CLAIMS

What is claimed is:

1. An isolated anti-LAG-3 construct comprising a single-domain antibody (sdAb) specifically recognizing LAG-3, wherein the sdAb comprises any one of the following:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 42; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194; or

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 43; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195.

2. The isolated anti-LAG-3 construct of claim 1, wherein the sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 275-278, 288, 295, 304 and

306, or a variant thereof having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 275-278, 288, 295, 304 and 306.

3. The isolated anti-LAG-3 construct of claim 1 or 2, wherein the sdAb comprises:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; a FR1 comprising the amino acid sequence of SEQ ID NO: 31; a FR2 comprising the amino acid sequence of SEQ ID NO: 107; a FR3 comprising the amino acid sequence of SEQ ID NO: 183; and a FR4 comprising the amino acid sequence of SEQ ID NO: 259;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; a FR1 comprising the amino acid sequence of SEQ ID NO: 15; a FR2 comprising the amino acid sequence of SEQ ID NO: 91; a FR3 comprising the amino acid sequence of SEQ ID NO: 167; and a FR4 comprising the amino acid sequence of SEQ ID NO: 243;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223; a FR1 comprising the amino acid sequence of SEQ ID NO: 33; a FR2 comprising the amino acid sequence of SEQ ID NO: 109; a FR3 comprising the amino acid sequence of SEQ ID NO: 185; and a FR4 comprising the amino acid sequence of SEQ ID NO: 261;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; a FR1 comprising the amino acid sequence of SEQ ID NO: 22; a FR2 comprising the amino acid sequence of SEQ ID NO: 98; a FR3 comprising the amino acid sequence of SEQ ID NO: 174; and a FR4 comprising the amino acid sequence of SEQ ID NO: 250;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192; a FR1 comprising the amino acid sequence of SEQ ID NO: 2; a FR2 comprising the amino acid sequence of SEQ ID NO: 78; a FR3 comprising the amino acid sequence of SEQ ID NO: 154; and a FR4 comprising the amino acid sequence of SEQ ID NO: 230;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193; a FR1 comprising the amino acid sequence of SEQ ID NO: 3; a FR2 comprising the amino acid sequence of SEQ ID NO: 79; a FR3 comprising the amino acid sequence of SEQ ID NO: 155; and a FR4 comprising the amino acid sequence of SEQ ID NO: 231;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 42; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194; a FR1 comprising the amino acid sequence of SEQ ID NO: 4; a FR2 comprising the amino acid sequence of SEQ ID NO: 80; a FR3 comprising the amino acid sequence of SEQ ID NO: 156; and a FR4 comprising the amino acid sequence of SEQ ID NO: 232; or

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 43; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195; a FR1 comprising the amino acid sequence of SEQ ID NO: 5; a FR2 comprising the amino acid sequence of SEQ ID NO: 81; a FR3 comprising the amino acid sequence of SEQ ID NO: 157; and a FR4 comprising the amino acid sequence of SEQ ID NO: 233.

4. The isolated anti-LAG-3 construct of any one of claims 1 to 3, wherein the K_D of the binding between the sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M, preferably about 10^{-9} M to about 10^{-11} M.

5. The isolated anti-LAG-3 construct of any one of claims 1 to 4, wherein the isolated anti-LAG-3 construct is a heavy chain-only antibody (HCAb) comprising the sdAb specifically recognizing LAG-3 fused to an Fc fragment, optionally wherein the HCAb is monomeric or dimeric,

optionally wherein the Fc fragment is a human IgG1 (hIgG1) Fc, effectorless hIgG1 Fc, hIgG4 Fc, or hIgG4 Fc (S228P).

6. The isolated anti-LAG-3 construct of claim 5, wherein the sdAb is fused to the Fc fragment via a peptide linker, optionally wherein the peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372.

7. The isolated anti-LAG-3 construct of claim 6, wherein the HCAb comprises the amino acid sequence of any one of SEQ ID NOs: 313-316, 326, 333, 342 and 344.

8. The isolated anti-LAG-3 construct of any one of claims 1 to 7, comprising: (a) a first antigen binding portion comprising the sdAb specifically recognizing LAG-3; and (b) a second antigen binding portion that specifically recognizes a second epitope.

9. The isolated anti-LAG-3 construct of claim 8, wherein the second antigen binding portion comprises 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 a second sdAb, optionally

wherein the first antigen binding portion and the second antigen binding portion are fused to each other via a peptide linker, preferably wherein the peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372.

10. The isolated anti-LAG-3 construct of any one of claims 8-9, wherein the second antigen binding portion specifically recognizes an immune checkpoint molecule selected from the group consisting of PD-1, 4-1BB, PD-L1, TIM-3, TIGIT, CTLA-4, VISTA, B7-1, B7-H3, CD47, OX40 and GITR.

