-16

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE
SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKPGGSGGS**EVQLL**
ESGGGEVQPGGSLRLSCAASGFSFSINAMGWYRQAPGKRREFVAAIYSGSSTVYAESVKGR
FTISRDNAKNTVYLQMSSLRAEDTAVYYCGLLKGNRVVSPSVAYWGQGTLVTVKPGGGGDK
THTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 451)

Bispecific PDL1 x 41BB: hz28A2v5 x hz4E01v16

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE
SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKPGGSGGSEVQLL
ESGGG**EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFVSRLAWQGG**
STDYVESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWG
QGTLVTVKPGGGGDKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT

TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 452)

Bispecific PDL1 x 41BB: hz28A2v5 x hz4E01v18

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKPGGSGGSEVQLL ESGGG**EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFVSRLAWGGG STDYVESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWG QGTLVTVKP**GGGGDKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 453)

Bispecific PDL1 x 41BB: hz28A2v5 x hz4E01v21

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKPGGSGGSEVQLL ESGGG**EVQLLESGGGEVQPGGSLRLSCAASGWAFSNYGMAWFRQAPGKEREFVSRLAWGGG STDYVESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWG QGTLVTVKP**GGGGDKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 454)

Bispecific PDL1 x 41BB: hz28A2v5 x hz4E01v22

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKPGGSGGSEVQLL ESGGG**EVQLLESGGGEVQPGGSLRLSCAASGWAFGNYGMAWFRQAPGKEREFVSRLAWSGG STDYVESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWG QGTLVTVKP**GGGGDKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT

TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 455)

Bispecific PDL1 x 41BB: hz28A2v5 x hz4E01v23

EVQLLESGGGEVQPGGSLRLSCAASGGIFAIKPISWYRQAPGKQREWVSTTTSSGATNYAE SVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCNVFEYWGQGTLVTVKPGGSGGSEVQLL ESGGG**EVQLLESGGGEVQPGGSLRLSCAASGWAFSNYGMAWFRQAPGKEREFVSRLAWSGG STDYVESVKGRFTISRDNAKNTLYLQMSSLRAEDTAVYYCARQRSYSRYDIRTPQTYDYWG QGTLVTVKP**GGGGDKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 456)

Folate Receptor Alpha (FRα) Targeting

[00208] 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.

[00209] Exemplary FRα -targeting single domain sequences are shown below:

Fra-5:

QLQLQESGGGLVQAGGSLRLSCAASGIMFYISDMGWYRQAPGKQREFVATITSGGTTNYAD SVEGRFSISRDNAKNTVYLQMNSLEPEDTAVYYCTAHGPTYGSTWDDLWGQGTQVTVKPGG (SEQ ID NO: 409)

CDR1: GIMFYISD (SEQ ID NO: 410)

CDR2: TITSGGTTNY (SEQ ID NO: 411)

CDR3: TAHGPTYGSTWDDL (SEQ ID NO: 412)

Fra-6:

QVQLQESGGGLVQAGGSLRLSCAASETFGVVFTLGWYRQTPGKQREFVARVTGTDTVDYAD SVKGRFTISSDFARNTVYLQMNNLKPEDTAVYYCNTGAYWGQGTQVTVKPGG (SEQ ID NO: 413)

CDR1: TFGVVFT (SEQ ID NO: 414)

CDR2: VTGTDTV (SEQ ID NO: 415)

CDR3: NTGAY (SEQ ID NO: 416)

Fra-57:

QVQLVQSGGGLVQTGGSLRLSCAASGRTASTYSMGWFRQAPGKERQFVARIIWSTGSTYYT NSVEGRFTISRDIAKNTLYLQMNSLEPEDTAVYYCTAREPTGYDYWGQGTQVTVKPGG (SEQ ID NO: 417)

CDR1: GRTASTYS (SEQ ID NO: 418)

CDR2: IWSTGST (SEQ ID NO: 419)

CDR3: TAREPTGYDY (SEQ ID NO: 420)

1A3:

QLQLQESGGGLVQAGGSLGLSCAASGSIFRFGARGWYRQAPGKQRELVAIITSGGSTNYAD SVQGRFTISRDNAKNMVYLQMNGLKSGDTAVYYCAADRSDAVGVGWDYWGQGTQVTVKPGG (SEQ ID NO: 421)

CDR1: GSIFRFGA (SEQ ID NO: 422)

CDR2: ITSGGST (SEQ ID NO: 423)

CDR3: AADRSDAVGVGWDY (SEQ ID NO: 424)

1F3:

QVQLQQSGGGLVQTGGSLRLSCAASGRTASTYSMGWFRQAPGKERQFVARIIWSTGSTYYT NSVEGRFTISRDIAKNTLYLQMNSLEPEDTAVYYCTARDPTGYDYWGQGTQVTVKPGG (SEQ ID NO: 425)

CDR1: GRTASTYS (SEQ ID NO: 418)

CDR2: IIWSTGST (SEQ ID NO: 426)

CDR3: TARDPTGYDY (SEQ ID NO: 427)

1G10:

QLQLQESGGGLVQAGGSLRLSCAASGSIFSIDATAWYRQAPGKQRELVAIITSSGSTNYPD
SVKGRFTISRDNAKNTVYLQMNSLNPEDTALYSCNAITRMGGSTYDFWGQGTQVTVKPGG
(SEQ ID NO: 428)

CDR1: GSIFSIDA (SEQ ID NO: 429)

CDR2: ITSSGST (SEQ ID NO: 430)

CDR3: NAITRMGGSTYDF (SEQ ID NO: 431)

[00210] 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. 41BB-Targeting Single Domain Antibodies Bind 41BB

[00211] The 41BB-targeting single domain antibodies (sdAbs) referred to herein as 1G3 (SEQ ID NO: 432), 1H4 (SEQ ID NO: 436), 1H1 (SEQ ID NO: 440), 4H4 (SEQ ID NO: 16), 1H8 (SEQ ID NO: 444), 4F5 (SEQ ID NO: 23), and 4E1 (SEQ ID NO: 20) bind recombinant human 41BB (Figure 2A), cynomolgus 41BB (Figure 2B). The 41BB-targeting single domain antibodies (sdAbs) referred to herein as 4F5 (SEQ ID NO: 23), 4H04 (SEQ ID NO: 16), 4E01 (SEQ ID NO: 20), RH03 (SEQ ID NO: 25), and D1 (SEQ ID NO: 29) bind human 41BB expressed on the cell surface of CHO cells (Figure 3). The 41BB-targeting sdAbs referred to herein as 4H04, RH03, and bind cynomolgus 41BB. For Figure 2A, Figure 2B, and Figure 4, binding was assessed by ELISA wherein recombinant 41BB-mFc fusion protein (a fusion protein containing 41BB operably linked to a mouse Fc region) was immobilized on a Medisorp 96 well plate. For Figure 3, binding was assessed by flow cytometry using 41BB expressing CHO cells, and the data is presented as median fluorescence intensity.

Example 2. 41BB-Targeting Single Domain Antibodies Block 41BB

[00212] The 41BB-targeting single domain antibodies (sdAbs) referred to herein as 4F05 (SEQ ID NO: 23), 4H04 (SEQ ID NO: 16), 4E01 (SEQ ID NO: 20), RH03 (SEQ ID

NO: 25), and D1 (SEQ ID NO: 29) block the interaction between 41BB and its ligand 41BBL. All single domain antibodies tested, with the exception of RH3 blocks the interaction between 41BB and 41BBL. Blocking was assessed by flow cytometry using a recombinant 41BB fusion protein and 41BB expressing CHO cells, data is presented as median fluorescence intensity.

[00213] In contrast to the 41BB sdAbs of the disclosure, conventional bivalent anti-41BB antibodies do not induce 41BB signaling unless further clustered with an exogenous crosslinking anti-human IgG antibody. Figure 6 demonstrates the inability of a conventional bivalent anti-41BB antibody PF-05082566, which is disclosed in US Patent No. 8,337,850, to induce 41BB signaling unless further clustered with an exogenous crosslinking anti-human IgG antibody. In Figure 6, 41BB signaling was monitored using a NF-kB reporter 293 cell line expressing 41BB.

Example 3. PDL1-Targeting Single Domain Antibodies Bind PDL1 and Block the Interaction Between PLD1 and PD1

[00214] The studies presented herein use an exemplary PDL1 single domain antibody (sdAb), referred to herein as 28A10 (SEQ ID NO: 100) to demonstrate that the PDL1-targeting sdAbs of the disclosure bind cell surface PDL1 (Figure 7A) and block the interaction of PDL1 with PD1 (Figure 7B). Binding was assessed by flow cytometry on PDL1 expressing CHO cells, and blocking was assessed by flow cytometry using a recombinant PD1 fusion protein and PDL1 expressing CHO cells. The data presented in Figures 7A and 7B are presented as median fluorescence intensity.

Example 4. PDL1-41BB Targeting Fusion Proteins

[00215] The disclosure provides fusion proteins that target at least PDL1 and 41BB. These bispecific PDL1-41BB targeting fusion proteins are agonists of PDL1-dependent 41BB mediated signaling. Figures 8A and 8B are conceptual schematics wherein the bispecific fusion proteins have minimal 41BB agonistic properties (Figure 8A) unless bound by a PD-L1 expressing cell (Figure 8B). Figure 8C demonstrates the ability of a PDL1-positive cell, in this case, a population of PDL1 transfected CHO cells, to mediate 41BB signaling and the inability of PDL1-negative cell, in this case, a population of

untransfected CHO cells, to mediate 41BB signaling. Two distinct bispecific fusion proteins are shown in this figure, each containing a distinct 41BB binding VHH (e.g., 4E01or RH3) and the same PD-L1 VHH, 28A10. 41BB signaling was monitored using a NF-kB reporter 293 cell line expressing 41BB. This reporter cell line implements an NF-kB driven secreted alkaline phosphatase, to monitor NF-kB signaling.

[00216] The PDL1-41BB targeting fusion proteins of the disclosure include a humanized anti-41BB sequence. In the studies presented herein, the PDL1-41BB targeting fusion proteins of the disclosure include a humanized anti-41BB sequence such as hzRH3v5-1 (SEQ ID NO: 30) and/or hzRH3v9 (SEQ ID NO: 82) bind both human and cynomolgus 41BB (Figures 9A, 9B), including human 41BB and cynomolgus 41BB expressed on the surface of CHO cells (Figures 9C, 9D). Binding was assessed by flow cytometry on 41BB expressing 293freestyle cells.

[00217] The humanized variants hzRH3v5-1 and hzRH3v9 do not block binding of 41BBL to cell surface 41BB as shown in Figure 9E. In these studies, a recombinant fusion protein 41BBL-mFc, containing a mouse Fc region, was used, and bound 41BBL was detected using an anti-mouse IgG-Fc specific secondary antibody.

[00218] The humanized variant hzRH3v5-1 specifically binds 41BB as compared to the other TNFRSF members OX40 and GITR (Figure 10). Binding was assessed by flow cytometry using CHO cells expressing the given TNFRSF member.

[00219] Additional humanized 41BB variants were analyzed. Figures 11A, 11B, 11C, and 11D demonstrate the binding to human (Figure 11A and Figure 11C) or cynomolgus monkey (Figure 11B) 41BB of the humanized 4E01 variants. Binding was assessed by flow cytometry on 41BB expressing 293freestyle cells. Figure 11D demonstrates that the humanized variants hz4E01v16, hz4E01v18, hz4E01v21, hz4E01v22 and hz4E01v23 block binding of 41BBL to cell surface 41BB. In these studies, a recombinant fusion protein 41BBL-mFc, containing a mouse Fc region was used and bound 41BBL was detected using an anti-mouse IgG-Fc specific secondary antibody.

[00220] The PDL1-41BB targeting fusion proteins of the disclosure also include a humanized anti-PDL1 sequence. In the studies presented herein, the PDL1-41BB targeting fusion proteins of the disclosure include a humanized anti-PDL1 sequence such as hz28A2v1 (SEQ ID NO: 120), hz28A2v2 (SEQ ID NO: 121), hz28A2v3 (SEQ ID NO: 122), and hz28A2v4-1 (SEQ ID NO: 123). Figure 12 demonstrates binding of

humanized single domain antibodies targeting PDL1. Binding was assessed by flow cytometry on PDL1-expressing CHO cells.

[00221] Figure 13 is a schematic of two exemplary formats of a PDL1x41BB bispecific fusion protein of the disclosure, referred to herein as INBRX-105-1. INBRX-105-1-A (left) has the PDL1 and 41BB binding domains located at opposing terminal positions with a central Fc region, whereas INBRX-105-1-B (right) has the PDL1 and 41BB binding domains positioned in tandem, N-terminal to an Fc region.

[00222] These two formats were further evaluated for their ability to bind human or cynomolgus monkey 41BB, to block the interaction between 41BB and 41BBL, to bind PDL1, and to block the interaction between PDL1 and PD1.

[00223] In particular, Figures 14A, 14B, and 14C demonstrate the equivalent binding to human (Figure 14A) or cynomolgus monkey (Figure 14B) 41BB by the two distinct formats of a bispecific fusion protein targeting PDL1 and 41BB referred to herein as INBRX-105-1-A and INBRX-105-1-B and illustrated in Figure 13. Binding was assessed by flow cytometry on 41BB expressing 293freestyle cells. In the studies presented herein, hzRH3v5-1 (SEQ ID NO: 124) is the 41BB binding domain used in both formats. As shown in Figure 14C, the bispecific fusion protein containing hzRh3v5-1 does not block 41BBL binding to cell surface 41BB. In these studies, a recombinant fusion protein of 41BBL and a mouse Fc region was used, and bound 41BBL was detected using an anti-mouse IgG-Fc specific secondary antibody.

[00224] Furthermore, Figures 15A, 15B, 15C, and 15D demonstrate the equivalent binding (Figure 15A and Figure 15C) and PD1 blocking (Figure 15B and Figure 15D) by the two distinct formats of a bispecific fusion protein targeting PDL1 and 41BB referred to herein as INBRX-105-1-A and INBRX-105-1-B. Binding was assessed by flow cytometry on human (Figure 15A) or cynomolgus monkey (Figure 15C) PDL1 expressing 293freestyle cells. Blocking was assessed by flow cytometry using on human (Figure 15B) or cynomolgus monkey (Figure 15D) PDL1 expressing 293freestyle cells with either recombinant human (Figure 15B) or cynomolgus monkey (Figure 15D) PD1-mFc fusion protein. Bound PD1 was detected using an anti-mouse IgG-Fc specific secondary antibody. In the studies presented herein, hz28A2v5 is the PDL1-binding domain used in both formats.

