Claims

1. An antigen-binding protein, or an antigen-binding fragment thereof, comprising a CD28-binding portion, wherein the CD28-binding portion binds to a CD28 epitope comprising (i) amino acid residues 51-122 of human CD28 (SEQ ID NO:1), or (ii) amino acid residues 51, 52, 54, 55, 98-101, 110-111, 113-114, and 118-122 of SEQ ID NO:1, and

optionally wherein the CD28-binding portion is cross-reactive with cynomolgus monkey and mouse CD28, and/or does not have superagonist activity.

2. The antigen-binding protein or fragment thereof of claim 1, wherein the CD28-binding portion comprises an antibody heavy chain variable domain (V_H) and an antibody light chain variable domain (V_L), and wherein the V_H and V_L comprise heavy chain complementarity-determining regions (CDRs) 1-3 and light chain CDR1-3 set forth in

SEQ ID NOs:5-10, respectively,

SEQ ID NOs:15, 6, 16, 17-19, respectively,

SEQ ID NOs:24, 6, 25, 26-28, respectively,

SEQ ID NOs:33, 6, 35-38, respectively,

SEQ ID NOs:43, 6, 44, 45, 9, and 46, respectively,

SEQ ID NOs:33, 51-53, 300, and 10, respectively,

SEQ ID NOs:24, 58, 59, 60, 300, and 61, respectively,

SEQ ID NOs:66-69, 300 and 70, respectively,

SEQ ID NOs:24, 6, 75, 76, 18, and 28, respectively,

SEQ ID NOs:24, 58, 81, 82, 27, and 83, respectively,

SEQ ID NOs:88-91, 300, and 70, respectively,

SEQ ID NOs:24, 96-98, 9, and 70, respectively,

SEQ ID NOs:103-106, 18, and 83, respectively,

SEQ ID NOs:111, 6, 112, 113, 18, and 114, respectively,

SEQ ID NOs:15, 6, 119, 120, 9, and 121, respectively,

SEQ ID NOs:126, 67, 127, 128, 18, and 129, respectively,

SEQ ID NOs:134, 6, 135, 136, 27, and 83, respectively,

SEQ ID NOs:43, 58, 141, 142, 300, and 143, respectively,

SEQ ID NOs:148, 6, 149, 150, 300, and 83, respectively,

SEQ ID NOs:15, 155, 16, 156, 27, and 70, respectively,

SEQ ID NOs:161, 6, 162, 163, 300, and 164, respectively,

SEQ ID NOs:5, 96, 307, 76, 308, and 46, respectively,

SEQ ID NOs:5, 96, 309, 310, 9, and 311, respectively,

SEQ ID NOs:43, 96, 312, 76, 9, and 28, respectively,

SEQ ID NOs:43, 96, 307, 310, 9, and 313, respectively,

SEQ ID NOs:314, 315, 309, 76, 9, and 313, respectively,

SEQ ID NOs:33, 316, 312, 8, 308, and 313, respectively,

SEQ ID NOs:314, 96, 317, 76, 308, and 318, respectively,

SEQ ID NOs:43, 316, 312, 8, 9, and 10, respectively,

SEQ ID NOs:43, 6, 319, 320, 9, and 10, respectively,

SEQ ID NOs:5, 67, 307, 310, 308, and 28, respectively,

SEQ ID NOs:43, 6, 321, 8, 9, and 10, respectively,

SEQ ID NOs:314, 316, 322, 8, 9, and 10, respectively,

SEQ ID NOs:33, 6, 321, 8, 9, and 10, respectively,

SEQ ID NOs:5, 6, 317, 8, 9, and 10, respectively,

SEQ ID NOs:5, 6, 323, 8, 9, and 10, respectively, or

SEQ ID NOs:5, 6, 324, 8, 9, and 10, respectively.

