

activation. Therefore, TIGIT acts as an important negative regulator in immune responses in infection and cancer, and blockade of TIGIT signaling has been proposed as an approach to enhance T cell and NK cell immunity for cancer treatment.

[6] Programmed Cell Death Receptor 1 (PD-1) is another inhibitory immune checkpoint molecule with important negative regulation on T cell functions. T-cell responses can be attenuated by PD-1 signaling when PD-1 binds to Programmed Cell Death Ligand 1 (PD-L1) and/or Programmed Cell Death Ligand 2 (PD-L2), which regulates T-cell receptor (TCR) signaling. Blockade of the PD-1/PD-L1 axis using antibodies targeting either PD-1 or PD-L1 has been shown to promote tumor-specific T cell immunity with significant clinical benefit to cancer patients. However, there are still huge unmet clinical needs due to the resistance or relapse upon PD-1/PD-L1 blockade.

[7] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

SUMMARY

[8] The present disclosure relates to anti-TIGIT constructs comprising an sdAb moiety that specifically recognizes TIGIT (hereinafter referred to as “anti-TIGIT sdAb”), such as anti-TIGIT sdAb, anti-TIGIT sdAb-Fc fusion protein comprising an anti-TIGIT sdAb fused to a crystalline fragment (Fc) fragment of human immunoglobulin G (IgG), and multispecific (such as bispecific) antigen binding proteins comprising an anti-TIGIT sdAb, for example fused to other sdAbs or fused to a full-length four-chain antibody, and methods of making and using thereof.

[9] One example of the present application provides an isolated anti-TIGIT construct comprising an sdAb moiety specifically recognizing TIGIT, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 36-42, 54, 56-59, 63, 65-67, 69-70, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 106-112, 124, 126-129, 133, 135-137, 139-140, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 176-182, 194, 196-199, 203, 205-207, 209-210, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some examples, the isolated anti-TIGIT construct comprises an sdAb moiety specifically recognizing TIGIT, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 36-42, 54, 56-59, 63, 65-67, 69-70, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 106-112, 124, 126-129, 133, 135-137, 139-140, or a variant

thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 176-182, 194, 196-199, 203, 205-207, 209-210. In some examples, the sdAb moiety specifically recognizing TIGIT comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 36-42, 54, 56-59, 63, 65-67, 69-70; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 106-112, 124, 126-129, 133, 135-137, 139-140; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 176-182, 194, 196-199, 203, 205-207, 209-210; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some examples, the amino acid substitutions are in CDR1 and/or CDR2, wherein CDR3 comprises the amino acid sequence of any one of SEQ ID NOs: 176-182, 194, 196-199, 203, 205-207, 209-210. In some examples, the sdAb moiety specifically recognizing TIGIT comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 36-42, 54, 56-59, 63, 65-67, 69-70; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 106-112, 124, 126-129, 133, 135-137, 139-140; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 176-182, 194, 196-199, 203, 205-207, 209-210.

[9a] In one aspect, the present disclosure refers to an isolated anti-TIGIT construct comprising a single-domain antibody (sdAb) moiety specifically recognizing TIGIT (“anti-TIGIT sdAb moiety”), wherein the anti-TIGIT sdAb moiety comprises any one of the following:

- (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 106, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 176, or a variant thereof comprising up to about 3 amino acid substitutions;
- (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 63, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 133, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 203, or a variant thereof comprising up to about 3 amino acid substitutions;

- (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 109, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 179, or a variant thereof comprising up to about 3 amino acid substitutions;
- (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 42, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 112, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 182, or a variant thereof comprising up to about 3 amino acid substitutions;
- (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 107, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 177, or a variant thereof comprising up to about 3 amino acid substitutions;
- (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 38, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 108, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 178, or a variant thereof comprising up to about 3 amino acid substitutions;
- (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 110, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 180, or a variant thereof comprising up to about 3 amino acid substitutions; and
- (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 111, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 181, or a variant thereof comprising up to about 3 amino acid substitutions.

[10] In some examples according to any one of the isolated anti-TIGIT constructs described above, the sdAb moiety specifically recognizing TIGIT comprises any one of the following:

- (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 36; a CDR2 comprising the amino acid sequence of SEQ ID NO: 106; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 176; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;
- (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 37; a CDR2 comprising the amino acid sequence of SEQ ID NO: 107; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 177; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;
- (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 38; a CDR2 comprising the amino acid sequence of SEQ ID NO: 108; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 178; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;
- (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 109; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 179; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 65; a CDR2 comprising the amino acid sequence of SEQ ID NO: 135; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 66; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 207; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(17) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 139; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 209; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions; or

(18) a CDR1 comprising the amino acid sequence of SEQ ID NO: 70; a CDR2 comprising the amino acid sequence of SEQ ID NO: 140; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 210; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions.

