

CLAIMS

What is claimed is:

1. A chimeric antigen receptor (CAR) comprising an antigen-binding domain capable of binding Fibroblast Activation Protein (FAP), a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises:
 - a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence YTITSYSLH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence EINPANGDHNFSKFEIK (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence LDDSRFWYFDV (SEQ ID NO: 3); and
 - a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASSSVSYMY (SEQ ID NO: 4), a LCDR2 comprises the amino acid sequence LTSNLA (SEQ ID NO: 5), and LCDR3 comprises the amino acid sequence QQWSGYPPIT (SEQ ID NO: 6).
2. The CAR of claim 1, wherein the antigen-binding domain comprises:
 - (a) a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7; and/or
 - (b) a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9; and/or
 - (c) a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7; and a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9; and/or
 - (d) an antigen-binding domain selected from the group consisting of a full-length antibody or antigen-binding fragment thereof, a Fab, a single-chain variable fragment (scFv), or a single-domain antibody; and/or

- (e) a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 11 or 13 .
3. The CAR of any one of claims 1-2, wherein the CAR:
- (a) is capable of binding to fibroblast activation protein (FAP); and/or
 - (b) is capable of binding human FAP; and/or
 - (c) is capable of binding canine FAP; and/or
 - (d) is capable of binding murine FAP; and/or
 - (e) is capable of binding human, canine, and murine FAP; and/or
 - (f) further comprises a hinge domain, wherein the hinge domain comprises a hinge domain of CD8 alpha.
4. The CAR of any one of claims 1-3, wherein the transmembrane domain:
- (a) is selected from the group consisting of an artificial hydrophobic sequence, and a transmembrane domain of a type I transmembrane protein, an alpha, beta, or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, OX40 (CD134), 4-1BB (CD137), ICOS (CD278), and CD154, or a transmembrane domain derived from a killer immunoglobulin-like receptor (KIR); and/or
 - (b) comprises a transmembrane domain of CD8; and/or
 - (c) comprises a transmembrane domain of CD8 alpha; and/or
 - (d) comprises a transmembrane domain derived from a killer immunoglobulin-like receptor (KIR).
5. The CAR of any one of claims 1-4, wherein the intracellular domain:
- (a) comprises a costimulatory signaling domain and an intracellular signaling domain; and/or
 - (b) comprises one or more of a costimulatory domain of a protein selected from the group consisting of proteins in the TNFR superfamily, CD28, 4-1BB (CD137), OX40 (CD134), PD-1, CD7, LIGHT, CD83L, DAP10, DAP12, CD27, CD2, CD5, ICAM-1,

LFA-1, Lck, TNFR-I, TNFR-II, Fas, CD30, CD40, ICOS (CD278), NKG2C, and B7-H3 (CD276), or a variant thereof, or an intracellular domain derived from a killer

immunoglobulin-like receptor (KIR); and/or

(c) comprises a costimulatory domain of 4-1BB; and/or

(d) comprises a costimulatory domain of DAP12; and/or

(e) comprises a costimulatory domain of CD28; and/or

(f) comprises a costimulatory domain of 4-1BB and a costimulatory domain of CD28; and/or

(g) comprises a costimulatory domain of DAP12 and a costimulatory domain of CD28; and/or

(h) comprises an intracellular domain selected from the group consisting of cytoplasmic signaling domains of a human CD3 zeta chain (CD3 ζ), Fc γ RIII, Fc ϵ RI, a cytoplasmic tail of an Fc receptor, an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic receptor, TCR zeta, FcR gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d, or a variant thereof; and/or

(i) the intracellular signaling domain comprises an intracellular domain of CD3 ζ .

6. A chimeric antigen receptor (CAR) capable of binding Fibroblast Activation Protein (FAP), comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or 25 .
7. A nucleic acid comprising a polynucleotide sequence encoding a CAR of any one of claims 1-6.
8. A nucleic acid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR) capable of binding FAP, comprising an antigen-binding domain, a transmembrane domain, and an intracellular domain, wherein the antigen-binding domain comprises:
 - a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence YTITSYSLH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence

EINPANGDHNHFSEKFEIK (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence LDDSRFHWYFDV (SEQ ID NO: 3); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASSSVSYMY (SEQ ID NO: 4), LCDR2 comprises the amino acid sequence LTSNLA (SEQ ID NO: 5), and LCDR3 comprises the amino acid sequence QQWSGYPPIT (SEQ ID NO: 6).

