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

[0006a] In one aspect, the present disclosure refers to an isolated polypeptide comprising at least one VHH domain that binds PDL1 and at least one VHH domain that binds OX40, wherein at least one VHH domain that binds PDL1 comprises a complementarity determining region 1 (CDR1), a complementarity determining region 2 (CDR2), and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from: SEQ ID NOs: 58, 59, and 60; SEQ ID NOs: 61, 62, and 63; SEQ ID NOs: 64, 65, and 66; SEQ ID NOs: 58, 65, and 67; SEQ ID NOs 58, 65, and 68; and SEQ ID NOs: 58, 69, and 70.

[0006b] In one aspect, the present disclosure refers to an isolated polynucleotide that encodes a polypeptide as described herein. In another aspect, the present disclosure refers to an isolated cell that comprises the polynucleotide as described herein. In yet another aspect, the present disclosure refers to an isolated cell that expresses the polypeptide as described herein.

[0006c] In one aspect, the present disclosure refers to a method of producing the polypeptide as described herein comprising culturing the host cell as described herein under conditions suitable for producing the polypeptide.

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

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

[0010] In other examples, the fusion proteins of the present disclosure bind OX40 and a second TNFRSF member such as, for example, GITR, CD137, CD27, TNFR2, and/or CD40. In these examples, the fusion proteins of the present disclosure modulate immune cells leading to enhanced tumor destruction. In other examples, the fusion proteins of the present disclosure have utility in treating inflammatory conditions. In these examples, 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.

[0010a] In another aspect, the present disclosure refers to an in vitro method of increasing T cell activation and/or proliferation comprising contacting T cells with a polypeptide as described herein.

[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 examples, the fusion protein will incorporate more than 2 TBDs, for example, three, four, five, or six.

[0012] In some examples, the fusion proteins are multispecific containing a TBD and a binding domain directed toward a second antigen. In these, examples, 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 examples, the TNFRSF signaling is enhanced and focused by the presence of the second antigen. These multispecific TBD containing fusion proteins are useful means to achieve conditional signaling of a given TNFRSF member.

[0013] The present disclosure provides isolated polypeptides that specifically bind OX40. In some examples, the isolated polypeptide is derived from antibodies or antibody fragments including scFv, Fabs, single domain antibodies (sdAb), V_{NAR}, or VHHs. In some examples, 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

What is claimed is:

1. An isolated polypeptide comprising at least one VHH domain that binds PDL1 and at least one VHH domain that binds OX40, wherein at least one VHH domain that binds PDL1 comprises a complementarity determining region 1 (CDR1), a complementarity determining region 2 (CDR2), and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from: SEQ ID NOs: 58, 59, and 60; SEQ ID NOs: 61, 62, and 63; SEQ ID NOs: 64, 65, and 66; SEQ ID NOs: 58, 65, and 67; SEQ ID NOs 58, 65, and 68; and SEQ ID NOs: 58, 69, and 70.

2. The isolated polypeptide of claim 1, wherein at least one VHH domain that binds PDL1 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 61; a CDR2 comprising the amino acid sequence of SEQ ID NO: 62; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 63.

3. The isolated polypeptide of claim 1 or claim 2, wherein at least one VHH domain that binds PDL1 is humanized.

4. The isolated polypeptide of any one of claims 1-3, 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: 52-57.

5. The isolated polypeptide of any one of claims 1-4, wherein at least one VHH domain that binds PDL1 comprises an amino acid sequence selected from SEQ ID NOs: 52-57.

6. The isolated polypeptide of any one of claims 1-5, wherein at least one VHH domain that binds PDL1 comprises the amino acid sequence of SEQ ID NO: 57.

7. The isolated polypeptide of any one of claims 1-6, wherein the polypeptide comprises one VHH domain that binds PDL1.

8. The isolated polypeptide of any one of claims 1-6, wherein the polypeptide comprises two VHH domains that bind PDL1.

9. The isolated polypeptide of any one of claims 1-8, wherein at least one VHH domain that binds OX40 comprises CDR1, CDR2, and CDR3 selected from: SEQ ID NOs: 30, 31, and 32; SEQ ID NOs: 30, 31, and 33; SEQ ID NOs: 30, 31, and 34; SEQ ID NOs: 30, 35, and 32; SEQ ID NOs: 36, 37, and 38; SEQ ID NOs: 36, 37, and 39; SEQ ID NOs: 36, 37, and 40; SEQ ID NOs: 36, 37, and 42; SEQ ID NOs: 36, 37, and 43; SEQ ID NOs: 44, 37, and 39; and SEQ ID NOs: 36, 37, and 45.

10. The isolated polypeptide of any one of claims 1-9, wherein at least one VHH domain that binds OX40 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 36, a CDR2 comprising the amino acid sequence of SEQ ID NO: 37, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 42; or comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 36, a CDR2 comprising the amino acid sequence of SEQ ID NO: 37, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 39.

11. The isolated polypeptide of any one of claim 1-10, wherein at least one VHH domain that binds OX40 is humanized.

12. The isolated polypeptide of any one of claims 1-11, wherein at least one VHH domain that binds OX40 comprises an amino acid sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NOs: 377-385 and 29.

13. The isolated polypeptide of any one of claims 1-12, wherein at least one VHH domain that binds OX40 comprises an amino acid sequence selected from SEQ ID NOs: 377-385 and 29.

14. The isolated polypeptide of any one of claims 1-13, wherein the polypeptide comprises one VHH domain that binds OX40.

15. The isolated polypeptide of any one of claims 1-13, wherein the polypeptide comprises two VHH domains that bind OX40.

16. The isolated polypeptide of any one of claims 1-15, wherein the isolated polypeptide comprises an Fc region.

17. The isolated polypeptide of claim 16, 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.

18. The isolated polypeptide of claim 16, wherein the Fc region comprises an amino acid sequence selected from SEQ ID NOs: 1-6.

19. The isolated polypeptide of any one of claims 16-18, which forms a homodimer under physiological conditions.

20. The isolated polypeptide of any one of claims 16-19, wherein the polypeptide has the structure VHH-Linker-VHH-Linker-Hinge-Fc.

21. The isolated polypeptide of claim 20, wherein at least one linker is, independently, 5-20 amino acids in length.

22. The isolated polypeptide of claim 20 or claim 21, wherein the hinge comprises a sequence selected from SEQ ID NOs: 7-9.

23. The isolated polypeptide of any one of claims 16-22, which forms a dimer at physiological conditions.

24. An isolated polynucleotide that encodes the polypeptide of any one of claims 1-23.

25. An isolated cell that comprises the polynucleotide of claim 24.

26. An isolated cell that expresses the polypeptide of any one of claims 1-23.

27. A method of producing the polypeptide of any one of claims 1-23 comprising culturing the host cell of claim 25 or claim 26 under conditions suitable for producing the polypeptide.

28. Use of a polypeptide of any one of claims 1-23 in the manufacture of a medicament for treating cancer.

29. The use of claim 28, wherein the cancer is carcinoma, sarcoma, lymphoma, or leukemia.

30. An in vitro method of increasing T cell activation and/or proliferation comprising contacting T cells with a polypeptide of any one of claims 1-23.

31. The use of claim 28, wherein the polypeptide increases T cell activation and/or proliferation.