[11] In some examples according to any one of the isolated anti-TIGIT constructs described above, the sdAb moiety specifically recognizing TIGIT comprises a V_HH domain comprising the amino acid sequence of any one of the following: a-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, V, L, A, H, S, I, W, C, N, G, D, T, and P (such as F, Y, L, I, or V, such as F or Y, or such as F); a-2) the amino acid residue at position 44 is selected from the group consisting of E, Q, G, D, A, K, R, L, P, S, V, H, T, N, W, M, and I (such as A, G, E, D, Q, R, S, or L, or such as G, E, or Q); a-3) the amino acid residue at position 45 is selected from the group consisting of L, R, P, H, F, G, Q, S, E, T, Y, C, I, D, and V (such as L, C, or R, or such as L or R); a-4) the amino acid residue at position 103 is selected from the group consisting of W, R, G, S, K, A, M, Y, I, F, T, N, V, Q, P, E, and C (such as W, G, or R, or such as W); and a-5) the amino acid residue at position 108 is selected from the group consisting of Q, L, R, P, E, K, S, T, M, A, and H (such as Q); or b-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V (such as F or Y, or such as F); b-2) the amino

acid residue at position 44 is selected from the group consisting of E and Q; b-3) the amino acid residue at position 45 is selected from the group consisting of R and L (such as R); b-4) the amino acid residue at position 103 is selected from the group consisting of W, R, G, and S (such as W); and b-5) the amino acid residue at position 108 is selected from the group consisting of Q and L (such as Q); or c-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V (such as F or Y, or such as F); c-2) the amino acid residue at position 44 is selected from the group consisting of A, G, E, D, Q, R, S and L (such as G, E, or Q); c-3) the amino acid residue at position 45 is selected from the group consisting of L, R and C (such as L or R); c-4) the amino acid residue at position 103 is selected from the group consisting of P, R and S (such as R or S); and c-5) the amino acid residue at position 108 is selected from the group consisting of Q and L (such as Q); wherein the amino acid position is according to Kabat numbering. In some examples, position 108 can be optionally humanized to L when position 108 is Q.

[12] In some examples according to any one of the isolated anti-TIGIT constructs described above, the sdAb moiety specifically recognizing TIGIT comprises a V_{HH} domain comprising the amino acid sequence of any one of SEQ ID NOs: 253-259, 271, 273-276, 280, 282-284, 286-287, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 253-259, 271, 273-276, 280, 282-284, 286-287. In some examples, the sdAb moiety specifically recognizing TIGIT comprises a V_{HH} domain comprising the amino acid sequence of any one of SEQ ID NOs: 253-259, 271, 273-276, 280, 282-284, 286-287, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the V_{HH} domain. In some examples, the amino acid substitutions are in the CDRs, such as the CDR1, and/or the CDR2, and/or the CDR3 of any one of SEQ ID NOs: 253-259, 271, 273-276, 280, 282-284, 286-287. In some examples, the amino acid substitutions are in the FRs, such as the FR1, and/or the FR2, and/or the FR3, and/or the FR4 of any one of SEQ ID NOs: 253-259, 271, 273-276, 280, 282-284, 286-287. In some examples, the amino acid substitutions are in both CDRs and FRs. In some examples, the sdAb moiety specifically recognizing TIGIT comprises a V_{HH} domain comprising the amino acid sequence of any one of SEQ ID NOs: 253-259, 271, 273-276, 280, 282-284, 286-287.

[13] In some examples according to any one of the isolated anti-TIGIT constructs described above, the K_d of the binding between the sdAb moiety specifically recognizing TIGIT and TIGIT is about 10^{-5} M to about 10^{-12} M (such as about 10^{-5} M to about 10^{-12} M, about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M).

[14] In some examples according to any one of the isolated anti-TIGIT constructs described above, the sdAb moiety specifically recognizing TIGIT is camelid, chimeric, human, partially humanized, or fully humanized.

[15] In some examples according to any one of the isolated anti-TIGIT constructs described above, the isolated anti-TIGIT construct is an sdAb-Fc fusion protein. In some examples, sdAb-Fc fusion protein is monomeric. In some examples, the sdAb-Fc fusion protein is dimeric. In some examples, the Fc fragment is a human IgG1 (hIgG1) Fc, effectorless (inert) hIgG1 Fc, or hIgG4 Fc. In some examples, the Fc fragment comprises the amino acid sequence of any one of SEQ ID NOs: 355, 356, and 389. In some examples, the sdAb moiety specifically recognizing TIGIT and the Fc fragment are optionally connected by a peptide linker, such as peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 324 and 370-378. In some examples, the sdAb-Fc fusion protein comprises the amino acid sequence of any one of SEQ ID NOs: 288-294, 306, 308-311, 315, 317-319, 321-322 and 365-367.