[00225] The PDL1x41BB bispecific fusion proteins were evaluated for their ability to induce PDL1-dependent 41BB agonism. Figure 16 demonstrates the ability of humanized

versions of a PDL1x41BB bispecific fusion protein (INBRX-105-1) to induce PDL1-dependent 41BB agonism. Compared herein are two distinct formats, INBRX-105-1-A vs INBRX-105-1-B, having the PDL1 and 41BB binding domains positioned at opposite termini or in tandem within the fusion protein, respectively. Notably, INBRX-105-1-A vs INBRX-105-1-B demonstrate equivalent PDL1-dependent agonistic activities. A 41BB-expressing HEK293 NF-kB reporter cell line was used to assess 41BB signaling and a PDL1-expressing CHO cell line was used as the source of PDL1. This reporter cell line implements an NF-kB driven secreted alkaline phosphatase, to monitor NF-kB signaling.

[00226] The ability of the 41BB-speciifc binding and the PDL1-speicifc binding by the binding domains in the PDL1x41BB bispecific fusion proteins was evaluated. Figures 17A and 17B demonstrate the 41BB-specific binding by the 41BB-binding portion of a PDL1x41BB bispecific fusion protein (INBRX-105-1) of the present disclosure. Binding was assessed on 41BB (Figure 17A) or the closest homolog, TNFRSF21/DR6 (Figure 17B), expressing 293freestyle cells by flow cytometry. An anti-DR6 antibody (Invitrogen) was used to as positive control for DR6 expression. In addition, Figures 18A, 18B, and 18C demonstrate the PDL1-specific binding by the PDL1-binding portion of a PDL1x41BB bispecific fusion protein (INBRX-105-1) of the present disclosure. Binding was assessed on PDL1 (Figure 18A), the closest homologs PDL2 (Figure 18B) or VISTA/PDL3 (Figure 18C), expressing 293freestyle cells by flow cytometry. An anti-PDL2 antibody and an anti-VISTA antibody known as VSTB174, which is disclosed in PCT Publication No. WO 2015/097536, were used to as positive controls for PDL2 and PDL3 expression respectively.

[00227] The ability of the PDL1x41BB bispecific fusion proteins to simultaneously bind both 41BB and PDL1 was evaluated. Figures 19A and 19B demonstrate the ability of a PDL1x41BB bispecific fusion protein to simultaneously bind PDL1 and 41BB. INBRX-105-1 was titrated onto PDL1 expressing K562 cells and 25nM recombinant 41BB-mFc proteins was added. Bound 41BB was detected using an anti-mouse IgG-Fc specific secondary antibody. Figure 19A. is a graph showing the binding of INBRX-105-1 to the PDL1 expressing K562 cells. Figure 19B is a graph showing the binding of recombinant 41BB to INBRX-105-1 on the PDL1 expressing cells.

[00228] Figure 20 demonstrates the ability of a PDL1x41BB bispecific fusion protein to simultaneously bind recombinant PDL1 and recombinant 41BB in an ELISA. INBRX-105-1 was titrated on to immobilized (Medisorp plate) recombinant PDL1, subsequently

either 2 or 10μg/ml biotinylated-recombinant 41BB (His-tagged) was added. Bound recombinant 41BB was detected via streptavidin-HRP.

[00229] The effect of the PDL1x41BB bispecific fusion proteins to on T-cell activation and proliferation was evaluated. Figures 21A, 21B, and 21C demonstrate the effect of a PDL1x41BB bispecific fusion protein (INBRX-105-1) of the present disclosure on T-cell activation and proliferation. Herein an autologous *in vitro* co-culture system implementing immature DC (iDC) and donor matched T-cells was conducted for 7 days. $PDL1^+$ iDC were derived by enriching the monocyte population (EasySep™ Human Monocyte Enrichment Kit, STEMCELL Technologies Inc.) from human donor PBMCs and culturing them in 500 U/ml GM-CSF and 250 U/ml IL-4 for 7 days. Autologous T-cells were enriched at the same time (EasySep™ Human T-cell Enrichment Kit, STEMCELL Technologies Inc.) and cryopreserved until iDC derivation was complete. Enriched T-cells were added to iDC at approximately 20:1 (T-cell:iDC) and co-cultured for at least 7 days in the presence of IL-7. The PDL1x41BB bispecific, INBRX-105-1, is superior to the monospecific PDL1 sdAb-Fc fusion protein (hz28A2v5-Fc), the 41BB sdAb-Fc fusion protein (hzRH3v5-1-Fc), the combination of the hz28A2v5-Fc and hzRH3v5-1-Fc, the anti-PDL1 antibody Atezolizumab, the anti-41BB antibody, Utomilumab (PF-05082566, disclosed in US8337850), or the anti-PD1 antibody Prembrolizumab, and combinations thereof, at inducing INFγ (Figure 21A) or mediating $CD8^+$ T-cell proliferation (Figure 21B) and activation (Figure 21C). INFγ production in the cell supernatant was monitored using an ELISA and normalized to the standard curve. T-cell proliferation was monitored by flow cytometry using CTV labeling of T-cells. T-cell activation was assessed by the presence of the activation marker CD25 monitored by flow cytometry. Antibodies were used at 10nM. INBRX-105-1 seemingly augments low level and/or tonic T-cell activation/signaling events that is dampened by the PDL1:PD1 interaction.

[00230] Figures 22A and 22B demonstrate PDL1-dependent 41BB agonism mediated by a PDL1x41BB bispecific fusion protein (INBRX-105-1) of the present disclosure. In these studies, T-cells were cultured alone or with autologous immature DCs (iDC, PDL1-expressing), a PDL1-expressing K562 cell line or the parental K562 cell line (PDL1-negative) in the presence or absence of 10nM INBRX-105-1 for 7 days. $CD8^+$ T-cell proliferation (Figure 22A) was monitored using CTV labeling and INFγ production (Figure

22B) in the cell supernatant was monitored using an ELISA and normalized to the standard curve.

[00231] Figure 23 demonstrates the capacity of a PDL1x41BB bispecific fusion protein (INBRX-105-1) of the present disclosure to enhance the Th1 lineage defining transcription factor, T-bet, expression in T-cell populations. Herein T-cells were co-cultured with autologous immature DCs for 7 days in the presence or absence of INBRX-105-1. T-bet expression was assessed on $CD4^+$ and $CD8^+$ T-cell population by flow cytometry via intracellular staining following fixation and permeabilization. INBRX-105-1 has a more dramatic effect on T-bet expression in $CD8^+$ T-cells.

[00232] The PDL1x41BB bispecific fusion proteins of the disclosure were compared to various known monospecific antibodies. Figures 24A and 24B contrast the capacity of a PDL1x41BB bispecific fusion protein (INBRX-105-1) of the present disclosure and the combination of monospecific antibodies Atezolizumab (anti-PDL1) and Utomilumab (anti-41BB) to induce INFγ (Figure 24A) or TNFα (Figure 24B) production from $CD4^+$ or $CD8^+$ T-cells. Herein T-cells were co-cultured with autologous immature DCs for 7 days in the presence or absence of INBRX-105-1 or the combination of the monospecific antibodies. INBRX-105-1 is far superior at T-cell co-stimulation compared to monospecific antibodies targeting the same antigens. Cytokine expression was assessed on $CD4^+$ and $CD8^+$ T-cell population by flow cytometry via intracellular staining following fixation and permeabilization.

[00233] Figures 25A and 25B demonstrate the agonistic capacity of a tetravalent 41BB-binding fusion protein and PDL1x41BB bispecific fusion proteins of the present disclosure in the presence of an additional PDL1 positive (Figure 25A) or negative (Figure 25B) cell line. Notably only the tetravalent 41BB binding fusion protein is capable of inducing 41BB signaling in the absence of a PDL1 expressing cell line. The bispecific PDL1x41BB fusion proteins (INBRX-105-1, INBRX-105-2 and INBRX-105-16) only induced 41BB signaling when bound to cell surface PDL1 as shown in Figure 25A. This demonstrates that bivalent engagement of 41BB, as is the case of INBRX-105, is insufficient to effectively cluster and mediate productive 41BB signaling. Engagement of a second cell surface antigen, PDL1 as in the present example, enables further clustering of 41BB and productive signaling. Herein a 41BB-expressing HEK293 NF-kB reporter cell was used and co-incubated with either the PDL1-negative K562 cell line (Figure 25B) or a stably transfected, PDL1-expressing K562 cell line (Figure 25A). INBRX-105-1

incorporates the 41BB-targeting sdAb: hzRH3v5-1, INBRX-105-2 incorporates the 41BB-targeting sdAb: hzRH3v5-2 and INBRX-105-16 incorporates the 41BB-targeting sdAb: hzRH3v5-16 and all incorporate the hz28A2v5 PDL1-targeting sdAb. The tetravalent 41BB-targeting fusion protein used herein has the following format comprising hzRH3v5-1-Fc-hzRH3v5-1.

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What is claimed is:

1. An isolated polypeptide that binds PDL1 and 41BB and comprises a plurality of polypeptide binding domains (BDs) and a plurality of Tumor Necrosis Factor receptor superfamily (TNFRSF) binding domain (TBDs), wherein each binding domain (BD) binds PDL1 and each TNFRSF binding domain (TBD) binds 41BB; wherein each TBD in the plurality of TBDs and each BD in the plurality of BDs comprises an antibody or antigen-binding fragment thereof.

2. The isolated polypeptide of claim 1, wherein at least one TBD is a VHH domain that binds 41BB comprising CDR1, CDR2, and CDR3 amino acid sequences selected from: SEQ ID NOs: 26, 61, and 28; SEQ ID NOs: 17, 18, and 19; SEQ ID NOs: 26, 27, and 28; SEQ ID NOs: 30, 31, and 32; SEQ ID NOs: 21, 18, and 22; SEQ ID NOs: 17, 18, and 24; SEQ ID NOs: 21, 42, and 22; SEQ ID NOs: 21, 44, and 22; SEQ ID NOs: 21, 48, and 19; SEQ ID NOs: 21, 48, and 22; SEQ ID NOs: 50, 48, and 22; SEQ ID NOs: 21, 52, and 22; SEQ ID NOs: 50, 52, and 22; SEQ ID NOs: 21, 48, and 55; SEQ ID NOs: 21, 48, 57; SEQ ID NOs: 26, 63, and 28; SEQ ID NOs: 65, 61, and 28; SEQ ID NOs: 67, 61, and 28; SEQ ID NOs: 69, 61, and 28; SEQ ID NOs: 26, 71, and 28; SEQ ID NOs: 26, 73, and 28; SEQ ID NOs: 26, 75, and 28; SEQ ID NOs: 26, 77, and 28; SEQ ID NOs: 26, 79, and 28; SEQ ID NOs: 433, 434, and 435; SEQ ID NOs: 437, 438, and 439; SEQ ID NOs: 441, 442, and 443; and SEQ ID NOs: 445, 446, and 447.

3. The isolated polypeptide of claim 2, wherein at least one TBD is a VHH domain that binds 41BB and comprises an amino acid sequence that is at least 95% identical to or is 100% identical to an amino acid sequence selected from SEQ ID NOs: 16, 20, 23, 25, 29, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80-83, 432, 436, 440, and 444.

4. The isolated polypeptide of any one of claims 1-3, wherein at least one BD is a VHH domain that binds PDL1 comprising CDR1, CDR2, and CDR3 amino acid sequences selected from: SEQ ID NOs: 105, 106, and 107; SEQ ID NOs: 101, 102, and 103; SEQ ID NOs: 109, 110, and 111; SEQ ID NOs: 101, 110, and 113; SEQ ID NOs: 101, 110, and 115; and SEQ ID NOs: 101, 117, and 118.

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5. The isolated polypeptide of claim 4, wherein at least one BD is a VHH domain that binds PDL1 and comprises an amino acid sequence that is at least 95% identical to or is 100% identical to an amino acid sequence selected from SEQ ID NOs: 100, 104, 108, 112, 114, 116, and 119-124.

6. The isolated polypeptide of any one of claims 1-5, wherein each TBD in the plurality of TBDs is a VHH domain that binds 41BB.

7. The isolated polypeptide of any one of claims 1-6, wherein each TBD in the plurality of TBDs binds the same epitope on 41BB.

8. The isolated polypeptide of any one of claims 1-6, wherein at least two TBDs in the plurality of TBDs bind different epitopes on 41BB.

9. The isolated polypeptide of any one of claims 1-8, wherein each BD in the plurality of BDs is a VHH domain that binds PDL1.

10. The isolated polypeptide of any one of claims 1-9, wherein each BD in the plurality of BDs binds the same epitope on PDL1.

11. The isolated polypeptide of any one of claims 1-9, wherein at least two BDs in the plurality of BDs bind different epitopes on PDL1.

12. The isolated polypeptide of any one of claims 1-11, wherein each TBD in the plurality of TBDs and each BD in the plurality of BDs are operably linked via a linker polypeptide.

13. The isolated polypeptide of any one of claims 1-12, wherein at least one TBD is a VHH domain that binds 41BB and comprises a complementarity determining region 1 (CDR1) comprising the amino acid sequence of SEQ ID NO: 26; a complementarity determining region 2 (CDR2) comprising the amino acid sequence of SEQ ID NO: 61; and a complementarity determining region 3 (CDR3) comprising the amino acid sequence of SEQ ID NO: 28.

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14. The isolated polypeptide of any one of claims 1-13, wherein at least one VHH domain that binds PDL1 comprises a complementarity determining region 1 (CDR1) comprising the amino acid sequence of SEQ ID NO: 105; a complementarity determining region 2 (CDR2) comprising the amino acid sequence of SEQ ID NO: 106; and a complementarity determining region 3 (CDR3) comprising the amino acid sequence of SEQ ID NO: 107.

15. The isolated polypeptide of any claim 1, wherein at least one TBD is a VHH domain that binds 41BB and comprises a complementarity determining region 1 (CDR1) comprising the amino acid sequence of SEQ ID NO: 26; a complementarity determining region 2 (CDR2) comprising the amino acid sequence of SEQ ID NO: 61; and a complementarity determining region 3 (CDR3) comprising the amino acid sequence of SEQ ID NO: 28; and wherein at least one BD is a VHH domain that binds PDL1 and comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 105; a CDR2 comprising the amino acid sequence of SEQ ID NO: 106; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 107.

16. The isolated polypeptide of any one of claims 1-15, wherein each VHH is a humanized VHH.

17. The isolated polypeptide of claim 1, wherein at least one TBD is a VHH domain that binds 41BB and comprises the amino acid sequence of SEQ ID NO: 60, and wherein at least one BD is a VHH domain that binds PDL1 and comprises the amino acid sequence of SEQ ID NO: 124.