9. The nucleic acid of claim 8, wherein the antigen-binding domain:

(a) comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8; and/or

(b) comprises a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10; and/or

(c) comprises a heavy chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8; and a light chain variable region encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10; and/or

(d) comprises a single-chain variable fragment (scFv) encoded by a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 12 or 14.

10. The nucleic acid of any one of claims 8-9, wherein:

(a) the transmembrane domain comprises a transmembrane domain of CD8 alpha; and/or

(b) the intracellular domain comprises a costimulatory signaling domain and an intracellular signaling domain; and/or

(c) the intracellular domain comprises a costimulatory signaling domain comprising a costimulatory domain of 4-1BB; and/or

- (d) the intracellular domain comprises a costimulatory domain of DAP12; and/or
 - (e) the intracellular domain comprises a costimulatory domain of CD28; and/or
 - (f) the intracellular domain comprises a costimulatory domain of 4-1BB and a costimulatory domain of CD28; and/or
 - (g) the intracellular domain comprises a costimulatory domain of DAP12 and a costimulatory domain of CD28; and/or
 - (h) the intracellular signaling domain comprises an intracellular domain of CD3ζ.
11. A nucleic acid comprising a polynucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 22 or 24.
 12. A vector comprising the nucleic acid of any one of claims 8-11.
 13. The vector of claim 12, wherein the vector is an expression vector and/or the vector is selected from the group consisting of a DNA vector, an RNA vector, a plasmid, a lentiviral vector, an adenoviral vector, an adeno-associated viral vector, and a retroviral vector.
 14. A modified immune cell or precursor cell thereof, comprising the CAR of any one of claims 1-7, the nucleic acid of any one of claims 8-11, or the vector of any one of claims 12-13.
 15. A modified immune cell or precursor cell thereof, comprising a chimeric antigen receptor (CAR) capable of binding fibroblast activation protein (FAP), wherein the CAR comprises:
 - a heavy chain variable region that comprises three heavy chain complementarity determining regions (HCDRs), wherein HCDR1 comprises the amino acid sequence YTITSYSLH (SEQ ID NO: 1), HCDR2 comprises the amino acid sequence EINPANGDHNFSKFEIK (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence LDDSRFHWYFDV (SEQ ID NO: 3); and

a light chain variable region that comprises three light chain complementarity determining regions (LCDRs), wherein LCDR1 comprises the amino acid sequence TASSSVSYMY (SEQ ID NO: 4), a LCDR2 comprises the amino acid sequence LTSNLA (SEQ ID NO: 5), and LCDR3 comprises the amino acid sequence QQWSGYPPIT (SEQ ID NO: 6).

16. The modified immune cell of claim 15, wherein the CAR comprises:
 - (a) a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7; and
a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9; and/or
 - (b) a single-chain variable fragment (scFv) comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 11 or 13; and/or
 - (c) an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or 25.
17. The modified cell of any one of claims 14-16, wherein the CAR:
 - (a) is capable of binding FAP; and/or
 - (b) is capable of binding human FAP; and/or
 - (c) is capable of binding canine FAP; and/or
 - (d) is capable of binding mouse FAP; and/or
 - (e) is capable of binding human, canine, and mouse FAP.
18. The modified cell of any one of claims 14-17, wherein the modified cell:
 - (a) is a modified T cell; and/or
 - (b) is a modified NK cell; and/or
 - (c) is an autologous cell; and/or
 - (d) is an autologous cell obtained from a human subject; and/or
 - (e) is an autologous cell obtained from a canine subject; and/or
 - (f) is an allogeneic cell.