[16] In some examples according to any one of the isolated anti-TIGIT construct described above, the isolated anti-TIGIT construct further comprises a second antibody moiety specifically recognizing a second epitope. In some examples, the second antibody moiety is a full-length antibody, a Fab, a Fab', a (Fab')₂, an Fv, a single chain Fv (scFv), an scFv-scFv, a minibody, a diabody, or an sdAb. In some examples, the anti-TIGIT construct is monospecific. In some examples, the anti-TIGIT construct is multispecific (such as bispecific). In some examples, the second epitope is not from TIGIT. In some examples, the second epitope is from TIGIT but different from that specifically recognized by the anti-TIGIT sdAb moiety. In some examples, the second epitope is the same as that specifically recognized by the anti-TIGIT sdAb moiety. In some examples, the sdAb moiety specifically recognizing TIGIT and the second antibody moiety are optionally connected by a peptide linker, such as peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 324 and 370-378. In some examples, the second antibody moiety is an sdAb. In some examples, the second antibody moiety is a Fab. In some examples, the second antibody moiety is an scFv. In some examples, the second antibody moiety is a full-length antibody consisting of two heavy chains and two light chains. In some examples, the Fc fragment of the heavy chain is IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, or IgG4 Fc. In some examples, the N-terminus of the sdAb moiety specifically recognizing TIGIT is fused to the C-terminus of at least one of the heavy chains of the full-length antibody. In some examples, the C-terminus of the sdAb moiety specifically recognizing TIGIT is fused to the N-terminus of at least one of the heavy chains of the full-length antibody. In some examples, the N-terminus of the sdAb moiety specifically recognizing TIGIT is fused to the C-

terminus of at least one of the light chains of the full-length antibody. In some examples, the C-terminus of the sdAb moiety specifically recognizing TIGIT is fused to the N-terminus of at least one of the light chains of the full-length antibody. In some examples, the isolated anti-TIGIT construct comprises four identical sdAb moieties specifically recognizing TIGIT as described above, the C-terminus of each anti-TIGIT sdAb moiety is fused to the N-terminus of each chain of the full-length antibody via an optional peptide linker. In some examples, the isolated anti-TIGIT construct comprises four identical sdAb moieties specifically recognizing TIGIT as described above, two anti-TIGIT sdAb moieties are fused to each other via an optional peptide linker, the other two anti-TIGIT sdAb moieties are fused to each other via an optional peptide linker, and the C-terminus of each of the anti-TIGIT sdAb moiety fusion polypeptide is fused to the N-terminus of each heavy chain of the full-length antibody via an optional peptide linker. In some examples the isolated anti-TIGIT construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L ; (2) anti-TIGIT sdAb- $V_H-C_{H1}-C_{H2}-C_{H3}$; (3) anti-TIGIT sdAb- $V_H-C_{H1}-C_{H2}-C_{H3}$; and (4) V_L-C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (e.g., PD-1, PD-L1), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (e.g., PD-1, PD-L1), and each anti-TIGIT sdAb specifically binds a copy of TIGIT. In some examples the isolated anti-TIGIT construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L ; (2) $V_H-C_{H1}-C_{H2}-C_{H3}$ -anti-TIGIT sdAb; (3) $V_H-C_{H1}-C_{H2}-C_{H3}$ -anti-TIGIT sdAb; and (4) V_L-C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (e.g., PD-1, PD-L1), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (e.g., PD-1, PD-L1), and each anti-TIGIT sdAb specifically binds a copy of TIGIT. In some examples the isolated anti-TIGIT construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) anti-TIGIT sdAb- V_L-C_L ; (2) $V_H-C_{H1}-C_{H2}-C_{H3}$; (3) $V_H-C_{H1}-C_{H2}-C_{H3}$; and (4) anti-TIGIT sdAb- V_L-C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (e.g., PD-1, PD-L1), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (e.g., PD-1, PD-L1), and each anti-TIGIT sdAb specifically binds a copy of TIGIT. In some examples the isolated anti-TIGIT construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L -anti-TIGIT sdAb; (2) $V_H-C_{H1}-C_{H2}-C_{H3}$; (3) $V_H-C_{H1}-C_{H2}-C_{H3}$; and (4) V_L-C_L -anti-TIGIT sdAb, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (e.g. PD-1, PD-L1), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (e.g. PD-1, PD-L1), and each anti-TIGIT sdAb specifically binds a copy of TIGIT. In some examples the isolated anti-TIGIT construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) anti-TIGIT sdAb- V_L-C_L ; (2) anti-TIGIT sdAb- $V_H-C_{H1}-C_{H2}-C_{H3}$; (3) anti-TIGIT sdAb- $V_H-C_{H1}-C_{H2}-C_{H3}$; and (4) anti-TIGIT sdAb- V_L-C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (e.g. PD-1, PD-L1), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that