18. The isolated polypeptide of any one of claims 1-17, wherein the isolated polypeptide comprises an immunoglobulin Fc region polypeptide.

19. The isolated polypeptide of claim 18, wherein the immunoglobulin Fc region polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-6.

20. The isolated polypeptide of claim 18 or claim 19, wherein the polypeptide is tetravalent.

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21. The isolated polypeptide of claim 20, wherein the polypeptide comprises the structure: VHH-Linker-VHH-Linker-Hinge-Fc, wherein each VHH is a humanized VHH sequence.

22. The isolated polypeptide of claim 18 or claim 19, wherein the polypeptide is hexavalent.

23. The isolated polypeptide of claim 22, wherein the polypeptide comprises the structure: VHH-Linker-VHH-Linker-VHH-Linker-Hinge-Fc, wherein each VHH is a humanized VHH sequence.

24. The isolated polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence selected from SEQ ID NOs: 449-456.

25. The isolated polypeptide of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 449.

26. An isolated polypeptide comprising a polypeptide binding domain (BD) and a Tumor Necrosis Factor receptor superfamily (TNFRSF) binding domain (TBD), wherein the BD binds PDL1 and the TBD binds 41BB; wherein the TBD and the BD each comprise an antibody or antigen-binding fragment thereof.

27. An isolated polynucleotide that encodes the polypeptide of any one of claims 1-26.

28. An isolated cell comprising the polynucleotide of claim 27.

29. An isolated cell that expresses the polypeptide of any one of claims 1-26.

30. A method of producing a polypeptide of any one of claims 1-26, comprising culturing the isolated cell of claim 28 or claim 29 under conditions suitable for expressing the polypeptide.

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31. A method for treating a neoplasm, comprising administering to a subject in need thereof a therapeutically effective amount of the isolated polypeptide of any one of claims 1-26.

32. The method of claim 31, wherein the neoplasm is cancer.

33. A method for modulating immune cells to enhance tumor destruction, comprising administering to a subject in need thereof a therapeutically effective amount of the isolated polypeptide of any one of claims 1-26.

FIG. 1

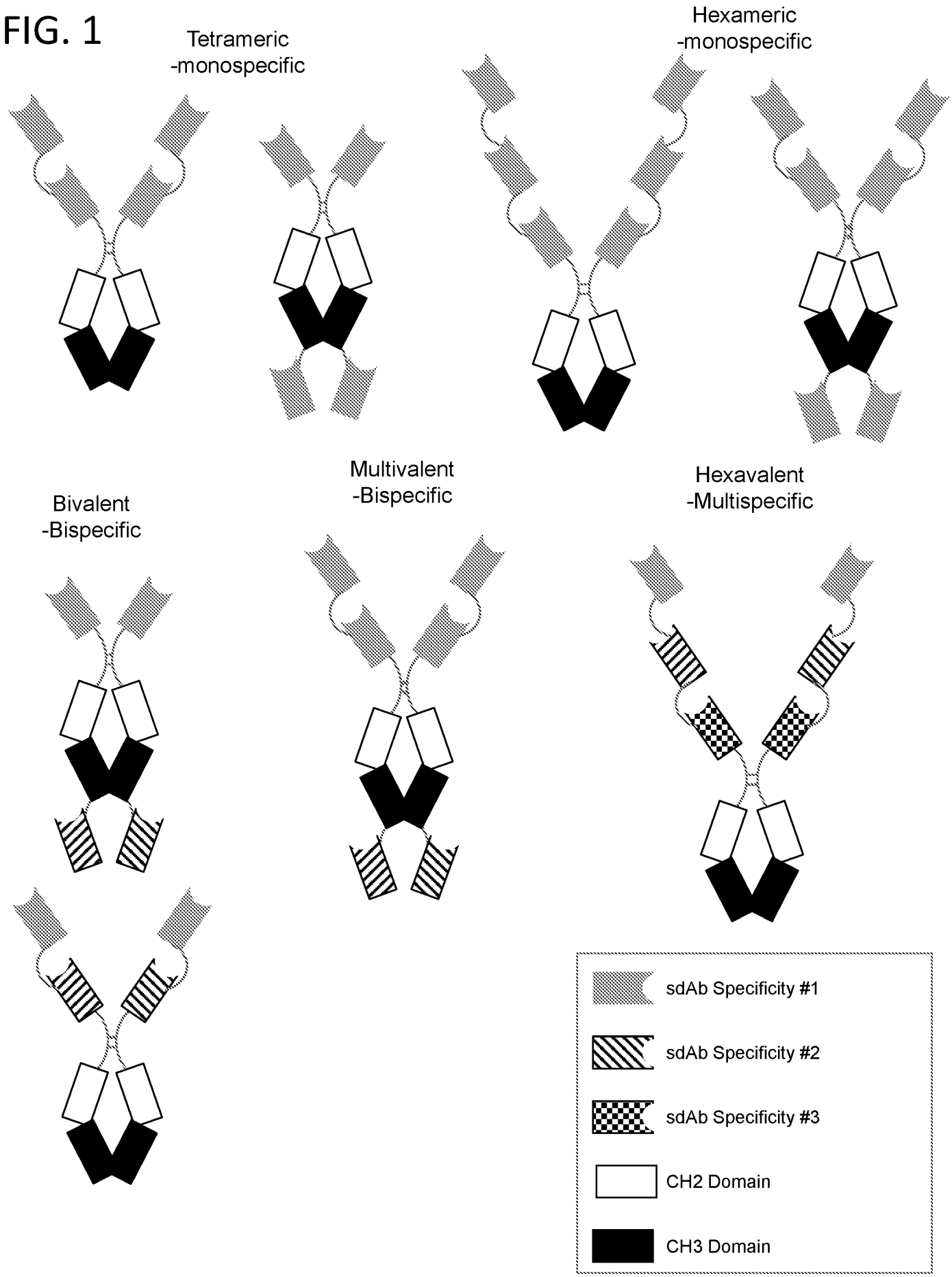

Tetrameric
-monospecific
Hexameric
-monospecific
Bivalent
-Bispecific
Multivalent
-Bispecific
Hexavalent
-Multispecific
sdAb Specificity #1
sdAb Specificity #2
sdAb Specificity #3
CH2 Domain
CH3 Domain

FIG. 2A

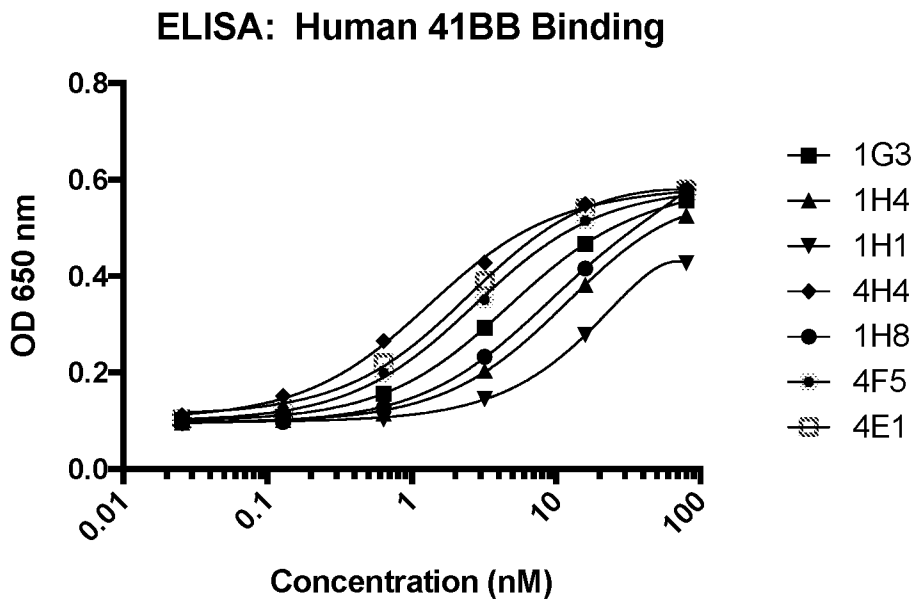

ELISA: Human 41BB Binding
OD 650 nm
0.8
0.6
0.4
0.2
0.0
0.01
0.1
1
10
100
Concentration (nM)
1G3
1H4
1H1
4H4
1H8
4F5
4E1

FIG. 2B

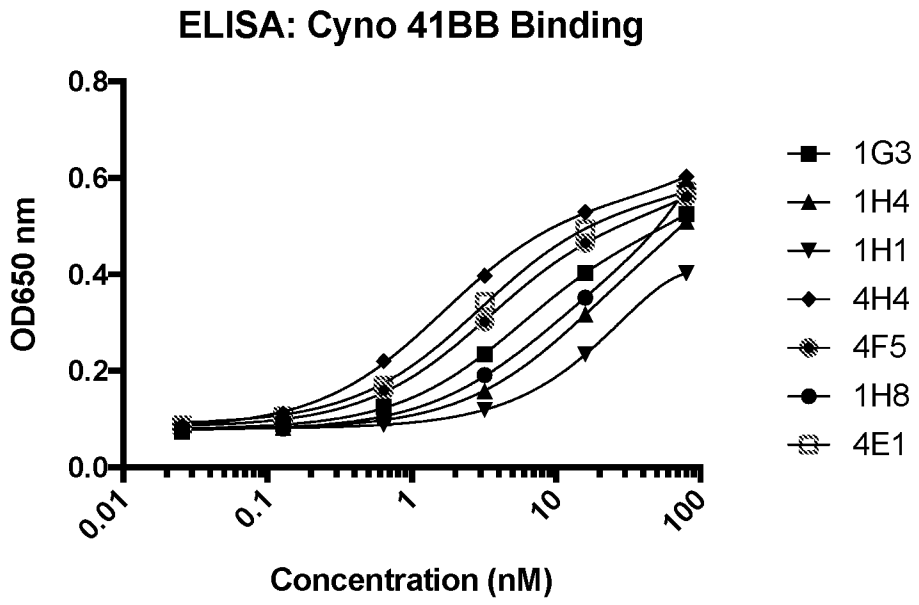

ELISA: Cyno 41BB Binding
OD650 nm
0.8
0.6
0.4
0.2
0.0
0.01
0.1
1
10
100
Concentration (nM)
1G3
1H4
1H1
4H4
4F5
1H8
4E1

FIG. 3

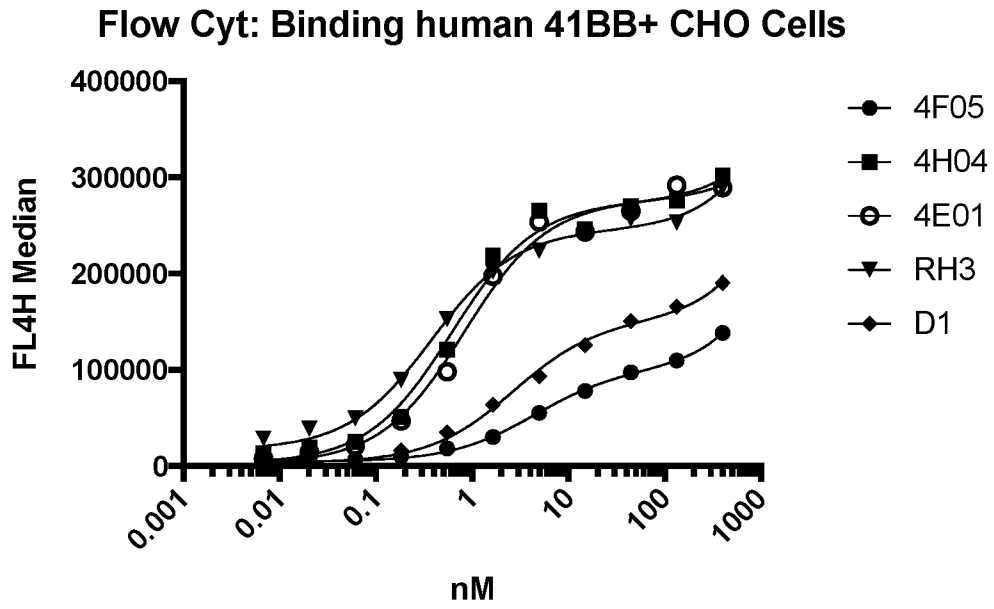

Flow Cyt: Binding human 41BB+ CHO Cells
FL4H Median
400000
300000
200000
100000
0
0.001
0.01
0.1
1
10
100
1000
nM
4F05
4H04
4E01
RH3
D1

FIG. 4

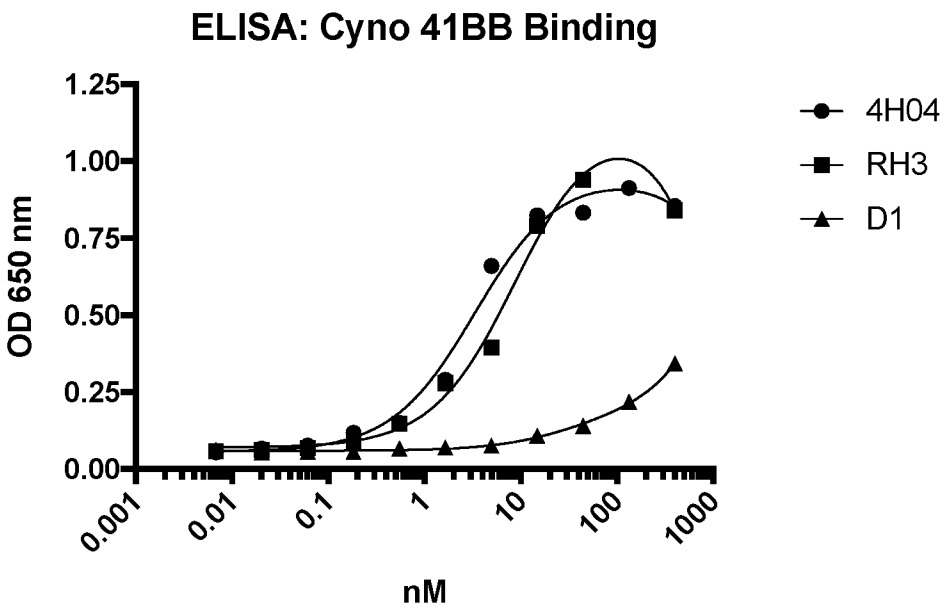

ELISA: Cyno 41BB Binding
OD 650 nm
1.25
1.00
0.75
0.50
0.25
0.00
0.001
0.01
0.1
1
10
100
1000
nM
4H04
RH3
D1

