

[0022] In some examples, 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 examples, 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 examples, 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 examples, the present disclosure provides isolated polypeptides that specifically bind at least 41BB and PDL1. In some examples, 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 examples, 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 examples, 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.

[0024a] In a first aspect, there is provided an isolated polypeptide comprising at least one binding domain that binds PDL1 and at least one binding domain that binds 41BB, wherein the polypeptide induces PDL1-dependent 41BB signaling.

[0024b] Also disclosed herein is an isolated polypeptide comprising at least one VHH domain that binds PDL1.

[0024c] Further disclosed herein is an isolated polypeptide comprising at least one VHH domain that binds 41BB.

What is claimed is:

1. An isolated polypeptide comprising at least one binding domain that binds PDL1 and at least one binding domain that binds 41BB, wherein the polypeptide induces PDL1-dependent 41BB signaling.
2. The isolated polypeptide of claim 1, wherein at least one binding domain that binds PDL1 is humanized.
3. The isolated polypeptide of claim 1 or claim 2, wherein at least one binding domain that binds 41BB is humanized.
4. The isolated polypeptide of any one of claims 1-3, comprising at least one VHH domain that binds PDL1 and at least one VHH domain that binds 41BB.
5. The isolated polypeptide of claim 4, wherein at least one VHH domain that binds PDL1 comprises CDR1, CDR2, and CDR3 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.
6. The isolated polypeptide of claim 4 or claim 5, wherein at least one VHH domain that binds PDL1 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.
7. The isolated polypeptide of claim 5 or claim 6, wherein at least one VHH domain that binds PDL1 comprises an amino acid sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NOs: 124, 100, 104, 108, 112-114, 116, and 119-123.
8. The isolated polypeptide of any one of claims 4-7, wherein at least one VHH domain that binds PDL1 comprises an amino acid sequence selected from SEQ ID NOs: 124, 100, 104, 108, 112-114, 116, and 119-123.
9. The isolated polypeptide of any one of claims 4-8, wherein at least one VHH domain that binds PDL1 comprises the amino acid sequence of SEQ ID NO: 124.
10. The isolated polypeptide of any one of claims 4-9, wherein the polypeptide comprises one VHH domain that binds PDL1.
11. The isolated polypeptide of any one of claims 4-10, wherein at least one VHH domain that binds 41BB comprises CDR1, CDR2, and CDR3 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.

12. The isolated polypeptide of any one of claims 4-11, wherein at least one VHH domain that binds 41BB comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a CDR2 comprising the amino acid sequence of SEQ ID NO: 61, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

13. The isolated polypeptide of claim 11 or claim 12, wherein at least one VHH domain that binds 41BB comprises an amino acid sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NOs: 60, 16, 20, 23, 25, 29, 33, 34-41, 43, 45-47, 49, 51, 53, 54, 56, 58, 59, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 81, 82, 83, 432, 436, 440, and 444.

14. The isolated polypeptide of any one of claims 4-13, wherein at least one VHH domain that binds 41BB comprises an amino acid sequence selected from SEQ ID NOs: 60, 16, 20, 23, 25, 29, 33, 34-41, 43, 45-47, 49, 51, 53, 54, 56, 58, 59, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 81, 82, 83, 432, 436, 440, and 444.

15. The isolated polypeptide of any one of claims 4-14, wherein at least one VHH domain that binds 41BB comprises the amino acid sequence of SEQ ID NO: 60.

16. The isolated polypeptide of any one of claims 4-15, wherein the polypeptide comprises one VHH domain that binds 41BB.

17. The isolated polypeptide of any one of claims 1-16, wherein the isolated polypeptide comprises an Fc region.

18. The isolated polypeptide of claim 17, wherein the Fc region comprises an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOs: 1-6.

19. The isolated polypeptide of claim 17, wherein the Fc region comprises an amino acid sequence selected from SEQ ID NOs: 1-6.
20. The isolated polypeptide of any one of claims 17-19, which forms a homodimer under physiological conditions.
21. The isolated polypeptide of any one of claims 17-20, wherein the polypeptide has the structure VHH-Linker-VHH-Linker-Hinge-Fc.
22. The isolated polypeptide of claim 21, wherein at least one linker is, independently, 5-20 amino acids in length.
23. The isolated polypeptide of claim 21 or claim 22, wherein the hinge comprises a sequence selected from SEQ ID NOs: 7-9.
24. The isolated polypeptide of any one of claims 1-23, comprising a first VHH domain (VHH₁) that binds PDL1 and a second VHH domain (VHH₂) that binds 41BB, wherein VHH₁ 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; and VHH₂ comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a CDR2 comprising the amino acid sequence of SEQ ID NO: 61, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 28; and wherein the polypeptide has the structure VHH₁-Linker-VHH₂-Linker-Hinge-Fc.
25. The isolated polypeptide of claim 24, wherein the first VHH domain comprises the amino acid sequence of SEQ ID NO: 124 and the second VHH domain comprises the amino acid sequence of SEQ ID NO: 60.
26. The isolated polypeptide of claim 24 or claim 25, wherein the Fc region comprises an amino acid sequence that is at least 97% identical to the sequence of SEQ ID NO: 2.
27. The isolated polypeptide of claim 24 or claim 25, wherein the Fc region comprises the amino acid sequence of SEQ ID NO: 2.
28. The isolated polypeptide of any one of claims 24-27, wherein the polypeptide comprises an amino acid sequence that is at least 97% identical to the sequence of SEQ ID NO: 449.
29. The isolated polypeptide of any one of claims 24-27, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 449.
30. The isolated polypeptide of any one of claims 24-29, which forms a dimer at physiological conditions.

31. An isolated polynucleotide that encodes the polypeptide of any one of claims 1-30.
32. An isolated cell that comprises the polynucleotide of claim 31.
33. An isolated cell that expresses the polypeptide of any one of claims 1-30.
34. A method of producing the polypeptide of any one of claims 1-30 comprising culturing the host cell of claim 32 or claim 33 under conditions suitable for producing the polypeptide.
35. Use of a polypeptide of any one of claims 1-30 in the manufacture of a medicament for treating cancer.
36. The use of claim 35, wherein the cancer is selected from carcinoma, sarcoma, lymphoma, and leukemia.
37. An in vitro method of increasing T cell activation and/or proliferation comprising contacting T cells with a polypeptide of any one of claims 1-30.
38. The method of claim 37, wherein the T cells are CD4+ T cells and/or CD8+ T cells.