specifically binds a second copy of the second epitope (e.g. PD-1, PD-L1), and each anti-TIGIT sdAb specifically binds a copy of TIGIT. In some examples the isolated anti-TIGIT construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L - C_L ; (2) anti-TIGIT sdAb-anti-TIGIT sdAb- V_H - C_H1 - C_H2 - C_H3 ; (3) anti-TIGIT sdAb-anti-TIGIT sdAb- V_H - C_H1 - C_H2 - C_H3 ; and (4) V_L - C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (e.g. PD-1, PD-L1), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (e.g. PD-1, PD-L1), and each anti-TIGIT sdAb specifically binds a copy of TIGIT. In some examples the isolated anti-TIGIT construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L - C_L ; (2) V_H - C_H1 -anti-TIGIT sdAb- C_H2 - C_H3 ; (3) V_H - C_H1 -anti-TIGIT sdAb- C_H2 - C_H3 ; and (4) V_L - C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (e.g. PD-1, PD-L1), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (e.g. PD-1, PD-L1), and each anti-TIGIT sdAb specifically binds a copy of TIGIT. In some examples the isolated anti-TIGIT construct consists of two polypeptide chains each with a structure from the N-terminus to the C-terminus as follows: V_L - V_H -anti-TIGIT sdAb- C_H2 - C_H3 , wherein V_H and V_L of each polypeptide chain forms a scFv domain that specifically binds a copy of the second epitope (e.g. PD-1, PD-L1), and each anti-TIGIT sdAb specifically binds a copy of TIGIT. In some examples the isolated anti-TIGIT construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L - C_L -anti-TIGIT sdAb- C_L ; (2) V_H - C_H1 -anti-TIGIT sdAb- C_H1 - C_H2 - C_H3 ; (3) V_H - C_H1 -anti-TIGIT sdAb- C_H1 - C_H2 - C_H3 ; and (4) V_L - C_L -anti-TIGIT sdAb- C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (e.g. PD-1, PD-L1), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (e.g. PD-1, PD-L1), and each anti-TIGIT sdAb specifically binds a copy of TIGIT. In some examples the isolated anti-TIGIT construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) anti-TIGIT sdAb- C_L ; (2) V_L - V_H -anti-TIGIT sdAb- C_H1 - C_H2 - C_H3 ; (3) V_L - V_H -anti-TIGIT sdAb- C_H1 - C_H2 - C_H3 ; and (4)

anti-TIGIT sdAb-CL, wherein V_H and V_L of polypeptide chains (2) and (3) each forms an scFv that specifically binds a copy of the second epitope (e.g. PD-1, PD-L1), and each anti-TIGIT sdAb specifically binds a copy of TIGIT. In some examples, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizes PD-1. In some examples, the anti-PD-1 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising the amino acid sequence of SEQ ID NO: 385, and a V_L comprising the amino acid sequence of SEQ ID NO: 386. In some examples, the anti-PD-1 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 325, and a light chain comprising the amino acid sequence of SEQ ID NO: 326. In some examples, the anti-PD-1 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising the amino acid sequence of SEQ ID NO: 387, and a V_L comprising the amino acid sequence of SEQ ID NO: 388. In some examples, the anti-PD-1 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 390, and a light chain comprising the amino acid sequence of SEQ ID NO: 391. In some examples, the anti-PD-1 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising the amino acid sequence of SEQ ID NO: 406, and a V_L comprising the amino acid sequence of SEQ ID NO: 407. In some examples, the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 390, and a light chain comprising the amino acid sequence of SEQ ID NO: 391, wherein at least one of the heavy chains of the full-length antibody is fused to the sdAb moiety specifically recognizing TIGIT described above, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 394 or 396. In some examples, the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 390, and a light chain comprising the amino acid sequence of SEQ ID NO: 391, wherein at least one of the light chains of the full-length antibody is fused to the sdAb moiety specifically recognizing TIGIT described above, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 399 or 401. In some examples, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizes PD-L1. In some examples, the anti-PD-L1 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises 1) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 349, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 350, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 351, and 2) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 352, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 353, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 354. In some examples, the anti-PD-L1 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising the amino acid sequence of SEQ ID NO: 339, and a V_L comprising the amino acid sequence of SEQ ID NO: 340. In some examples,