FIG. 5

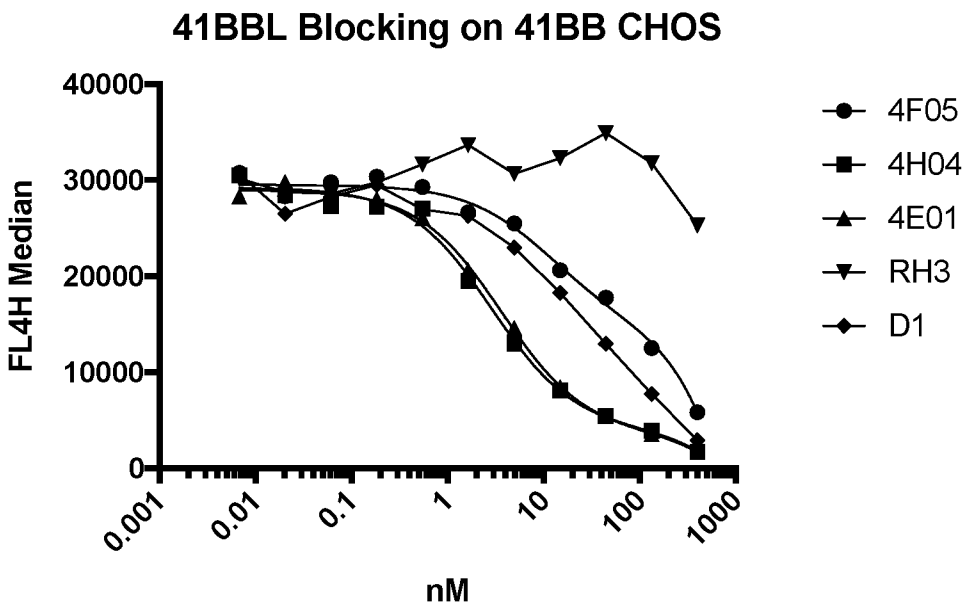

41BBL Blocking on 41BB CHOS
FL4H Median
40000
30000
20000
10000
0
0.001
0.01
0.1
1
10
100
1000
nM
4F05
4H04
4E01
RH3
D1

FIG. 6

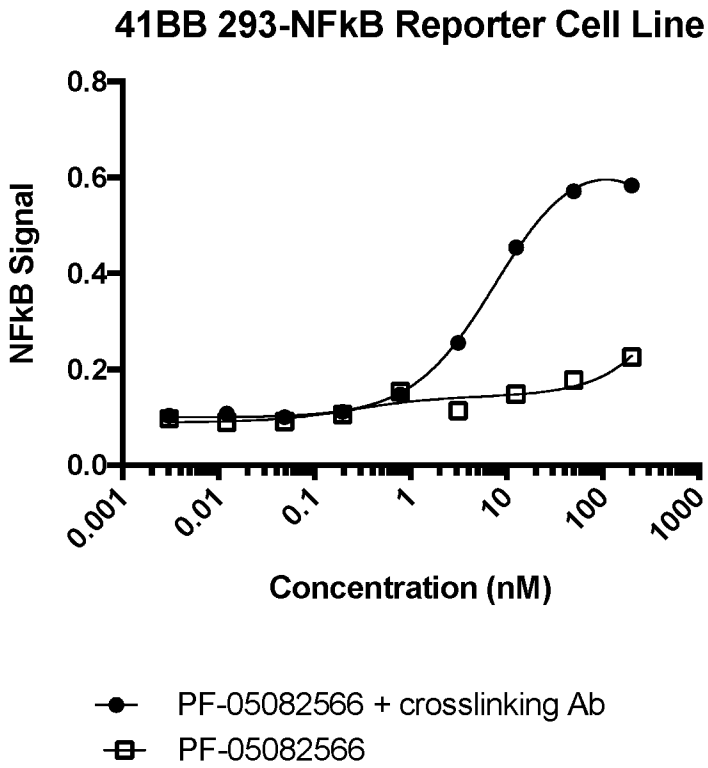

41BB 293-NFkB Reporter Cell Line
NFkB Signal
0.8
0.6
0.4
0.2
0.0
0.001
0.01
0.1
1
10
100
1000
Concentration (nM)
PF-05082566 + crosslinking Ab
PF-05082566

FIG. 7A

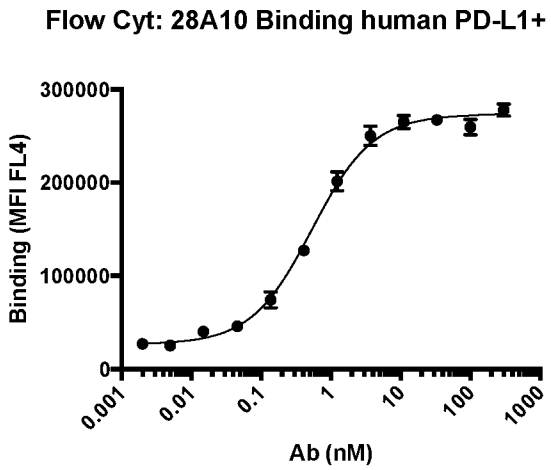

Flow Cyt: 28A10 Binding human PD-L1+ Cells
Binding (MFI FL4)
300000
200000
100000
0
0.001
0.01
0.1
1
10
100
1000
Ab (nM)

FIG. 7B

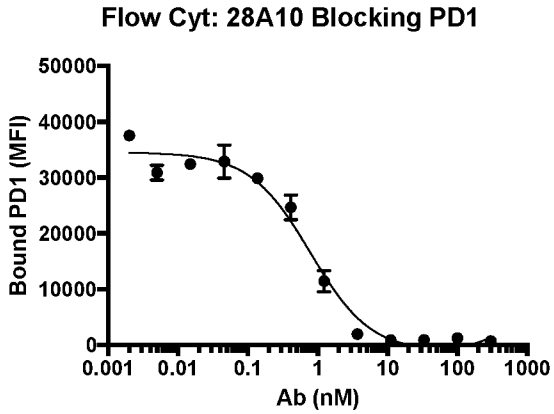
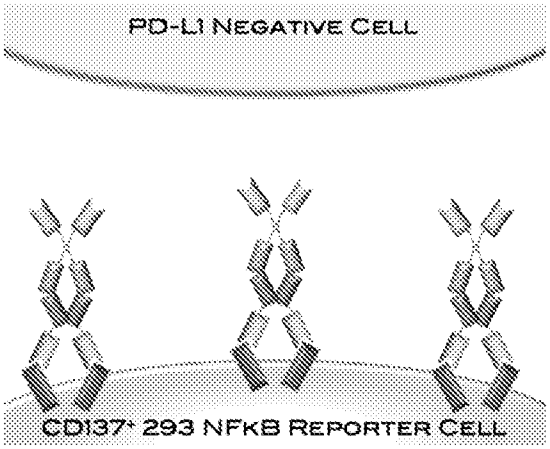
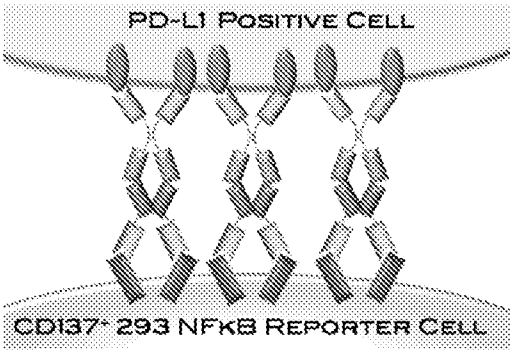

Flow Cyt: 28A10 Blocking PD1
Bound PD1 (MFI)
50000
40000
30000
20000
10000
0
0.001
0.01
0.1
1
10
100
1000
Ab (nM)

FIG. 8A



No 41BB signaling

FIG. 8B



PDL1-dependent 41BB signaling

FIG. 8C

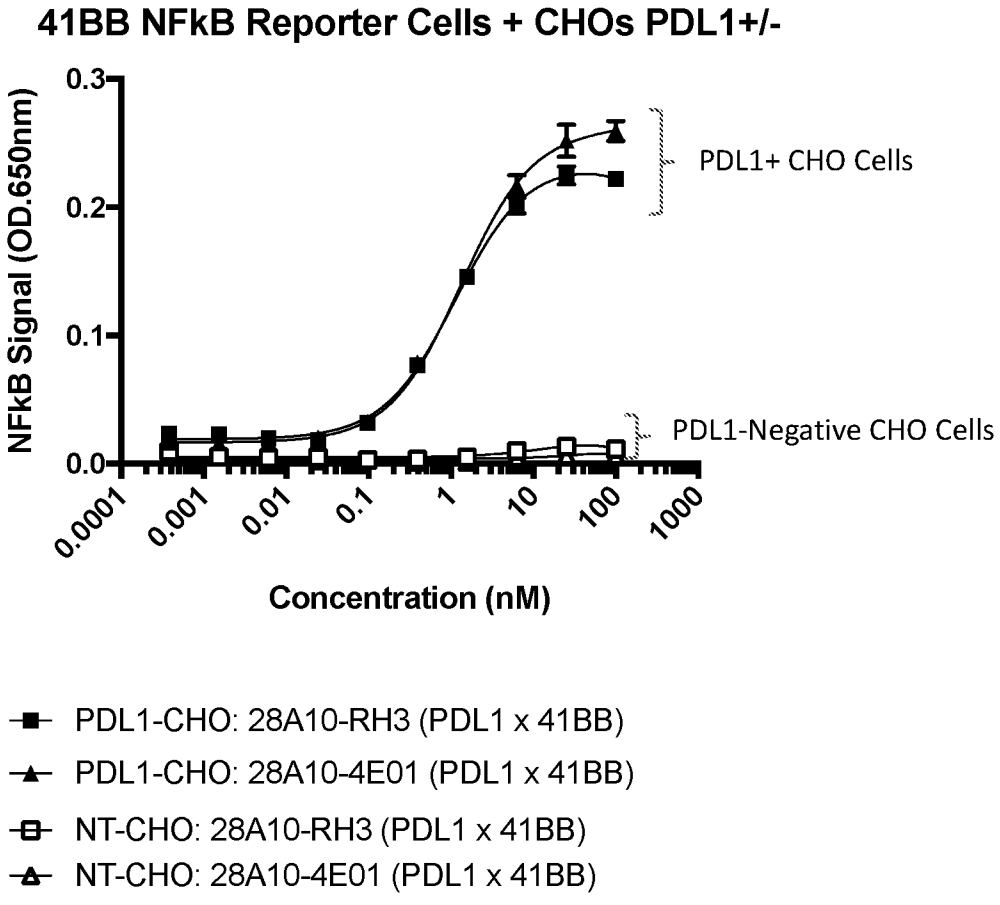


FIG. 9A

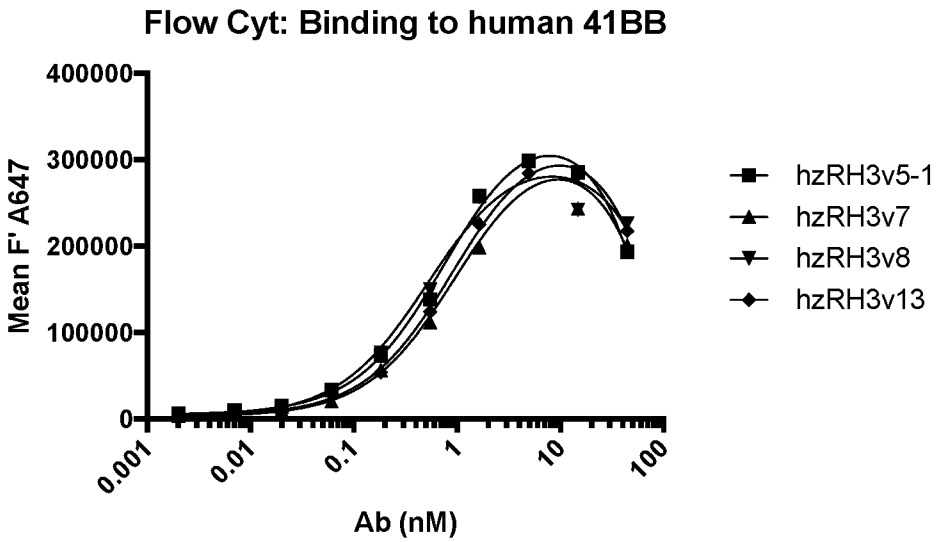

Flow Cyt: Binding to human 41BB
Mean F' A647
400000
300000
200000
100000
0
0.001
0.01
0.1
1
10
100
Ab (nM)
hzRH3v5-1
hzRH3v7
hzRH3v8
hzRH3v13

FIG. 9B

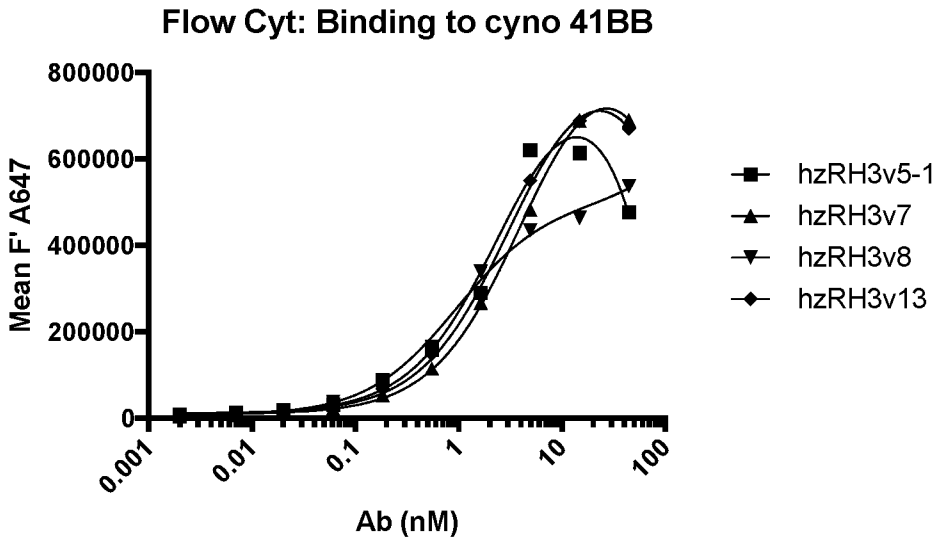

Flow Cyt: Binding to cyno 41BB
Mean F' A647
800000
600000
400000
200000
0
0.001
0.01
0.1
1
10
100
Ab (nM)
hzRH3v5-1
hzRH3v7
hzRH3v8
hzRH3v13

