

CLAIMS

What is claimed is:

1. An isolated binding polypeptide comprising an antigen-binding domain that specifically binds to an epitope of human and canine, and/or murine fibroblast activation protein (FAP).
2. The binding polypeptide of claim 1, wherein the antigen-binding domain comprises:
 - (a) 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
 - (b) 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).
3. The binding polypeptide of claim 1 or 2, wherein the binding polypeptide:
 - (a) binds a fibroblast activation protein (FAP); and/or
 - (b) comprises an antibody or an antigen-binding fragment thereof; and/or
 - (c) comprises a heavy chain variable region comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 96%, 97%, 98%, 99% identity to the amino acid sequence of the heavy chain variable region set forth in SEQ ID NO: 7; and/or
 - (d) comprises a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 7; and/or
 - (e) consists of a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 7; and/or
 - (f) comprises a light chain variable region comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to the amino acid sequence set forth in SEQ ID NO: 9; and/or

- (g) comprises a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 9; and/or
 - (h) consists of a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 9.
- 4. The binding polypeptide of any one of claims 1-3, wherein:
 - (a) the antigen-binding fragment is selected from the group consisting of a Fab, a single-chain variable fragment (scFv), or a single-domain antibody; and/or
 - (b) the antigen-binding fragment is selected from the group consisting of a Fab, a single-chain variable fragment (scFv), or a single-domain antibody and wherein the antibody is a full-length antibody; and/or
 - (c) the antigen-binding fragment is selected from the group consisting of a Fab, a single-chain variable fragment (scFv), or a single-domain antibody and wherein the antibody or antigen-binding fragment is a humanized antibody or an antigen-binding fragment thereof.
- 5. An isolated binding polypeptide comprising:
 - (a) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 7; and
 - (b) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9.
- 6. A single-chain variable fragment (scFv) comprising an antigen-binding domain that specifically binds to an epitope of human and canine, and/or murine fibroblast activation protein (FAP).
- 7. The scFv of claim 6, wherein the antigen-binding domain comprises:
 - (a) 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 EINPANGDHNHSEKFEIK (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence LDDSRFHWYFDV (SEQ ID NO: 3); and
 - (b) 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),

wherein the heavy chain variable region and the light chain variable region are separated by a linker.

8. A single-chain variable fragment (scFv) comprising:

(a) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 7; and

(b) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9,

wherein the heavy chain variable region and the light chain variable region are separated by a linker, and optionally wherein the linker comprises the amino acid sequence set forth in SEQ ID NO: 15.

9. A single chain variable fragment (scFv):

(a) comprising an amino acid sequence set forth in SEQ ID NOs: 11 or 13; or

(b) consisting of an amino acid sequence set forth in SEQ ID NOs: 11 or 13.

10. An isolated nucleic acid encoding the binding polypeptide or scFv of any one of claims 1-9.

11. An isolated nucleic acid encoding a binding polypeptide comprising an antigen-binding domain that specifically binds an epitope of human and canine, and/or murine Fibroblast Activation Protein (FAP).

12. The nucleic acid of claim 11, wherein the antigen binding domain comprises:

(a) 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

(b) 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).

13. The nucleic acid of claim 11 or 12, wherein:
 - (a) the binding polypeptide comprises an antibody or an antigen-binding fragment thereof, and optionally wherein the antibody is a full-length antibody; and/or
 - (b) the antigen-binding fragment is selected from the group consisting of a Fab, a single-chain variable fragment (scFv), or a single-domain antibody; and/or
 - (c) the antibody or antigen-binding fragment is a humanized antibody or a fragment thereof.
14. The nucleic acid of any one of 11-13, wherein:
 - (a) the heavy chain variable region is encoded by a nucleic acid comprising a polynucleotide sequence having at least 80%, 85%, 90%, 95%, 96%, 96%, 97%, 98%, 99% identity to SEQ ID NO: 8; and/or
 - (b) the heavy chain variable region is encoded by a nucleic acid comprising the polynucleotide sequence set forth in SEQ ID NO: 8; and/or
 - (c) the heavy chain variable region is encoded by a nucleic acid consisting of the polynucleotide sequence set forth in SEQ ID NO: 8; and/or
 - (d) the light chain variable region is encoded by a nucleic acid comprising a polynucleotide sequence having at least 80%, 85%, 90%, 95%, 96%, 96%, 97%, 98%, 99% identity to the amino acid sequence of the light chain variable region set forth in SEQ ID NO: 10; and/or
 - (e) the light chain variable region is encoded by a nucleic acid comprising the polynucleotide sequence set forth in SEQ ID NO: 10; and/or
 - (f) the light chain variable region is encoded by a nucleic acid consisting of a polynucleotide sequence set forth in SEQ ID NO: 10.
15. An isolated nucleic acid encoding a binding polypeptide comprising:
 - (a) a heavy chain variable region encoded by a nucleic acid sequence comprising the polynucleotide sequence set forth in SEQ ID NO: 8; and

- (b) a light chain variable region encoded by a nucleic acid sequence comprising the polynucleotide sequence set forth in SEQ ID NO: 10.
- 16. An isolated nucleic acid:
 - (a) encoding a single-chain variable fragment (scFv) comprising:
 - (i) 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 EINPANGDHNHSEKFEIK (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence LDDSRFHWYFDV (SEQ ID NO: 3); and
 - (ii) 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); or
 - (b) encoding a single-chain variable fragment (scFv) comprising:
 - (i) a heavy chain variable region comprising a nucleotide sequence set forth in SEQ ID NO: 8; and
 - (ii) a light chain variable region comprising a nucleotide sequence set forth in SEQ ID NO: 10,
 wherein the heavy chain variable region and the light chain variable region are separated by a linker, and optionally wherein the linker comprises the amino acid sequence set forth in SEQ ID NO: 15; and/or
 - (c) encoding a single-chain variable fragment (scFv) comprising a polynucleotide sequence set forth in SEQ ID NOs: 12 or 14; and/or
 - (d) encoding a single-chain variable fragment (scFv) consisting of a polynucleotide sequence set forth in SEQ ID NOs: 12 or 14.
- 17. A vector comprising the isolated nucleic acid of any one of claims 10-16.
- 18. The vector of claim 17, wherein:
 - (a) the vector is an expression vector; and/or
 - (b) 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.