the anti-PD-L1 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 323 or 327, and a light chain comprising the amino acid sequence of SEQ ID NO: 328. In some examples, the anti-PD-L1 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 329, and a light chain comprising the amino acid sequence of SEQ ID NO: 330. In some examples, the anti-PD-L1 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising the amino acid sequence of SEQ ID NO: 379, and a V_L comprising the amino acid sequence of SEQ ID NO: 380. In some examples, the anti-PD-L1 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising the amino acid sequence of SEQ ID NO: 383, and a V_L comprising the amino acid sequence of SEQ ID NO: 384. In some examples, the anti-PD-L1 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising the amino acid sequence of SEQ ID NO: 381, and a V_L comprising the amino acid sequence of SEQ ID NO: 382. In some examples, the anti-PD-L1 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 331, and a light chain comprising the amino acid sequence of SEQ ID NO: 332. In some examples, the anti-PD-L1 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 333, and a light chain comprising the amino acid sequence of SEQ ID NO: 334. In some examples, the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 327, and a light chain comprising the amino acid sequence of SEQ ID NO: 328, wherein at least one of the heavy chains of the full-length antibody is fused to the sdAb moiety specifically recognizing TIGIT described above, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 343. In some examples, the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 323, and a light chain comprising the amino acid sequence of SEQ ID NO: 328, wherein at least one of the heavy chains of the full-length antibody is fused to the sdAb moiety specifically recognizing TIGIT described above, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 357 or 359. In some examples, the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 329, and a light chain comprising the amino acid sequence of SEQ ID NO: 330, wherein at least one of the heavy chains of the full-length antibody is fused to the sdAb moiety specifically recognizing TIGIT described above, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 341 or 402. In some examples, the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 323, and a light chain comprising the amino acid sequence of SEQ ID NO: 328, wherein at least one of the light chains of the full-length antibody is fused to the sdAb moiety specifically recognizing TIGIT described above, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 362 or 364. In some examples, the full-length

antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 329, and a light chain comprising the amino acid sequence of SEQ ID NO: 330, wherein at least one of the light chains of the full-length antibody is fused to the sdAb moiety specifically recognizing TIGIT described above, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 405. In some examples, the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 331, and a light chain comprising the amino acid sequence of SEQ ID NO: 332, wherein at least one of the heavy chains of the full-length antibody is fused to the sdAb moiety specifically recognizing TIGIT described above, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 347. In some examples, the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 333, and a light chain comprising the amino acid sequence of SEQ ID NO: 334, wherein at least one of the heavy chains of the full-length antibody is fused to the sdAb moiety specifically recognizing TIGIT described above, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 345.

[17] In some examples according to any one of the isolated anti-TIGIT constructs described above, the isolated anti-TIGIT construct further comprises a biologically active protein or fragments thereof.

[18] Further provided is an isolated anti-TIGIT construct comprising an sdAb moiety specifically recognizing TIGIT, wherein the sdAb moiety comprises CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 253-259, 271, 273-276, 280, 282-284, 286-287.

[19] Further provided is an isolated anti-TIGIT construct (e.g., anti-TIGIT sdAb, anti-TIGIT sdAb-Fc fusion, PD-1×TIGIT BABP, or PD-L1×TIGIT BABP) that specifically binds to TIGIT competitively with the any of the isolated anti-TIGIT construct described above.

[19a] In another aspect, the present disclosure refers to an isolated anti-TIGIT construct that specifically binds to TIGIT competitively with the isolated anti-TIGIT construct as disclosed herein.

[20] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the isolated anti-TIGIT construct as disclosed herein, and optionally a pharmaceutical acceptable carrier.

[21] Another example of the present application provides a method of treating an individual having a TIGIT-related disease (such as cancer, or immune-related disease), comprising administering to the individual an effective amount of any one of the pharmaceutical compositions described above. In some examples, the TIGIT-related disease is cancer. In some examples, the cancer is a solid tumor, such as a colon cancer. In some examples, the TIGIT-related disease is an immune-related disease. In some examples, immune-related disease is associated with a T cell dysfunctional disorder. In some examples, the T cell dysfunctional disorder is characterized by T cell exhaustion. In some examples, the immune-related disease is selected from the group consisting of unresolved acute

infection, chronic infection, and tumor immunity. In some examples, the TIGIT related disease is a pathogenic infection. In some examples, the method further comprises administering to the individual an additional therapy (e.g., cancer therapy), such as surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or a combination thereof. In some examples, the additional therapy is immunotherapy. In some examples, the immunotherapy comprises administering to the individual an effective amount of a second pharmaceutical composition comprising an immunomodulator, such as an immune checkpoint inhibitor (e.g., antibody specifically recognizing PD-1 or PD-L1). In some examples, the pharmaceutical composition is administered systemically, such as intravenously (i.v.) or intraperitoneally (i.p.). In some examples, the pharmaceutical composition is administered locally, such as intratumorally. In some examples, the individual is a human.

[21a] In another aspect, the present disclosure refers to use of an effective amount of the isolated anti-TIGIT construct as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a cancer, a pathogenic infection, or an immune-related disease.

[22] In another aspect, the present disclosure refers to an isolated nucleic acid encoding the isolated anti-TIGIT construct as disclosed herein. In some examples, the isolated nucleic acid comprises the nucleic acid sequence of any one of SEQ ID NOs: 246-252.

[23] In another aspect, the present disclosure refers to a vector comprising the isolated nucleic acids as disclosed herein.

[24] In another aspect, the present disclosure refers to an isolated host cell comprising the isolated nucleic acid as disclosed herein, or the vector as disclosed herein.