FIG. 9C

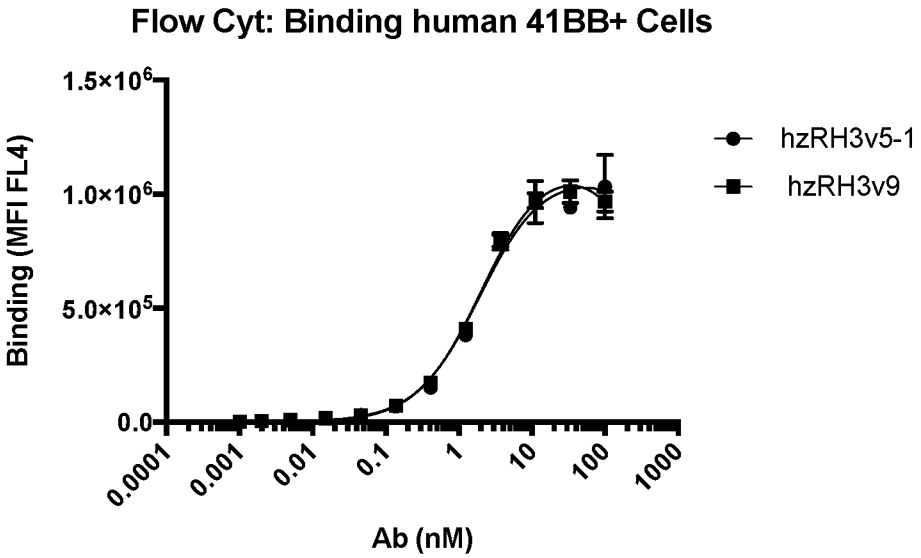


FIG. 9D

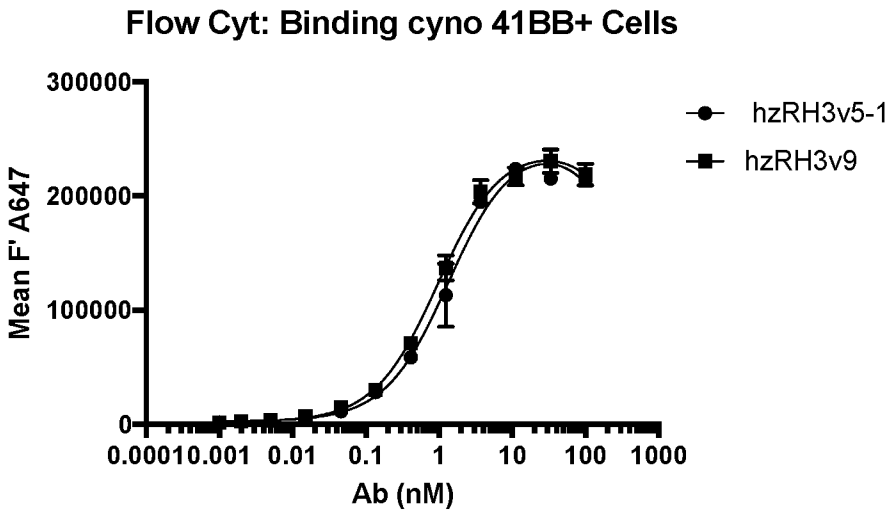


FIG. 9E

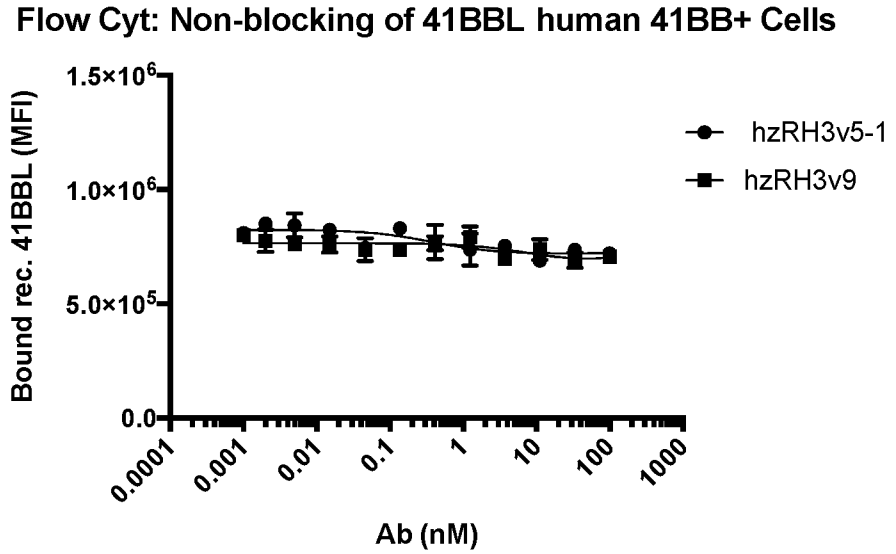


FIG. 10

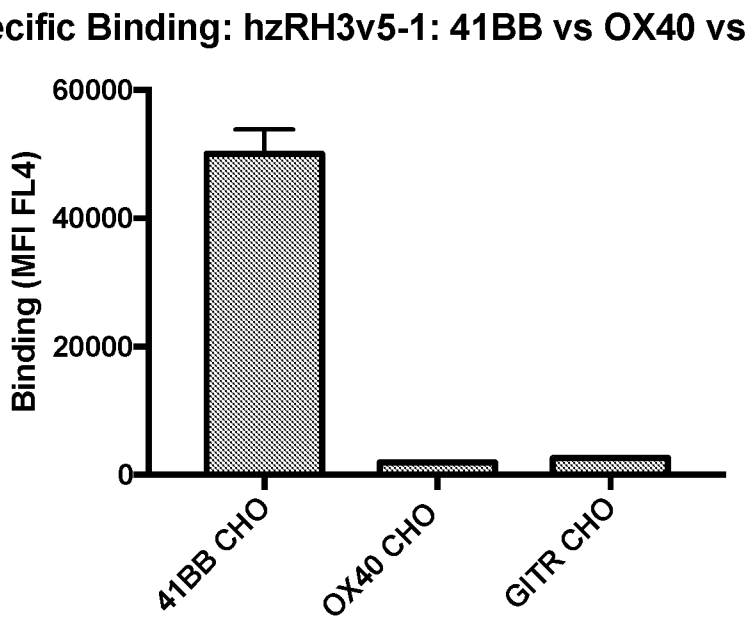

Specific Binding: hzRH3v5-1: 41BB vs OX40 vs GITR CHO
Binding (MFI FL4)
60000
40000
20000
0
41BB CHO
OX40 CHO
GITR CHO