19. A pharmaceutical composition comprising a therapeutically effective amount of the modified cell of any one of claims 14-18, and a pharmaceutically acceptable excipient.
20. A method of treating a disease in a subject in need thereof, comprising administering to the subject an effective amount of the modified cell of any one of claims 14-18, or the pharmaceutical composition of claim 19.
21. The method of claim 20, wherein
 - (a) the disease is selected from the group consisting of an immunological disease, a hematological disease, an autoimmune disease, fibrosis, and cancer; and/or
 - (b) the disease is cancer; and/or
 - (c) the disease is cardiac fibrosis; and/or
 - (d) the disease is cancer, and the cancer comprises a FAP-expressing cancer-associated cell.
22. The method of 21, wherein
 - (a) the FAP-expressing cancer-associated cell is a cancer-associated fibroblast (CAF); and/or
 - (b) the FAP-expressing cancer-associated cell is a FAP-expressing adipocyte; and/or
 - (c) the FAP-expressing cancer-associated cell is a tumor-associated macrophage (TAM); and/or
 - (d) the FAP-expressing cancer-associated cell is a tumor-associated neutrophil (TAN) ; and/or
 - (e) the FAP-expressing cancer-associated cell is a myeloid-derived suppressor cell (MDSC) ; and/or
 - (f) the FAP-expressing cancer-associated cell is a cancer-initiating cell.

23. A method of treating a cancer in a subject in need thereof, comprising administering to the subject an effective amount of a modified immune cell comprising a chimeric antigen receptor (CAR) capable of binding FAP, wherein the CAR comprises:
- a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7; and
 - a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9;
- wherein the cancer comprises fibroblast activation protein (FAP)-expressing cancer-associated cells.
24. The method of claim 23, wherein the CAR binds to FAP-expressing cancer-associated cells and does not bind to cells that do not express FAP.
25. A method of treating a cancer in a subject in need thereof, wherein the cancer is associated with fibroblast activation protein (FAP)-expressing cancer-associated fibroblasts, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding FAP, wherein the CAR comprises:
- a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7; and
 - a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9.
26. A method of treating a cancer in a subject in need thereof, wherein the cancer is associated with fibroblast activation protein (FAP)-expressing cancer-associated macrophages, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding FAP, wherein the CAR comprises:
- a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7; and

a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9.

27. A method of treating a cancer in a subject in need thereof, wherein the cancer is associated with fibroblast activation protein (FAP)-expressing cancer-associated neutrophils, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding FAP, wherein the CAR comprises:
 - a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7; and
 - a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9.
28. A method of treating a cancer in a subject in need thereof, wherein the cancer is associated with fibroblast activation protein (FAP)-expressing cancer-associated fibroblasts, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding FAP, wherein the CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or 25.
29. A method of treating a cancer in a subject in need thereof, wherein the cancer is associated with fibroblast activation protein (FAP)-expressing cancer-associated macrophages, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding FAP, wherein the CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or 25.
30. A method of treating a cancer in a subject in need thereof, wherein the cancer is associated with fibroblast activation protein (FAP)-expressing cancer-associated neutrophils, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding FAP, wherein the

CAR comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 23 or 25.

31. A method of treating a solid tumor in a subject in need thereof, wherein the tumor is associated with fibroblast activation protein (FAP)-expressing cancer-associated fibroblasts, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding FAP, wherein the CAR comprises:
 - a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7; and
 - a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9.
32. A method of treating a solid tumor in a subject in need thereof, wherein the tumor is associated with fibroblast activation protein (FAP)-expressing cancer-associated macrophages, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding FAP, wherein the CAR comprises:
 - a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7; and
 - a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9.
33. A method of treating a solid tumor in a subject in need thereof, wherein the tumor is associated with fibroblast activation protein (FAP)-expressing cancer-associated neutrophils, comprising administering to the subject an effective amount of a modified T cell comprising a chimeric antigen receptor (CAR) capable of binding FAP, wherein the CAR comprises:
 - a heavy chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7; and

a light chain variable region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9.

34. The method of any one of claims 20-33, further comprising administering an immune checkpoint inhibitor, tumor antigen vaccine, or neoplastic cell targeted therapies.
35. The method of any one of claims 20-34, wherein the subject is human.
36. The method of any one of claims 20-35, wherein the subject is a non-human animal.