[25] Further provided is a kit comprising any one of the isolated anti-TIGIT construct, isolated nucleic acid, vector, or isolated host cell described above.

[26] Another example of the present application provides a method of producing any one of isolated anti-TIGIT constructs described above, comprising culturing a host cell comprising any one of the isolated nucleic acid or vector described above, or culturing any one of the isolated host cell described above, under conditions effective to express the encoded anti-TIGIT construct; and obtaining the expressed anti-TIGIT construct from said host cell. In some examples, the method further comprises producing a host cell comprising any one of the isolated nucleic acid or vector described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[27] FIG. 1 depicts immune response evaluation of pre-immune serum and post-immune serum after final boost.

[28] FIG. 2 depicts the immune response evaluation of regular antibodies (IgG1) and heavy chain antibodies (IgG2 and IgG3) after final boost. Corresponding immunoglobulin fragment isolated from pre-immune serum were used as negative controls.

CLAIMS

What is claimed is:

1. An isolated anti-TIGIT construct comprising a single-domain antibody (sdAb) moiety specifically recognizing TIGIT (“anti-TIGIT sdAb moiety”), wherein the anti-TIGIT sdAb moiety comprises any one of the following:
 - (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 106, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 176, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 63, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 133, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 203, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 109, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 179, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 42, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 112, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 182, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 107, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 177, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 38, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 108, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3

- comprising the amino acid sequence of SEQ ID NO: 178, or a variant thereof comprising up to about 3 amino acid substitutions;
- (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 110, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 180, or a variant thereof comprising up to about 3 amino acid substitutions; and
 - (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 111, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 181, or a variant thereof comprising up to about 3 amino acid substitutions.
2. The isolated anti-TIGIT construct of claim 1, wherein the anti-TIGIT sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of the following:
- a-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, V, L, A, H, S, I, W, C, N, G, D, T, and P;
 - a-2) the amino acid residue at position 44 is selected from the group consisting of E, Q, G, D, A, K, R, L, P, S, V, H, T, N, W, M, and I;
 - a-3) the amino acid residue at position 45 is selected from the group consisting of L, R, P, H, F, G, Q, S, E, T, Y, C, I, D, and V;
 - a-4) the amino acid residue at position 103 is selected from the group consisting of W, R, G, S, K, A, M, Y, I, F, T, N, V, Q, P, E, and C; and
 - a-5) the amino acid residue at position 108 is selected from the group consisting of Q, L, R, P, E, K, S, T, M, A, and H; or
 - b-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V;
 - b-2) the amino acid residue at position 44 is selected from the group consisting of E and Q;
 - b-3) the amino acid residue at position 45 is selected from the group consisting of R and L;
 - b-4) the amino acid residue at position 103 is selected from the group consisting of W, R, G, and S; and
 - b-5) the amino acid residue at position 108 is selected from the group consisting of Q and L; or
 - c-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V;
 - c-2) the amino acid residue at position 44 is selected from the group consisting of A, G, E, D, Q, R, S and L;
 - c-3) the amino acid residue at position 45 is selected from the group consisting of L, R and C;

c-4) the amino acid residue at position 103 is selected from the group consisting of P, R and S; and
c-5) the amino acid residue at position 108 is selected from the group consisting of Q and L;
wherein the amino acid position is according to Kabat numbering, and wherein position 108 can be optionally humanized to L when position 108 is Q.

3. The isolated anti-TIGIT construct of claim 1 or 2, wherein the anti-TIGIT sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 253-259, 271, 273-276, 280, 282-284, 286, and 287, or a variant thereof having at least about 80% sequence identity to any one of SEQ ID NOs: 253-259, 271, 273-276, 280, 282-284, 286, and 287.
4. The isolated anti-TIGIT construct of any one of claims 1-3, wherein the anti-TIGIT sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.
5. The isolated anti-TIGIT construct of any one of claims 1-4, wherein the isolated anti-TIGIT construct is an anti-TIGIT sdAb-Fc fusion protein.
6. The isolated anti-TIGIT construct of claim 5, wherein the anti-TIGIT sdAb-Fc fusion protein comprises the amino acid sequence of any one of SEQ ID NOs: 288-294, 306, 308-311, 315, 317-319, 321-322, and 365-367.
7. The isolated anti-TIGIT construct of any one of claims 1-4, wherein the isolated anti-TIGIT construct further comprises a second antibody moiety specifically recognizing a second epitope.
8. The isolated anti-TIGIT construct of claim 7, wherein the second antibody moiety is a full-length antibody, a Fab, a Fab', a (Fab')₂, an Fv, a single chain Fv (scFv), an scFv-scFv, a minibody, a diabody, or an sdAb.
9. The isolated anti-TIGIT construct of claim 7 or 8, wherein the anti-TIGIT sdAb moiety and the second antibody moiety are connected by a peptide linker.
10. The isolated anti-TIGIT construct of claim 9, wherein the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 324 and 370-378.
11. The isolated anti-TIGIT construct of any one of claims 7-10, wherein the second antibody moiety is a full-length antibody consisting of two heavy chains and two light chains.
12. The isolated anti-TIGIT construct of claim 11, wherein the isolated anti-TIGIT construct comprises a configuration selected from the group consisting of:
 - (i) the N-terminus of the anti-TIGIT sdAb moiety is fused to the C-terminus of at least one of the heavy chains of the full-length antibody;
 - (ii) the C-terminus of the anti-TIGIT sdAb moiety is fused to the N-terminus of at least one of the heavy chains of the full-length antibody;
 - (iii) the N-terminus of the anti-TIGIT sdAb moiety is fused to the C-terminus of at least one of the light chains of the full-length antibody;