FIG. 11A

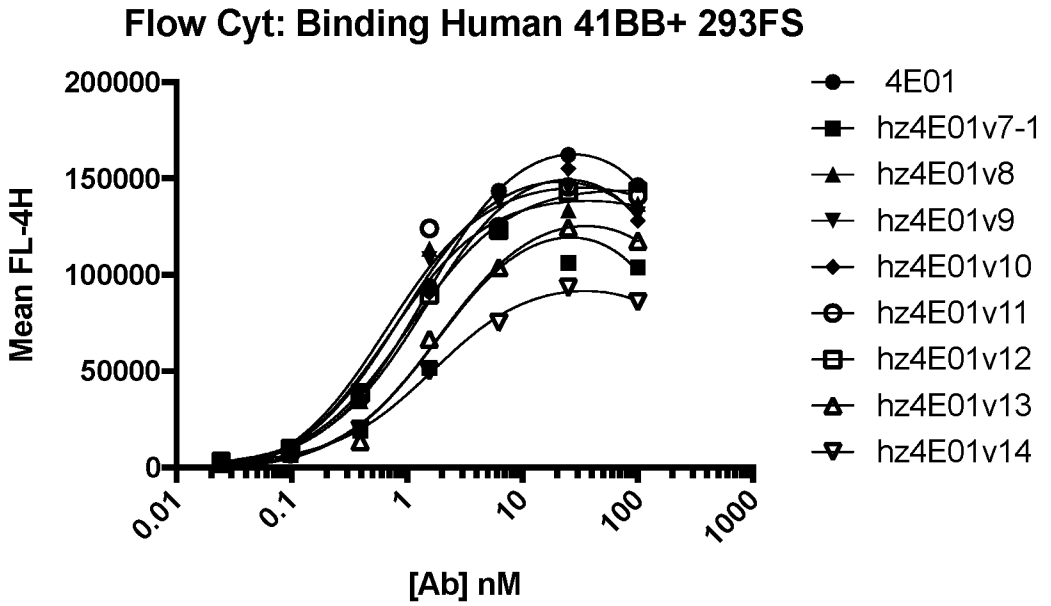


FIG. 11B

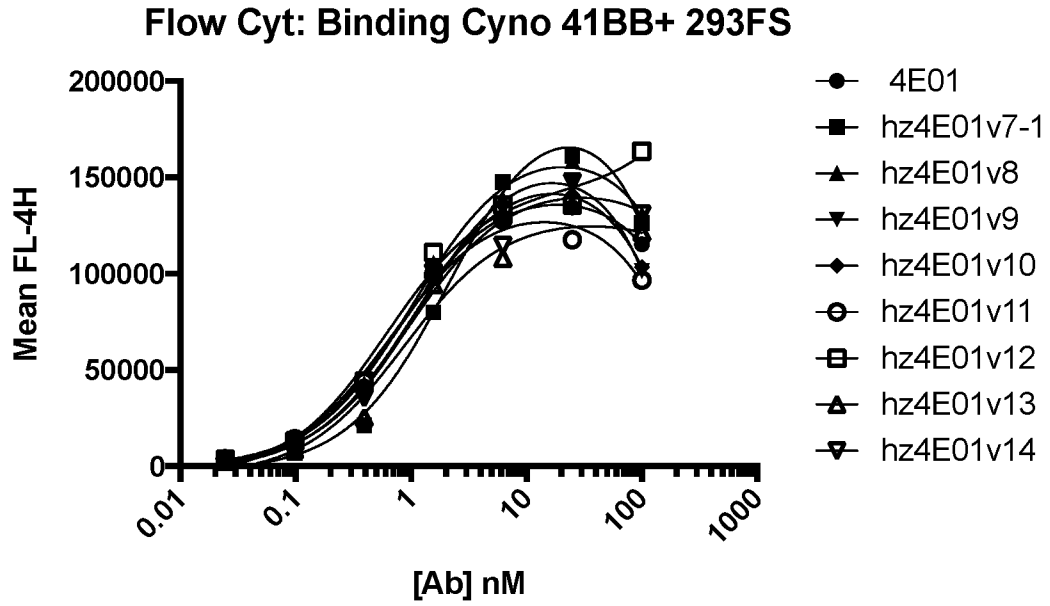


FIG. 11C

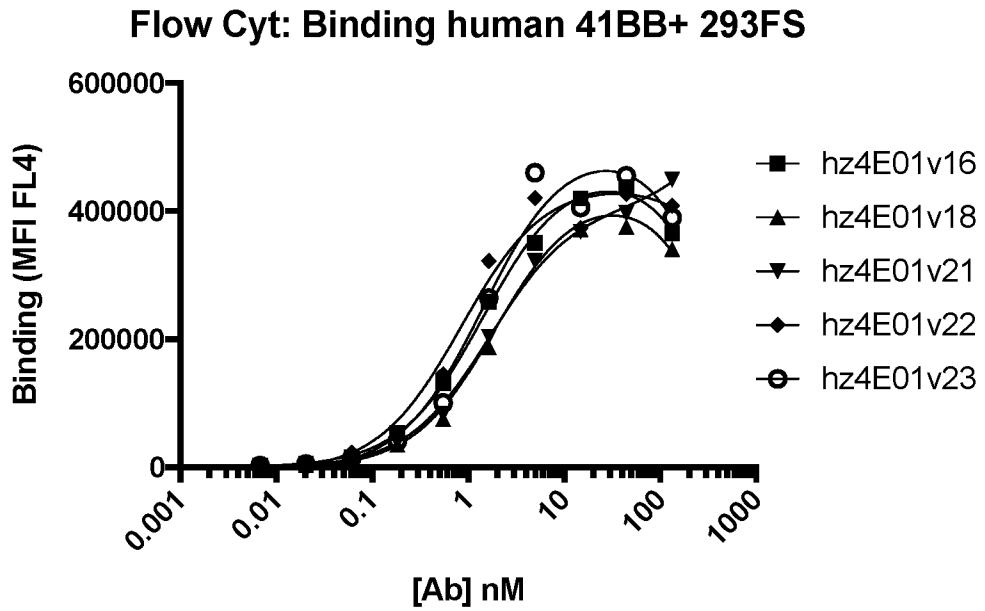


FIG. 11D

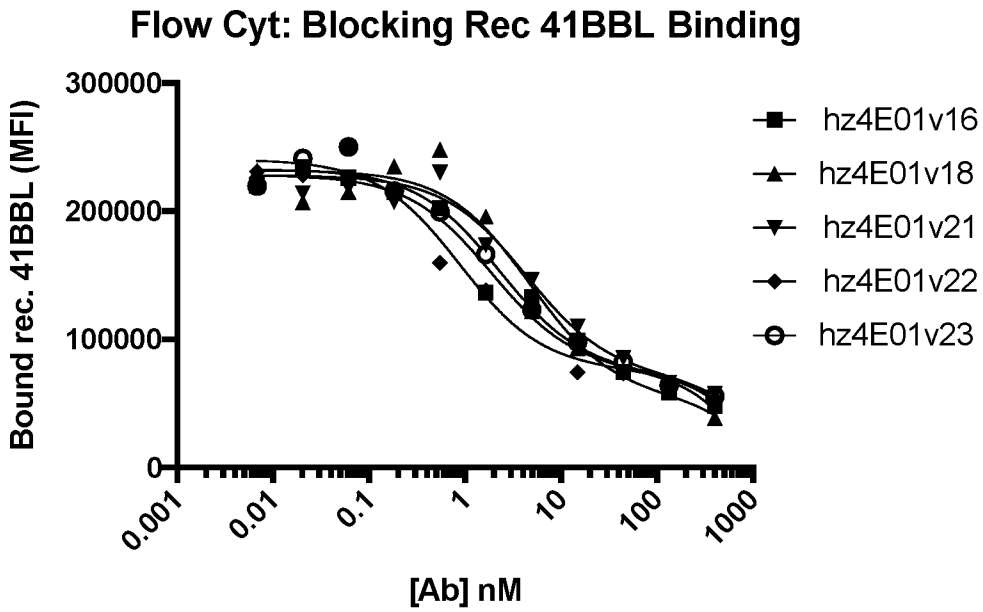


FIG. 12

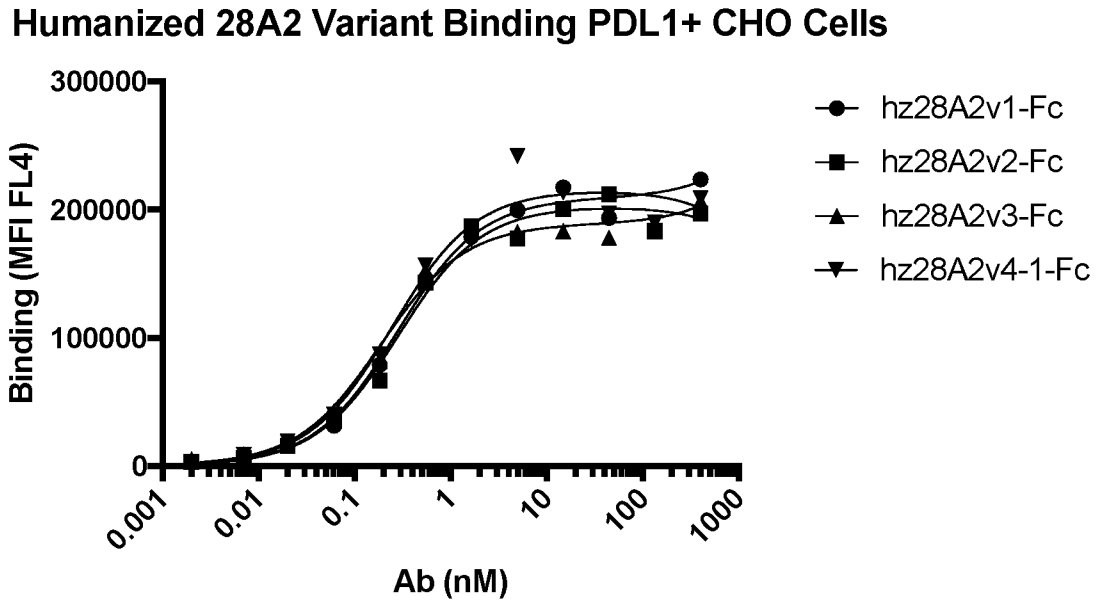

Humanized 28A2 Variant Binding PDL1+ CHO Cells
Binding (MFI FL4)
300000
200000
100000
0
0.001
0.01
0.1
1
10
100
1000
Ab (nM)
hz28A2v1-Fc
hz28A2v2-Fc
hz28A2v3-Fc
hz28A2v4-1-Fc

FIG. 13

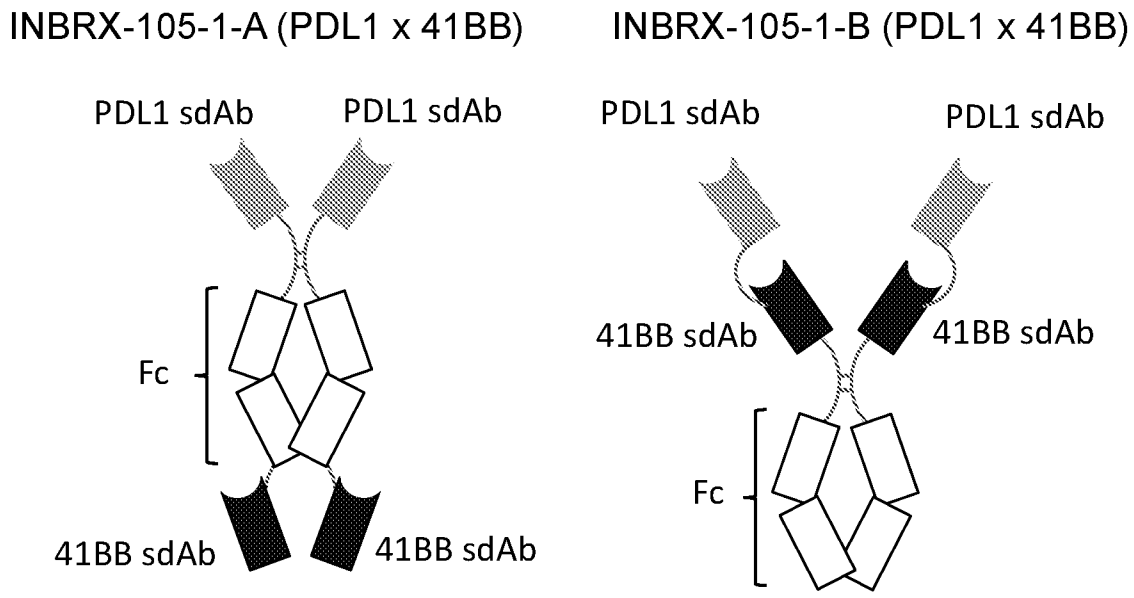

INBRX-105-1-A (PDL1 x 41BB)
PDL1 sdAb
PDL1 sdAb
Fc
41BB sdAb
41BB sdAb
INBRX-105-1-B (PDL1 x 41BB)
PDL1 sdAb
PDL1 sdAb
41BB sdAb
41BB sdAb
Fc

FIG. 14A

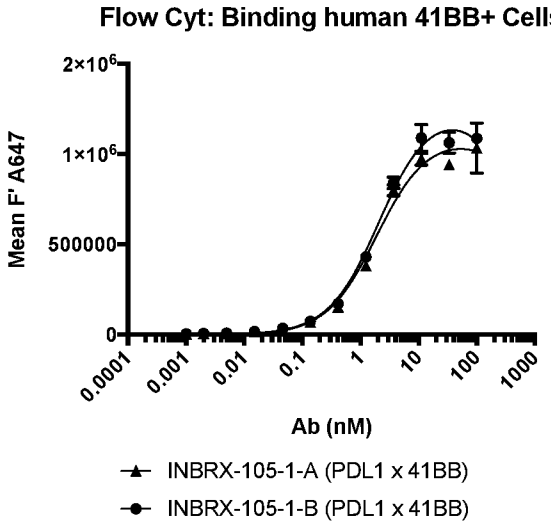


FIG. 14B

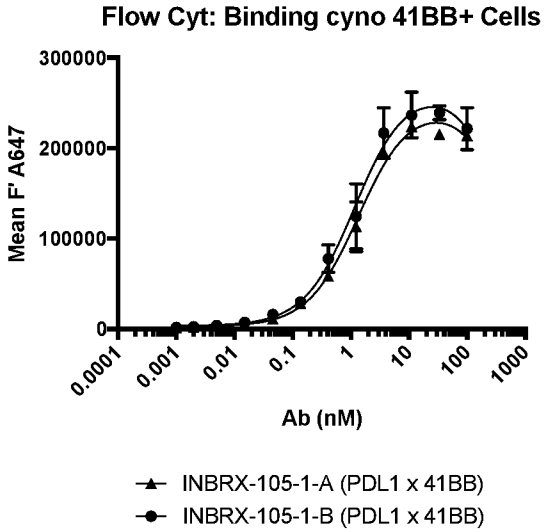


FIG. 14C

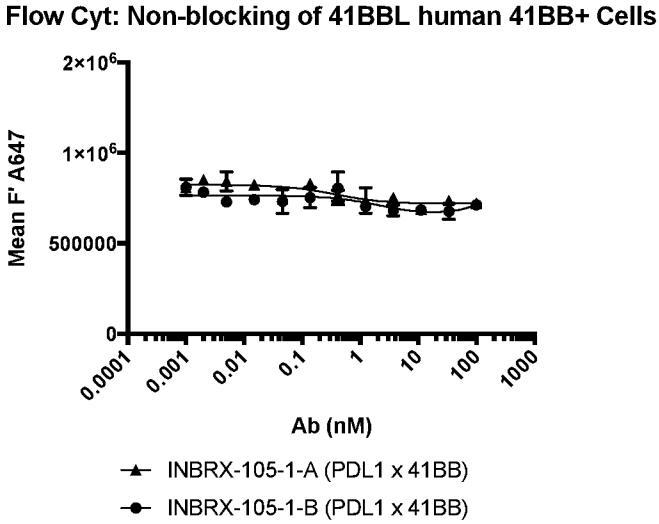


FIG. 15A

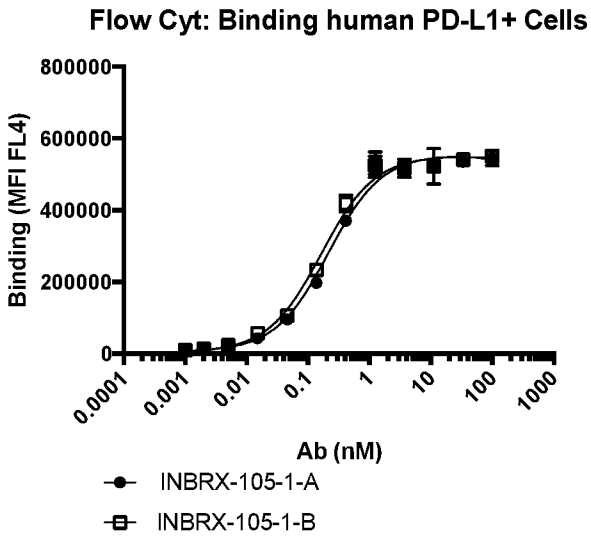


FIG. 15B

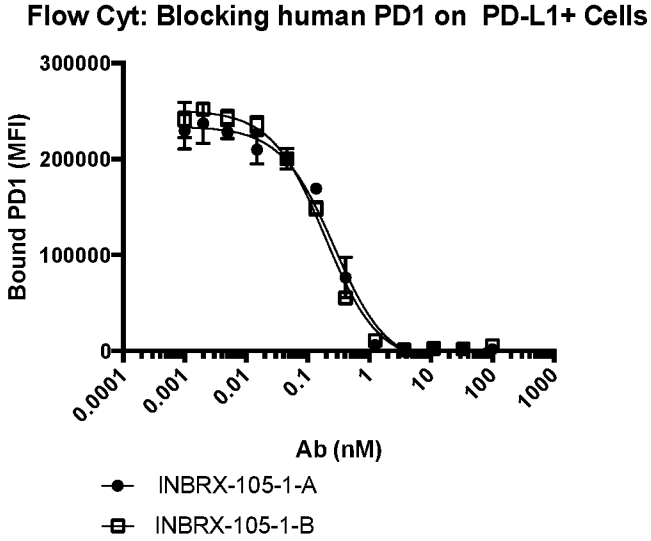


FIG. 15C

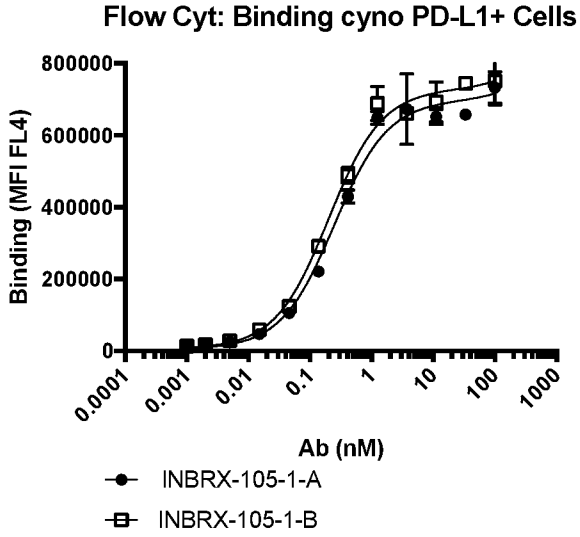


FIG. 15D

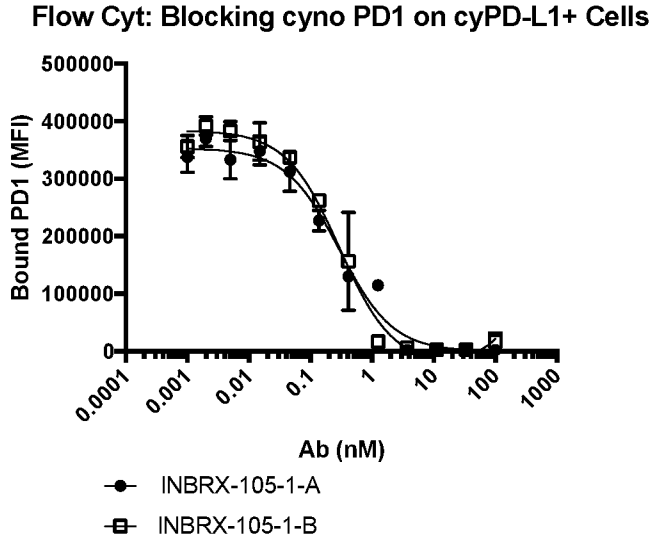


FIG. 16

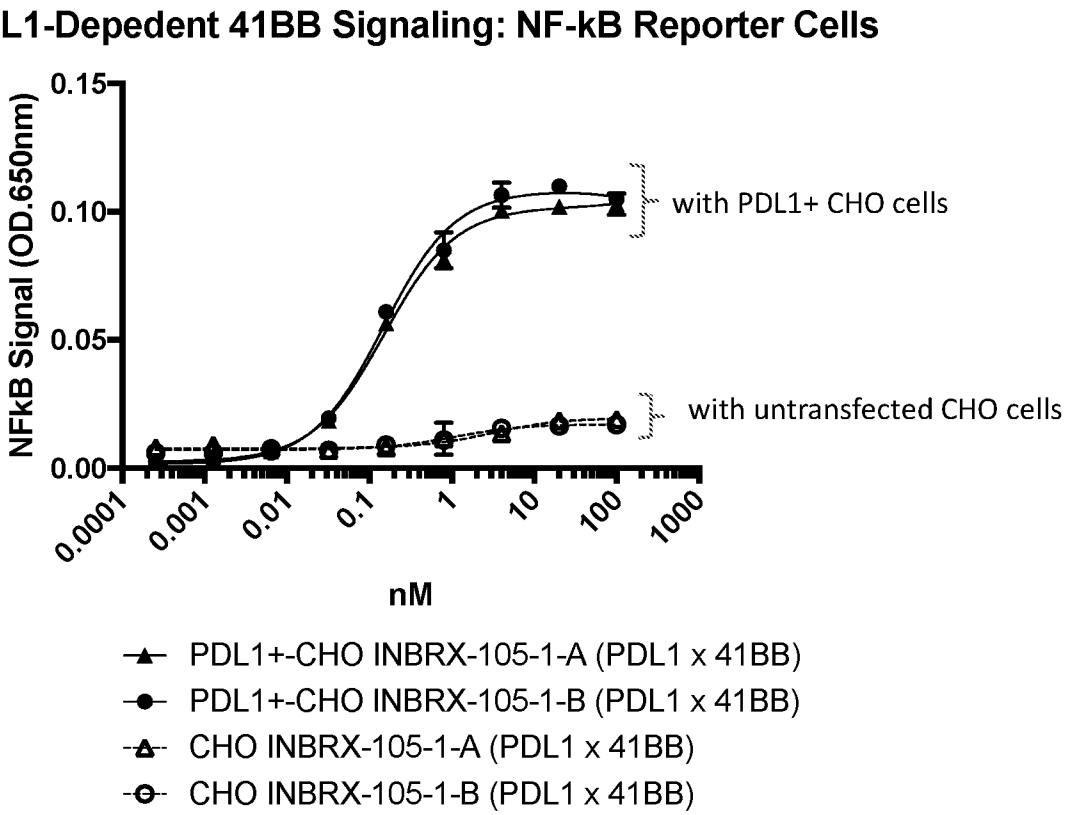

PD-L1-Depedent 41BB Signaling: NF-kB Reporter Cells
NFkB Signal (OD.650nm)
0.15
0.10
0.05
0.00
0.0001
0.001
0.01
0.1
1
10
100
1000
nM
with PDL1+ CHO cells
with untransfected CHO cells
PDL1+-CHO INBRX-105-1-A (PDL1 x 41BB)
PDL1+-CHO INBRX-105-1-B (PDL1 x 41BB)
CHO INBRX-105-1-A (PDL1 x 41BB)
CHO INBRX-105-1-B (PDL1 x 41BB)