3. The antigen-binding protein or fragment thereof of claim 2, wherein the V_H and V_L comprise

SEQ ID NOs:11 and 12, respectively,

SEQ ID NOs:20 and 21, respectively,

SEQ ID NOs:29 and 30, respectively,

SEQ ID NOs:39 and 40, respectively,

SEQ ID NOs:47 and 48, respectively,

SEQ ID NOs:54 and 55, respectively,

SEQ ID NOs:62 and 63, respectively,

SEQ ID NOs:71 and 72, respectively,

SEQ ID NOs:77 and 78, respectively,

SEQ ID NOs:84 and 85, respectively,

SEQ ID NOs:92 and 93, respectively,

SEQ ID NOs:99 and 100, respectively,

SEQ ID NOs:107 and 108, respectively,

SEQ ID NOs:115 and 116, respectively,

SEQ ID NOs:122 and 123, respectively,

SEQ ID NOs:130 and 131, respectively,

SEQ ID NOs:137 and 138, respectively,

SEQ ID NOs:144 and 145, respectively,

SEQ ID NOs:151 and 152, respectively,

SEQ ID NOs:157 and 158, respectively,

SEQ ID NOs:165 and 166, respectively,

SEQ ID NOs:362 and 363, respectively,

SEQ ID NOs:364 and 365, respectively,

SEQ ID NOs:366 and 367, respectively,

SEQ ID NOs:368 and 369, respectively,

SEQ ID NOs:370 and 371, respectively,

SEQ ID NOs:372 and 373, respectively,

SEQ ID NOs:374 and 375, respectively,

SEQ ID NOs:376 and 12, respectively,

SEQ ID NOs:377 and 378, respectively,

SEQ ID NOs:379 and 380, respectively,

SEQ ID NOs:381 and 12, respectively,

SEQ ID NOs:382 and 12, respectively,

SEQ ID NOs:383 and 12, respectively,

SEQ ID NOs:384 and 12, respectively,

SEQ ID NOs:385 and 12, respectively, or

SEQ ID NOs:386 and 12, respectively.

4. The antigen-binding protein of claim 2 or 3, comprising an antibody heavy chain and an antibody light chain comprising

SEQ ID NOs:13 and 14, respectively,

SEQ ID NOs:22 and 23, respectively,

SEQ ID NOs:31 and 32, respectively,

SEQ ID NOs:41 and 42, respectively,

SEQ ID NOs:49 and 50, respectively,

SEQ ID NOs:56 and 57, respectively,

SEQ ID NOs:64 and 65, respectively

SEQ ID NOs:73 and 74, respectively,

SEQ ID NOs:79 and 80, respectively,

SEQ ID NOs:86 and 87, respectively,

SEQ ID NOs:94 and 95, respectively,

SEQ ID NOs:101 and 102, respectively,

SEQ ID NOs:109 and 110, respectively,

SEQ ID NOs:117 and 118, respectively,

SEQ ID NOs:124 and 125, respectively,

SEQ ID NOs:132 and 133, respectively,

SEQ ID NOs:139 and 140, respectively,

SEQ ID NOs:146 and 147, respectively,

SEQ ID NOs:153 and 154, respectively,

SEQ ID NOs:159 and 160, respectively, or

SEQ ID NOs:167 and 168, respectively.

5. The antigen-binding protein or fragment thereof of any one of claims 1-4, further comprising a second antigen-binding portion targeting a tumor-associated antigen (TAA), optionally wherein the TAA is HER2, B7-H3, or TROP-2, further optionally wherein one or each of the CD28-binding portion and the TAA-binding portion is a single chain Fv (scFv), Fv, scFab, or Fab.

6. The antigen-binding protein or fragment thereof of claim 5, wherein the TAA-binding portion is:

a) a HER2-binding portion comprising HCDR1-3 and LCDR1-3 set forth in SEQ ID NOs:262-264 and 258-260, respectively, or V_H and V_L set forth in SEQ ID NOs:265 and 261, respectively;

b) a B7-H3-binding portion comprising HCDR1-3 and LCDR1-3 set forth in SEQ ID NOs:290-292 and 287, 18, and 288, respectively, or V_H and V_L set forth in SEQ ID NOs:293 and 289, respectively; or

c) a TROP-2-binding portion comprising HCDR1-3 and LCDR1-3 set forth in SEQ ID NOs:280-282 and 277, 259, and 278, respectively, or V_H and V_L set forth in SEQ ID NOs:283 and 279, respectively.