- (iv) the C-terminus of the anti-TIGIT sdAb moiety is fused to the N-terminus of at least one of the light chains of the full-length antibody;
 - (v) the isolated anti-TIGIT construct comprises four anti-TIGIT sdAb moieties, wherein the C-terminus of each anti-TIGIT sdAb moiety is fused to the N-terminus of each chain of the full-length antibody; and
 - (vi) the isolated anti-TIGIT construct comprises four anti-TIGIT sdAb moieties, wherein two of the four anti-TIGIT sdAb moieties are fused together, which is further fused to the N-terminus of each heavy chain of the full-length antibody.
13. The isolated anti-TIGIT construct of any one of claims 8-12, wherein the full-length antibody specifically recognizes PD-1.
 14. The isolated anti-TIGIT construct of claim 13, wherein the full-length antibody comprises heavy chain complementarity determining regions (HC-CDRs) and light chain complementarity determining regions (LC-CDRs) of:
 - (i) a heavy chain variable domain (V_H) comprising the amino acid sequence of SEQ ID NO: 385, and a light chain variable domain (V_L) comprising the amino acid sequence of SEQ ID NO: 386;
 - (ii) a V_H comprising the amino acid sequence of SEQ ID NO: 387, and a V_L comprising the amino acid sequence of SEQ ID NO: 388; or
 - (iii) a V_H comprising the amino acid sequence of SEQ ID NO: 406, and a V_L comprising the amino acid sequence of SEQ ID NO: 407.
 15. The isolated anti-TIGIT construct of claim 13 or 14, wherein:
 - (i) the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 390, and a light chain comprising the amino acid sequence of SEQ ID NO: 391, wherein at least one of the heavy chains of the full-length antibody is fused to the anti-TIGIT sdAb moiety, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 394 or 396; or
 - (ii) the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 390, and a light chain comprising the amino acid sequence of SEQ ID NO: 391, wherein at least one of the light chains of the full-length antibody is fused to the anti-TIGIT sdAb moiety, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 399 or 401.
 16. The isolated anti-TIGIT construct of any one of claims 8-12, wherein the full-length antibody specifically recognizes PD-L1.
 17. The isolated anti-TIGIT construct of claim 16, wherein the full-length antibody comprises:
 - (i) an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 349, an HC-CDR2 comprising

the amino acid sequence of SEQ ID NO: 350, an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 351, an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 352, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 353, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 354;

(ii) a VH comprising the amino acid sequence of SEQ ID NO: 339, and a VL comprising the amino acid sequence of SEQ ID NO: 340;

(iii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 323 or 327, and a light chain comprising the amino acid sequence of SEQ ID NO: 328;

(iv) a heavy chain comprising the amino acid sequence of SEQ ID NO: 329, and a light chain comprising the amino acid sequence of SEQ ID NO: 330;

(v) HC-CDRs of a VH comprising the amino acid sequence of SEQ ID NO: 379, and LC-CDRs of a VL comprising the amino acid sequence of SEQ ID NO: 380;

(vi) HC-CDRs of a VH comprising the amino acid sequence of SEQ ID NO: 383, and LC-CDRs of a VL comprising the amino acid sequence of SEQ ID NO: 384;

(vii) HC-CDRs of a VH comprising the amino acid sequence of SEQ ID NO: 381, and LC-CDRs of a VL comprising the amino acid sequence of SEQ ID NO: 382;

(viii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 331, and a light chain comprising the amino acid sequence of SEQ ID NO: 332; or

(ix) a heavy chain comprising the amino acid sequence of SEQ ID NO: 333, and a light chain comprising the amino acid sequence of SEQ ID NO: 334.