FIG. 17A

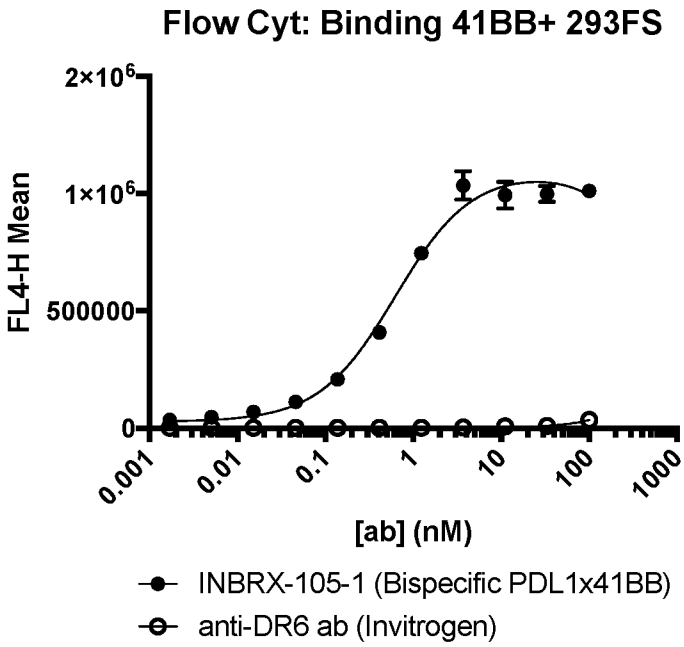


FIG. 17B

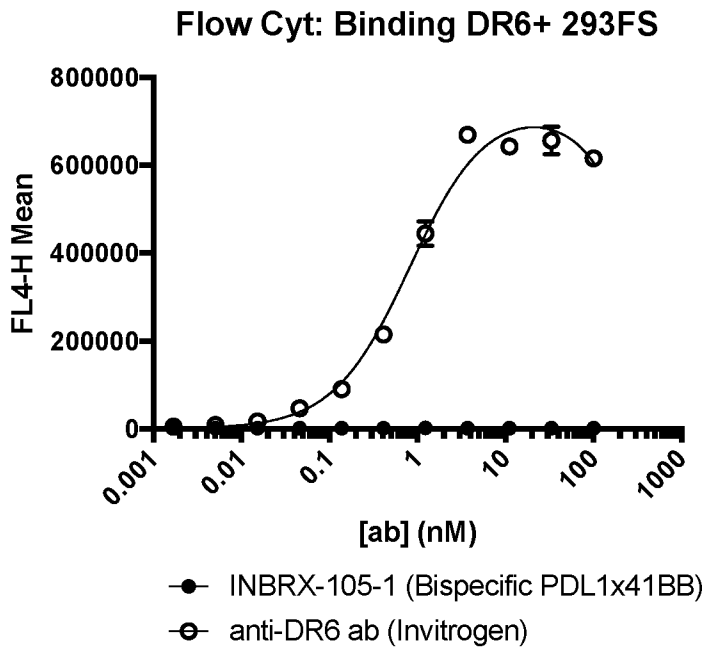


FIG. 18A

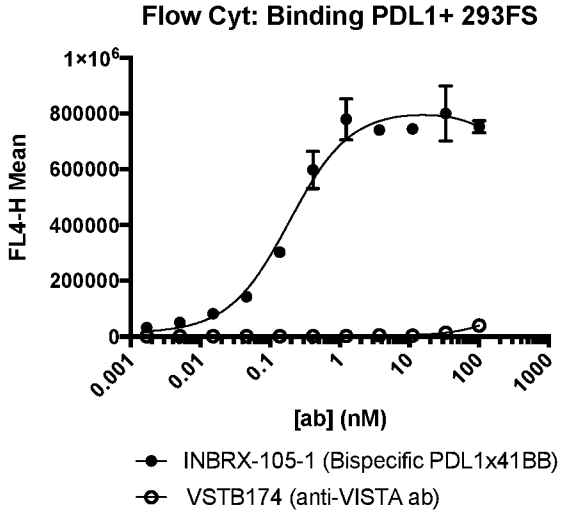


FIG. 18B

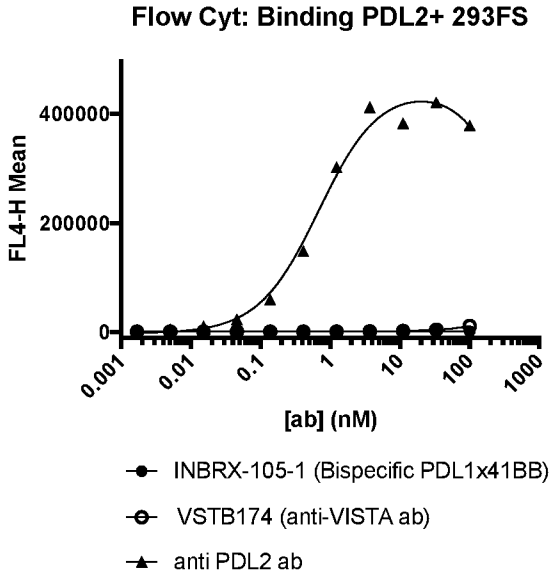


FIG. 18C

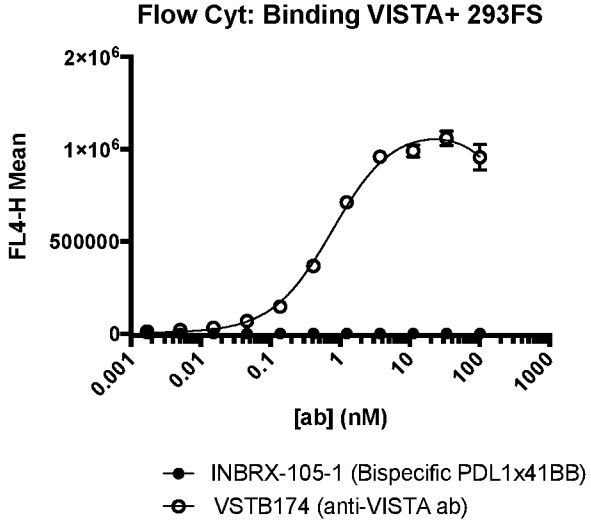


FIG. 19A

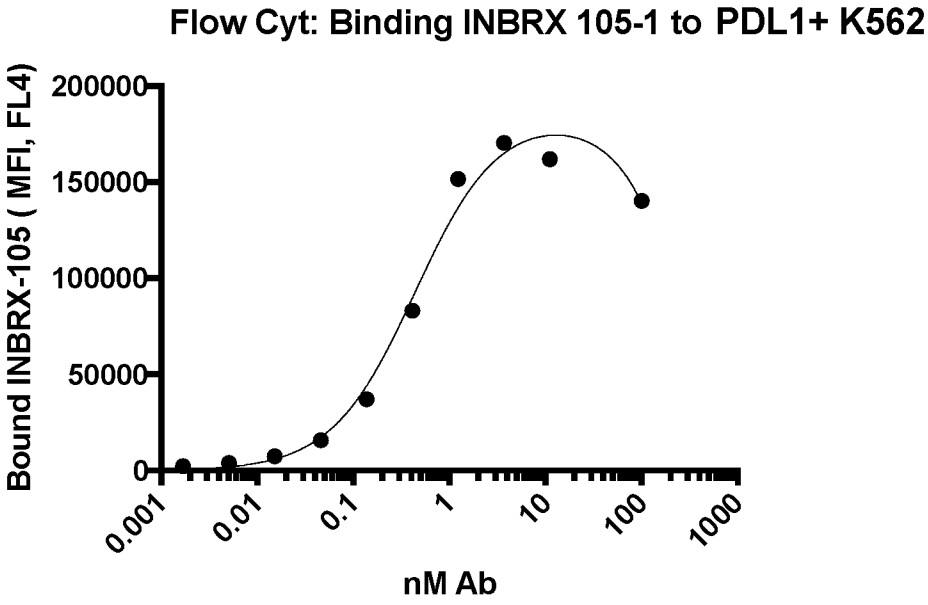


FIG. 19B

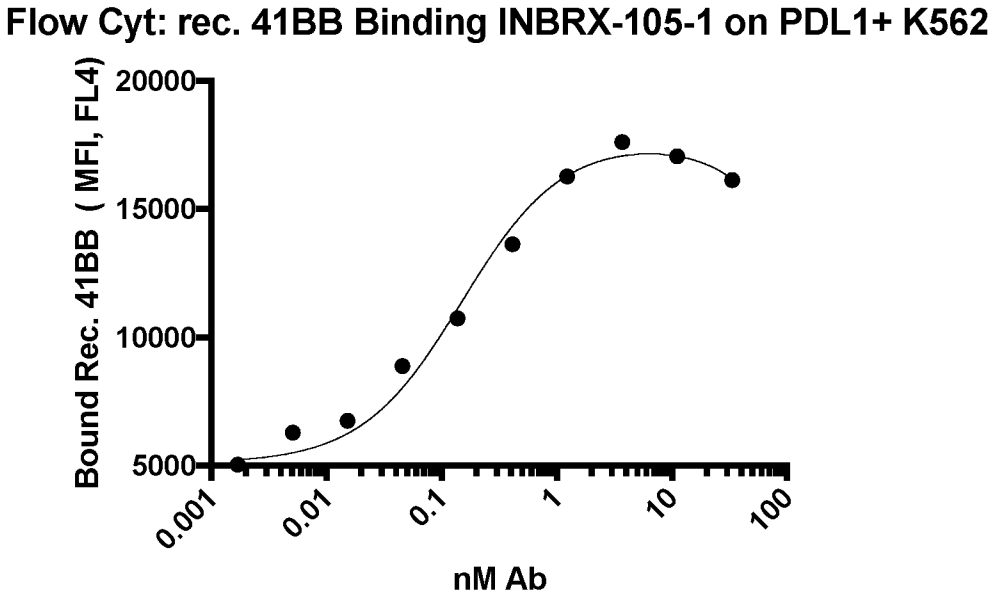


FIG. 20

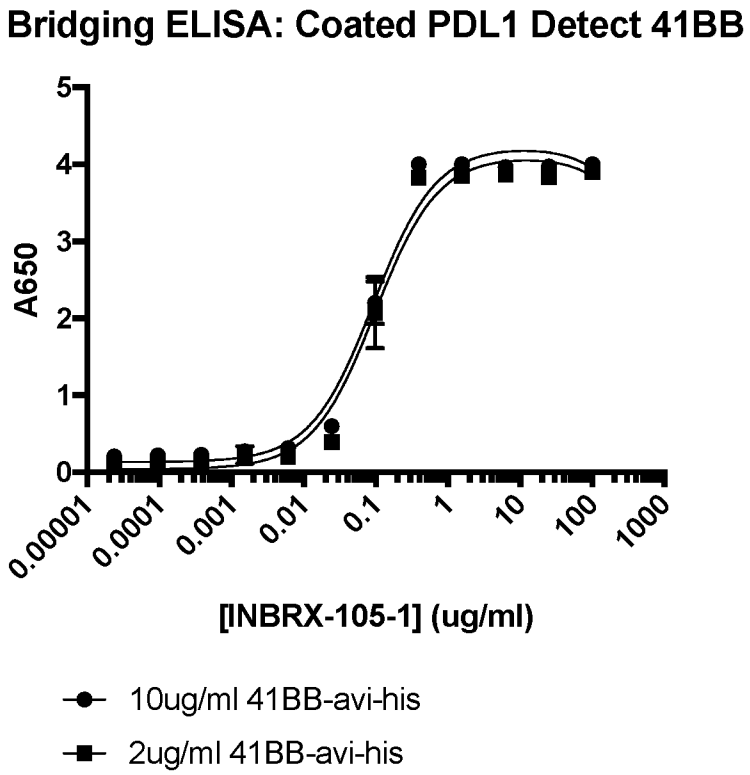

Bridging ELISA: Coated PDL1 Detect 41BB
A650
5
4
3
2
1
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
[INBRX-105-1] (ug/ml)
10ug/ml 41BB-avi-his
2ug/ml 41BB-avi-his