11. The isolated anti-LAG-3 construct of claim 10, wherein the second antigen binding portion specifically recognizes PD-1.

12. The isolated anti-LAG-3 construct of claim 11, wherein the second antigen binding portion comprises:

(i) HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 356, and LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 357; or

(ii) HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 373, and LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 374.

13. The isolated anti-LAG-3 construct of claim 11 or 12, comprising:

(i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 356, and a light chain comprising the amino acid sequence of SEQ ID NO: 357; or

(ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 373, and a light chain comprising the amino acid sequence of SEQ ID NO: 374.

14. The isolated anti-LAG-3 construct of any one of claims 8 to 13, wherein

(1) the N-terminus of the sdAb specifically recognizing LAG-3 is fused to the C-terminus of the heavy chain of the second antigen binding portion,

(2) the C-terminus of the sdAb specifically recognizing LAG-3 is fused to the N-terminus of the heavy chain of the second antigen binding portion,

(3) the N-terminus of the sdAb specifically recognizing LAG-3 is fused to the C-terminus of the light chain of the second antigen binding portion, or

(4) the C-terminus of the sdAb specifically recognizing LAG-3 is fused to the N-terminus of the light chain of the second antigen binding portion.

15. An isolated anti-LAG-3 construct, comprising

(1) a first polypeptide having the amino acid of SEQ ID NO: 441, and a second polypeptide having the amino acids of SEQ ID NO: 442;

(2) a first polypeptide having the amino acid of SEQ ID NO: 433, and a second polypeptide having the amino acids of SEQ ID NO: 434;

(3) a first polypeptide having the amino acid of SEQ ID NO: 403, and a second polypeptide having the amino acids of SEQ ID NO: 404;

(4) a first polypeptide having the amino acid of SEQ ID NO: 411, and a second polypeptide having the amino acids of SEQ ID NO: 412;

(5) a first polypeptide having the amino acid of SEQ ID NO: 381, and a second polypeptide having the amino acids of SEQ ID NO: 382;

(6) a first polypeptide having the amino acid of SEQ ID NO: 377, and a second polypeptide having the amino acids of SEQ ID NO: 378;

(7) a first polypeptide having the amino acid of SEQ ID NO: 379, and a second polypeptide having the amino acids of SEQ ID NO: 380;

(8) a first polypeptide having the amino acid of SEQ ID NO: 375, and a second polypeptide having the amino acids of SEQ ID NO: 376;

(9) a first polypeptide having the amino acid of SEQ ID NO: 383, and a second polypeptide having the amino acids of SEQ ID NO: 384;

(10) a first polypeptide having the amino acid of SEQ ID NO: 385, and a second polypeptide having the amino acids of SEQ ID NO: 386;

(11) a first polypeptide having the amino acid of SEQ ID NO: 387, and a second polypeptide having the amino acids of SEQ ID NO: 388;

(12) a first polypeptide having the amino acid of SEQ ID NO: 389, and a second polypeptide having the amino acids of SEQ ID NO: 390;

(13) a first polypeptide having the amino acid of SEQ ID NO:391, and a second polypeptide having the amino acids of SEQ ID NO: 392;

(14) a first polypeptide having the amino acid of SEQ ID NO: 393, and a second polypeptide having the amino acids of SEQ ID NO: 394;

(15) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 395, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 396;

(16) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 397, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 398;

(17) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 399, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 400;

(18) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 401, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 402;

(19) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 405, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 406;

(20) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 407, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 408;

(21) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 409, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 410;

(22) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 413, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 414;

(23) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 415, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 416;

(24) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 417, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 418;

(25) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 419, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 420;

(26) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 421, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 422;

(27) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 423, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 424;

(28) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 425, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 426;

(29) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 427, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 428;

(30) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 429, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 430;

(31) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 431, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 432;

(32) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 435, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 436;

(33) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 437, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 438;

(34) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 439, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 440;

(35) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 443, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 444; or

(36) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 445, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 446.

16. A pharmaceutical composition comprising an effective amount of the isolated anti-LAG-3 construct of any one of claims 1 to 15, and a pharmaceutically acceptable carrier.

17. Use of an effective amount of the isolated anti-LAG-3 construct of any one of claims 1-15 or the pharmaceutical composition of claim 16 in the manufacture of a medicament for treating a cancer or an immune-related disease, wherein the immune-related disease is associated with a T cell dysfunctional disorder, preferably wherein the cancer is a solid tumor, more preferably wherein the cancer is a colon cancer.

18. An isolated nucleic acid encoding the isolated anti-LAG-3 construct of any one of claims 1-15.

19. A vector comprising the isolated nucleic acid of claim 18.

20. An isolated host cell comprising the isolated nucleic acid of claim 18, or the vector of claim 19.