

[0024d] In a second aspect, the present disclosure refers to an isolated polynucleotide that encodes any of the polypeptide disclosed herein.

[0024e] In a third aspect, the present disclosure refers to an isolated cell that comprises the polynucleotide of the second aspect.

[0024f] In a fourth aspect, the present disclosure refers to an isolated cell that expresses the polypeptide of the first aspect.

[0024g] In a fifth aspect, the present disclosure refers to a method of producing the polypeptide of the first aspect comprising culturing the host cell of third or fourth aspect under conditions suitable for producing the polypeptide.

[0024h] In a sixth aspect, the present disclosure refers to use of any of the polypeptide disclosed herein in the manufacture of a medicament for treating cancer.

[0024i] In a seventh aspect, the present disclosure refers to an in vitro method of increasing T cell activation and/or proliferation comprising contacting T cells with any of the polypeptide disclosed herein.

[0024j] In an example, the present disclosure refers to use of any of the polypeptide disclosed herein in the manufacture of a medicament for increasing T cell activation and/or proliferation.

[0025] In some examples, 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.

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.
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 any one of claims 4-6, 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 a CDR1 comprising an amino acid sequence selected from SEQ ID NOs: 26, 65, 67, and 69; a CDR2 comprising an amino acid sequence

selected from SEQ ID NOs: 61, 27, 63, 71, 73, 75, 77, and 79; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

12. The isolated polypeptide of any one of claims 4-11, 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.

13. The isolated polypeptide of any one of claims 4-12, 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.

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 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.

15. The isolated polypeptide of any one of claims 4-14, 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.

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

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

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

19. The isolated polypeptide of claim 18, 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.
20. The isolated polypeptide of claim 18, wherein the Fc region comprises an amino acid sequence selected from SEQ ID NOs: 1-6.
21. The isolated polypeptide of any one of claims 18-20, which forms a homodimer under physiological conditions.
22. The isolated polypeptide of any one of claims 18-21, wherein the polypeptide has the structure VHH-Linker-VHH-Linker-Hinge-Fc.
23. The isolated polypeptide of claim 22, wherein at least one linker is, independently, 5-20 amino acids in length.
24. The isolated polypeptide of claim 22 or claim 23, wherein the hinge comprises a sequence selected from SEQ ID NOs: 7-9.
25. The isolated polypeptide of any one of claims 1-24, 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.
26. The isolated polypeptide of claim 25, 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.
27. The isolated polypeptide of claim 25 or claim 26, wherein the Fc region comprises an amino acid sequence that is at least 97% identical to the sequence of SEQ ID NO: 2.
28. The isolated polypeptide of claim 25 or claim 26, wherein the Fc region comprises the amino acid sequence of SEQ ID NO: 2.
29. The isolated polypeptide of any one of claims 25-28, wherein the polypeptide comprises an amino acid sequence that is at least 97% identical to the sequence of SEQ ID NO: 449.

30. The isolated polypeptide of any one of claims 25-28, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 449.
31. The isolated polypeptide of any one of claims 25-30, which forms a dimer at physiological conditions.
32. An isolated polynucleotide that encodes the polypeptide of any one of claims 1-31.
33. An isolated cell that comprises the polynucleotide of claim 32.
34. An isolated cell that expresses the polypeptide of any one of claims 1-31.
35. A method of producing the polypeptide of any one of claims 1-31 comprising culturing the host cell of claim 33 or claim 34 under conditions suitable for producing the polypeptide.
36. Use of a polypeptide of any one of claims 1-31 in the manufacture of a medicament for treating cancer.
37. The use of claim 36, wherein the cancer is selected from carcinoma, sarcoma, lymphoma, and leukemia.
38. An in vitro method of increasing T cell activation and/or proliferation comprising contacting T cells with a polypeptide of any one of claims 1-31.
39. The method of claim 38, wherein the T cells are CD4+ T cells and/or CD8+ T cells.