7. The antigen-binding protein or fragment thereof of claim 6, wherein the TAA-binding portion is a HER2-binding portion comprising:

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:169 and 170, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:171 or an scFv fusion polypeptide comprising SEQ ID NO:266;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:169 and 170, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:172 or an scFv fusion polypeptide comprising SEQ ID NO:266;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:271 and 276, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:171 or an scFv fusion polypeptide comprising SEQ ID NO:266;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:327;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:333;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:334;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:335;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:336;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:337;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:338;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:339;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:340;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:341;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:342;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:343;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:344;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:345;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:346;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:347;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:348;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:349;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:350;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:351;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:352; or

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:331 and 332, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:353.

8. The antigen-binding protein or fragment thereof of claim 6, wherein the TAA-binding portion is a B7-H3-binding portion comprising:

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:175 and 176, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:171 or an scFv fusion polypeptide comprising SEQ ID NO:266;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:354 and 355, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:327;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:354 and 355, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:356;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:354 and 355, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:357; or

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:354 and 355, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:358.

9. The antigen-binding protein or fragment thereof of claim 6, wherein the TAA-binding portion is a TROP2-binding portion comprising an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:173 and 174, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:171 or an scFv fusion polypeptide comprising SEQ ID NO:266.

10. The antigen-binding protein or fragment thereof of any one of claims 1-9, further comprising another antigen-binding portion targeting an immune checkpoint inhibitor, optionally wherein the immune checkpoint inhibitor is PD-L1, further optionally wherein the PD-L1-binding portion comprises

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:325 and 326, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:327;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:325 and 326, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:328;

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:325 and 326, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:329; or

an antibody light chain and an antibody heavy chain comprising SEQ ID NOs:325 and 326, respectively, and wherein the CD28-binding portion comprises a heavy chain comprising SEQ ID NO:330.

11. The antigen-binding protein or fragment thereof of any one of claims 1-10, further comprising an Fc region, optionally wherein

the Fc region is of the human IgG1 subclass or human IgG4 subclass, and/or

the Fc region has reduced or no effector function, reduced or no antibody-dependent cell cytotoxicity (ADCC) effect, and/or reduced or no crosslinking effects,

further optionally wherein the Fc domain further comprises an N297A substitution (Eu numbering).

12. The antigen-binding protein or fragment thereof of any one of claims 1-11, comprising a first CH3 domain and a second CH3 domain, wherein:

i) the first CH3 domain comprises a cysteine (C) residue at position 390 and the second CH3 domain comprises a cysteine residue at position 400, or the first

CH3 domain comprises a cysteine residue at position 400 and the second CH3 domain comprises a cysteine residue at position 390; or

ii) the first CH3 domain comprises a cysteine residue at position 392 and the second CH3 domain comprises a cysteine residue at position 397, or the first CH3 domain comprises a cysteine residue at position 397 and the second CH3 domain comprises a cysteine residue at position 392; or

iii) the first CH3 domain comprises a cysteine residue at position 392 and the second CH3 domain comprises a cysteine residue at position 400, or the first CH3 domain comprises a cysteine residue at position 400 and the second CH3 domain comprises a cysteine residue at position 392;

and wherein the amino acid residue numbering is based on Eu numbering;

optionally wherein

iv) the first CH3 domain further comprises a positively charged residue at position 357 and the second CH3 domain further comprises a negatively charged residue at position 351, or the first CH3 domain further comprises a negatively charged residue at position 351 and the second CH3 domain further comprises a positively charged residue at position 357; or

v) the first CH3 domain further comprises a positively charged residue at position 411 and the second CH3 domain further comprises a negatively charged residue at position 370, or the first CH3 domain further comprises a negatively charged residue at position 370 and the second CH3 domain further comprises a positively charged residue at position 411; or