FIG. 21A

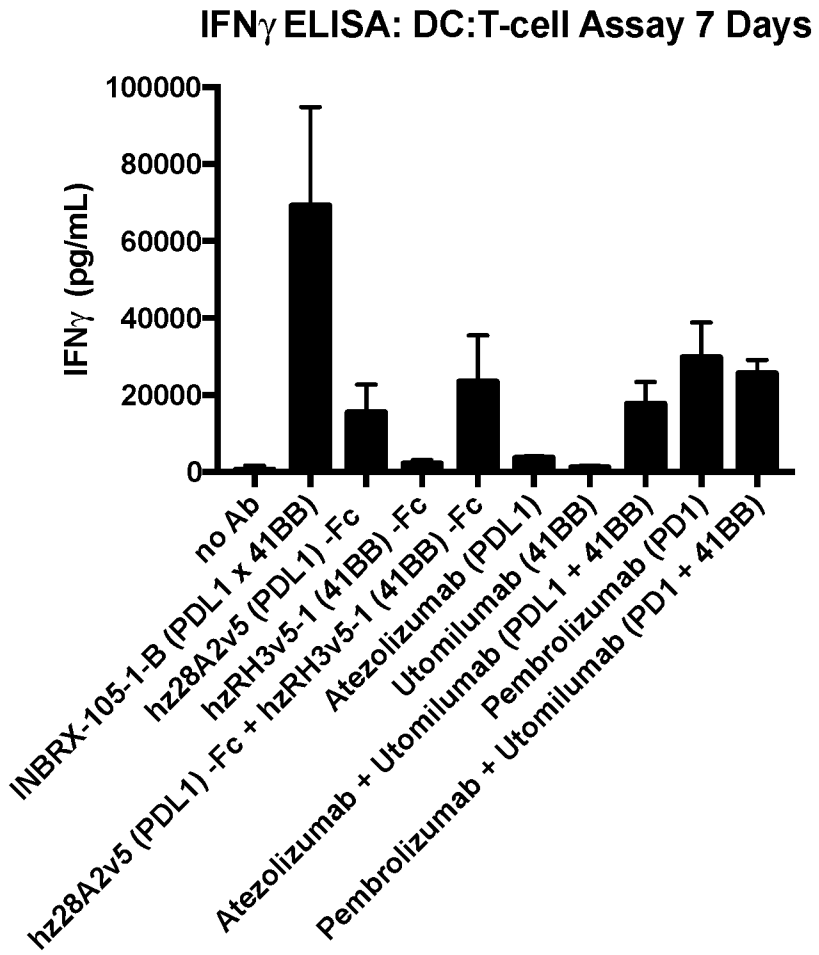

IFNγ ELISA: DC:T-cell Assay 7 Days
IFNγ (pg/mL)
100000
80000
60000
40000
20000
0
no Ab
INBRX-105-1-B (PDL1 x 41BB)
hz28A2v5 (PDL1) -Fc
hzRH3v5-1 (41BB) -Fc
hz28A2v5 (PDL1) -Fc + hzRH3v5-1 (41BB) -Fc
Atezolizumab (PDL1)
Utomilumab (41BB)
Atezolizumab + Utomilumab (PDL1 + 41BB)
Pembrolizumab (PD1)
Pembrolizumab + Utomilumab (PD1 + 41BB)

FIG. 21B

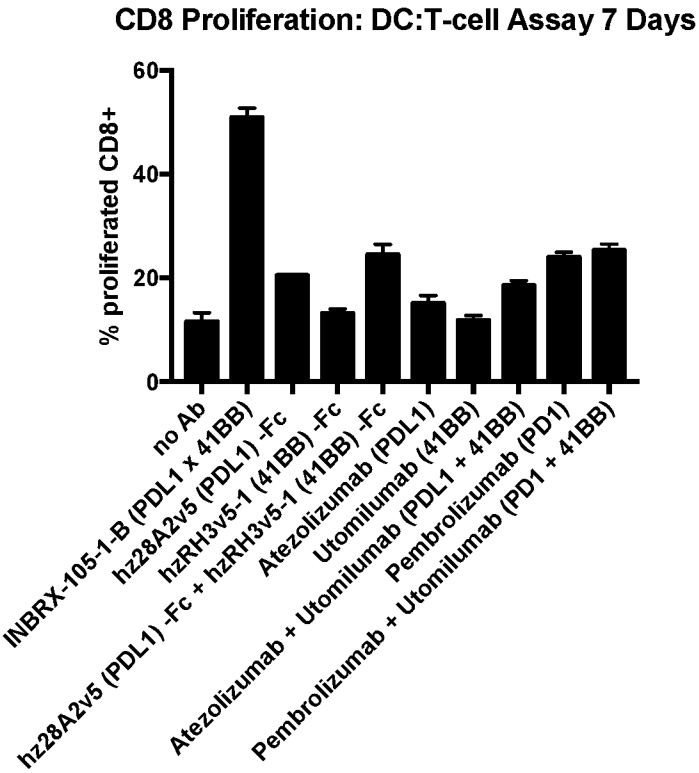


FIG. 21C

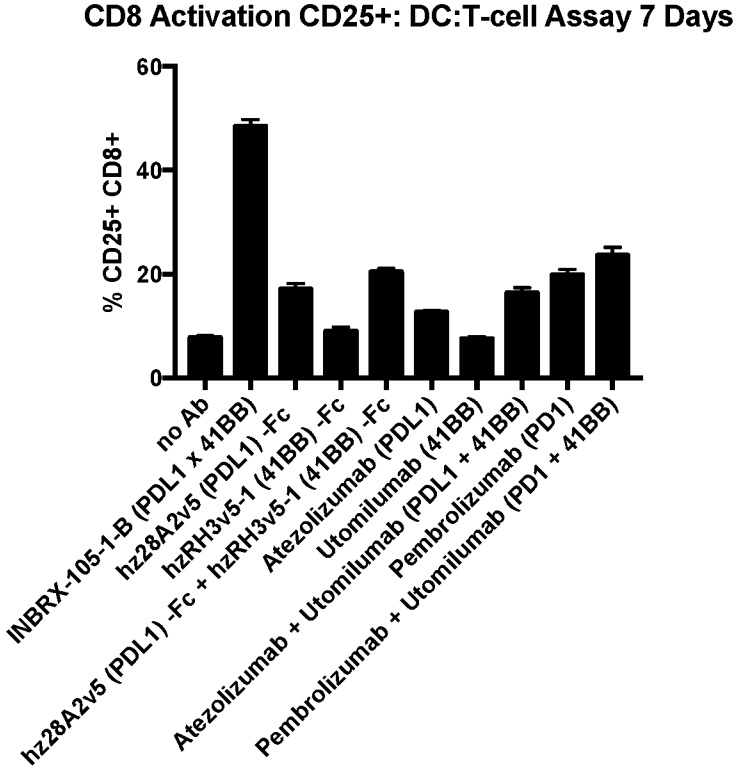


FIG. 22A

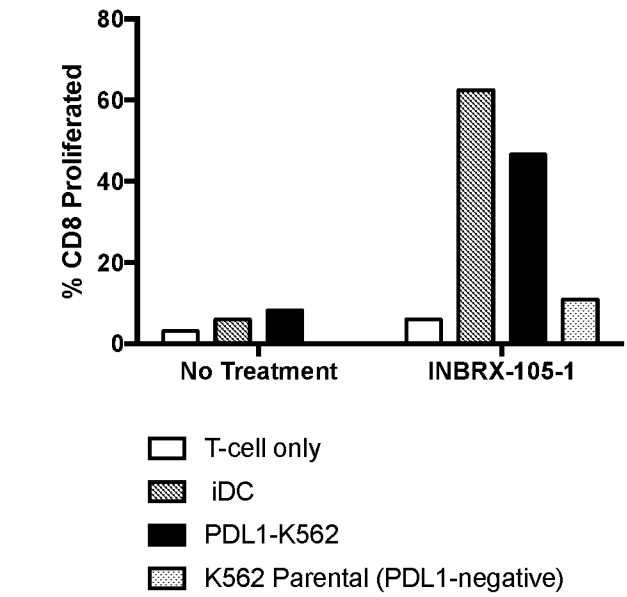


FIG. 22B

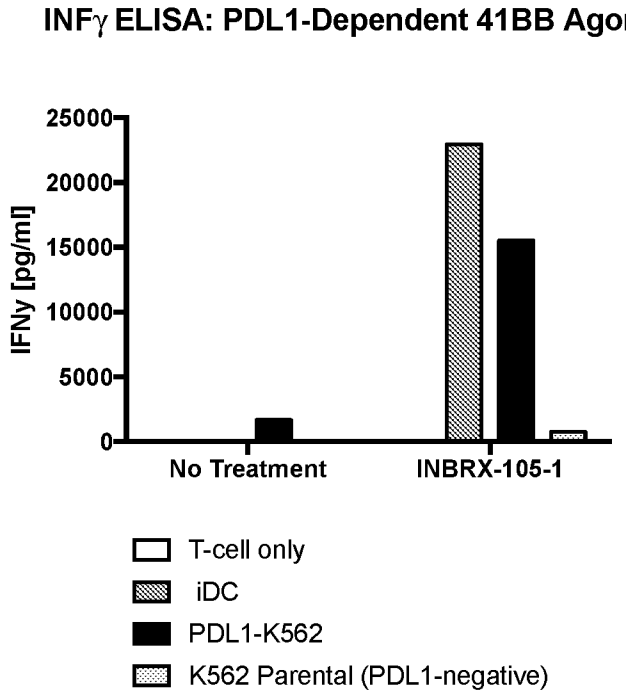


FIG. 23

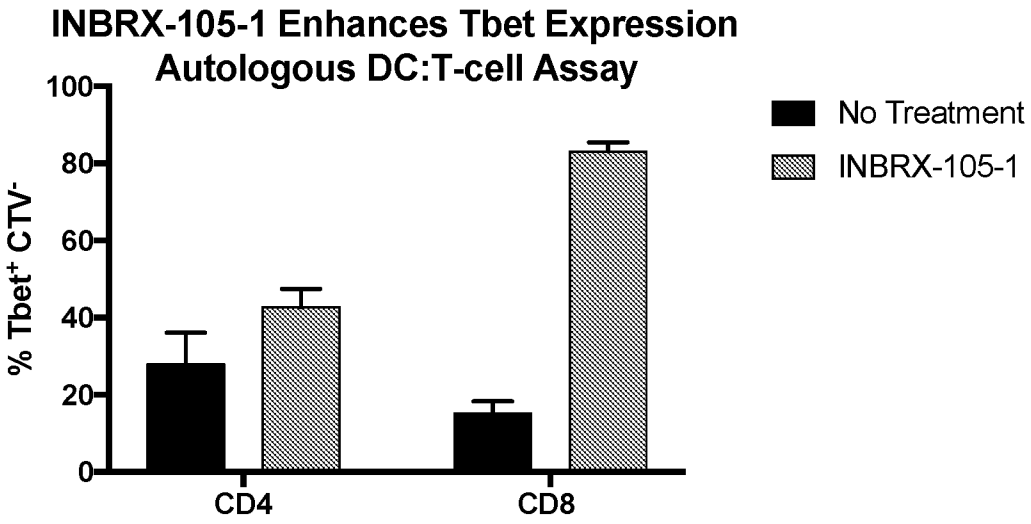

INBRX-105-1 Enhances Tbet Expression
Autologous DC:T-cell Assay
% Tbet+ CTV-
100
80
60
40
20
0
CD4
CD8
No Treatment
INBRX-105-1

FIG. 24A

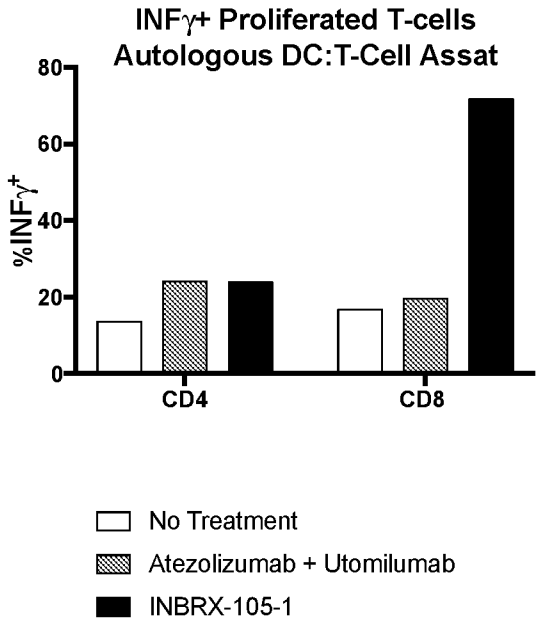


FIG. 24B

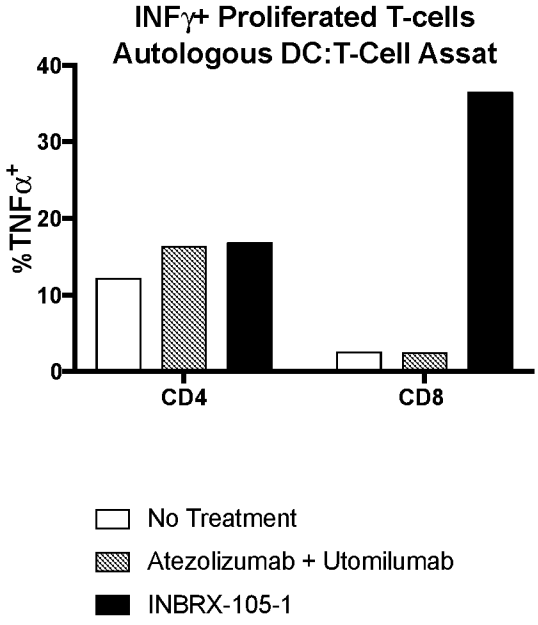


FIG. 25A

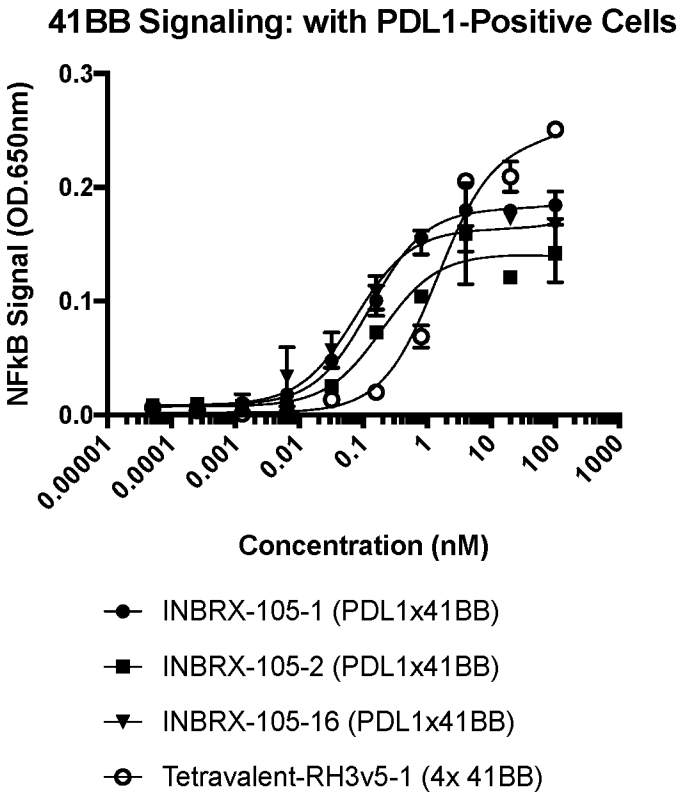


FIG. 25B