18. The isolated anti-TIGIT construct of claim 16 or 17,

(i) wherein the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 329, and a light chain comprising the amino acid sequence of SEQ ID NO: 330, wherein at least one of the heavy chains of the full-length antibody is fused to the anti-TIGIT sdAb moiety, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 341 or 402;

(ii) wherein the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 323, and a light chain comprising the amino acid sequence of SEQ ID NO: 328, wherein at least one of the heavy chains of the full-length antibody is fused to the anti-TIGIT sdAb moiety, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 357 or 359;

(iii) wherein the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 329, and a light chain comprising the amino acid sequence of SEQ ID NO: 330, wherein at least one of the light chains of the full-length antibody is fused to the anti-TIGIT sdAb moiety, and

- wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 405;
- (iv) wherein the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 327, and a light chain comprising the amino acid sequence of SEQ ID NO: 328, wherein at least one of the heavy chains of the full-length antibody is fused to the sdAb moiety specifically recognizing TIGIT, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 343;
- (v) wherein the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 333, and a light chain comprising the amino acid sequence of SEQ ID NO: 334, wherein at least one of the heavy chains of the full-length antibody is fused to the sdAb moiety specifically recognizing TIGIT, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 345;
- (vi) wherein the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 331, and a light chain comprising the amino acid sequence of SEQ ID NO: 332, wherein at least one of the heavy chains of the full-length antibody is fused to the sdAb moiety specifically recognizing TIGIT, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 347; or
- (vii) wherein the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 323, and a light chain comprising the amino acid sequence of SEQ ID NO: 328, wherein at least one of the light chains of the full-length antibody is fused to the sdAb moiety specifically recognizing TIGIT, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 362 or 364.
19. The isolated anti-TIGIT construct of any one of claims 7-10, comprising a configuration selected from the group consisting of:
- (a) the isolated anti-TIGIT construct comprises four polypeptides from N-terminus to C-terminus:
- (i) V_L-C_L , (ii) V_H-C_{H1} -optional peptide linker-first anti-TIGIT sdAb moiety- $C_{H2}-C_{H3}$, (iii) V_H-C_{H1} -optional peptide linker-second anti-TIGIT sdAb moiety- $C_{H2}-C_{H3}$, and (iv) V_L-C_L ; wherein V_L-C_L of polypeptide (i) and V_H-C_{H1} of polypeptide (ii) form a second antibody moiety specifically recognizing a second epitope, and V_L-C_L of polypeptide (iv) and V_H-C_{H1} of polypeptide (iii) form a third antibody moiety specifically recognizing a third epitope;
- (b) the isolated anti-TIGIT construct comprises two polypeptides from N-terminus to C-terminus:
- (i) first scFv specifically recognizing a second epitope-optional peptide linker-first anti-TIGIT sdAb moiety- $C_{H2}-C_{H3}$, and (ii) second scFv specifically recognizing a third epitope-optional peptide linker-second anti-TIGIT sdAb moiety- $C_{H2}-C_{H3}$;
- (c) the isolated anti-TIGIT construct comprises four polypeptides from N-terminus to C-terminus:

- (i) V_L - C_L -optional peptide linker-first anti-TIGIT sdAb moiety- C_L , (ii) V_H - C_H1 -optional peptide linker-second anti-TIGIT sdAb moiety- C_H1 - C_H2 - C_H3 , (iii) V_H - C_H1 -optional peptide linker-third anti-TIGIT sdAb moiety- C_H1 - C_H2 - C_H3 , and (iv) V_L - C_L -optional peptide linker-fourth anti-TIGIT sdAb moiety- C_L ; wherein V_L - C_L of polypeptide (i) and V_H - C_H1 of polypeptide (ii) form a second antibody moiety specifically recognizing a second epitope, and V_L - C_L of polypeptide (iv) and V_H - C_H1 of polypeptide (iii) form a third antibody moiety specifically recognizing a third epitope; and
- (d) the isolated anti-TIGIT construct comprises four polypeptides from N-terminus to C-terminus: (i) first anti-TIGIT sdAb moiety- C_L , (ii) first scFv specifically recognizing a second epitope-optional peptide linker-second anti-TIGIT sdAb moiety- C_H1 - C_H2 - C_H3 , (iii) second scFv specifically recognizing a third epitope-optional peptide linker-third anti-TIGIT sdAb moiety- C_H1 - C_H2 - C_H3 , and (iv) fourth anti-TIGIT sdAb moiety- C_L .
20. An isolated anti-TIGIT construct that specifically binds to TIGIT competitively with the isolated anti-TIGIT construct of any one of claims 1-19.
 21. A pharmaceutical composition comprising the isolated anti-TIGIT construct of any one of claims 1-20, and optionally a pharmaceutical acceptable carrier.
 22. Use of an effective amount of the isolated anti-TIGIT construct of any one of claims 1-20, or the pharmaceutical composition of claim 21 in the manufacture of a medicament for treating a cancer, a pathogenic infection, or an immune-related disease.
 23. The use of claim 22, wherein the cancer is a colon cancer.
 24. An isolated nucleic acid encoding the isolated anti-TIGIT construct of any one of claims 1-20.
 25. A vector comprising the isolated nucleic acid of claim 24.
 26. An isolated host cell comprising the isolated nucleic acid of claim 24, or the vector of claim 25.