vi) the first CH3 domain further comprises a positively charged residue at position 364 and the second CH3 domain further comprises a negatively charged residue at position 370, or the first CH3 domain further comprises a negatively charged residue at position 370 and the second CH3 domain further comprises a positively charged residue at position 364; or a combination of i) and ii), or a combination of i) and iii);

and wherein the amino acid residue numbering is based on Eu numbering;

further optionally wherein

the first CH3 domain comprises D/E356K, E357K, S364K and S400C substitutions and the second CH3 domain comprises L351D, K370D, N390C and K439D substitutions (Eu numbering), or

the first CH3 domain comprises L351D, K370D, N390C and K439D substitutions and the second CH3 domain comprises D/E356K, E357K, S364K and S400C substitutions (Eu numbering).

13. The antigen-binding protein or fragment thereof of any one of claims 1-12, further comprising at least one masking peptide, wherein the at least one masking peptide (MP) is linked to an N-terminus of the VL, wherein the MP comprises, from N-terminus to C-terminus, a masking unit (MU) and a linkage unit (LU) with or without cleavage sites,

optionally wherein the MU comprises a sequence selected from SEQ ID NOs:173-206;

further optionally wherein

the MP further comprises an N-terminal unit (NU) linked to the N-terminus of the MU, optionally wherein the N-terminal unit is about 1-10 amino acid residues long, further optionally wherein the N-terminal unit comprises E or EVGSY;

the LU comprises a cleavage site, optionally wherein the cleavage site is a protease cleavage site for a protease selected from urokinase-type plasminogen activator/uPA, matrix metalloproteinase-1/MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-14, Tobacco Etch Virus protease/TEV protease, plasmin, Thrombin, Factor X, PSA, PSMA, Cathepsin D, Cathepsin K, Cathepsin S, ADAM10, ADAM12, ADAMTS, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, and TACE;

further optionally wherein at least one masking peptide is linked to the B7-H3-, HER2-, or TROP2-binding portion or CD28-binding portion, optionally wherein the antigen-binding protein or fragment thereof comprises two masking peptides that bind respectively the B7-H3-, HER2-, or TROP2-binding portion and the CD28-binding portion.

14. The antigen-binding protein or fragment thereof of any one of claims 1-13, further comprising a conjugated therapeutic moiety, optionally wherein the therapeutic moiety is a radioactive moiety or a cytotoxic moiety.

15. A pharmaceutical composition comprising the antigen-binding protein or fragment thereof of any one of claims 1-14 and a pharmaceutically acceptable carrier.

16. One or more nucleic acid molecules encoding the antigen-binding protein or fragment thereof of any one of claims 1-14.

17. A host cell comprising the nucleic acid molecule(s) claim 16.

18. A method of producing an antigen-binding protein or antigen-binding fragment thereof, comprising:

culturing the host cell of claim 17 under conditions that allow expression of the antigen-binding protein or fragment thereof, optionally further comprising isolating the antigen-binding protein or fragment thereof from the culture.

19. Use of the antigen-binding protein or fragment thereof of any one of claims 1-14 or the pharmaceutical composition of claim 15 in the manufacture of a medicament for treating cancer in a patient in need thereof.

20. The use of claim 19, wherein the medicament is to be administered to the patent with another anti-cancer therapeutic, optionally wherein the additional anti-cancer therapeutic is

a bispecific antibody targeting CD3 and a tumor antigen, optionally wherein the tumor antigen is the same as or different from the TAA, further optionally wherein the TAA is B7-H3, HER2, or TROP2; or

an immune checkpoint inhibitor, optionally an anti-PD-1, anti-CTLA-4, or anti-PD-L1 antibody;

further optionally wherein the patient has a solid tumor or a hematological malignancy, optionally selected from breast cancer, gastric cancer, lung cancer, ovarian cancer, kidney cancer, pancreatic cancer, and colon cancer.