19. A host cell:
 - (a) comprising the vector of claim 17 or 18; and/or
 - (b) wherein the host cell is of eukaryotic or prokaryotic origin; and/or
 - (c) wherein the host cell is of mammalian origin; and/or
 - (d) wherein the host cell is of bacterial origin.
20. A method of producing a binding polypeptide or scFv that binds to FAP, the method comprising culturing the host cell of claim 19.
21. A pharmaceutical composition comprising the binding polypeptide or scFv of any one of claims 1-9.
22. A method for identifying a subject suitable for an adoptive cell therapy directed to Fibroblast Activation Protein (FAP), wherein the method comprises:
 - (a) isolating a diseased tissue from the subject;
 - (b) contacting the isolated tissue with a binding polypeptide that specifically binds FAP; and
 - (c) detecting FAP-expressing cells in the isolated tissue, thereby identifying a suitable subject for the adoptive cell therapy.
23. The method of claim 22, wherein the binding polypeptide comprises:
 - (a) 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
 - (b) 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).

24. The method of claim 22 or 23, wherein:
- (a) the binding polypeptide comprises an antibody or an antigen-binding fragment thereof; and/or
 - (b) the antigen-binding fragment is selected from the group consisting of a Fab, a single-chain variable fragment (scFv), or a single-domain antibody, and optionally wherein the antibody is a full-length antibody; and/or
 - (c) the antibody or antigen-binding fragment is a humanized antibody or an antigen-binding fragment thereof; and/or
 - (d) the binding polypeptide is conjugated to a therapeutic molecule or a diagnostic molecule; and/or
 - (e) the binding polypeptide is conjugated to a diagnostic molecule, wherein the diagnostic molecule comprises a detectable label; and/or
 - (f) the binding polypeptide is conjugated to a diagnostic molecule, wherein the diagnostic molecule comprises a detectable label, and further wherein the detectable label is a radiolabel, a fluorophore, an enzyme, a hapten, biotin, or a chromophore.
25. Use of a modified immune cell comprising a chimeric antigen receptor (CAR) in the manufacture of a medicament for the treatment of cancer~~The method of any one of claims 22-24~~, wherein the medicament is for adoptive cell therapy and wherein the subject is administered the adoptive cell therapy modified immune cell is to be administered to the subject after the subject is identified as a suitable subject according to the method of any one of claims 22-24.
26. The ~~method-use~~ of claim 25, wherein:
- ~~(a) — the adoptive cell therapy comprises a modified immune cell comprising a chimeric antigen receptor (CAR); and/or~~
 - ~~(ba) the adoptive cell therapy comprises a modified immune cell comprising a chimeric antigen receptor (CAR) and wherein~~ the immune cells are T lymphocytes; and/or
 - ~~(cb) the adoptive cell therapy comprises a modified immune cell comprising a chimeric antigen receptor (CAR) and wherein~~ the immune cells are NK cells; and/or

(dc) ~~the adoptive cell therapy comprises a modified immune cell comprising a chimeric antigen receptor (CAR), wherein~~ the CAR specifically binds to FAP.

27. The method of any one of claims 22-24 or the use of claim 25 or claim 26, wherein the binding polypeptide:

(a) comprises a heavy chain variable region comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 96%, 97%, 98%, 99% identity to the amino acid sequence of the heavy chain variable region set forth in SEQ ID NO: 7; and/or

(b) comprises a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 7; and/or

(c) consists a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 7; and/or

(d) comprises a light chain variable region comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to the amino acid sequence set forth in SEQ ID NO: 9; and/or

(e) comprises a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 9; and/or

(f) consists of a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 9.

28. The method of any one of claims 22-24 or the use of claim 25 or claim 26, wherein the antigen-binding domain comprises:

(a) 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

(b) 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).

2829. Use of an isolated binding polypeptide in the manufacture of a medicament for the treatment of~~A method for treating~~ a cancer in a subject in need thereof, wherein the comprising administering to the subject an isolated binding polypeptide comprising~~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

(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 wherein an effective amount of the isolated binding polypeptide is to be administered to the subject.

30. Use of an isolated binding polypeptide in the manufacture of a medicament for the treatment of a cancer in a subject in need thereof, wherein the antigen-binding domain comprises:

(a) 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

(b) 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), and wherein an effective amount of the isolated binding polypeptide is to be administered to the subject.

2931. The ~~method-use~~ of claim 2829 or claim 30, wherein:

(a) the cancer is associated with fibroblast activation protein (FAP)-expressing cells; and/or

(b) the FAP-expressing cell is a cancer-associated cell; and/or

(c) the FAP-expressing cell is a cancer-associated cell, wherein the cancer-associated cell is a cancer-associated fibroblast (CAF); and/or

- (d) the FAP-expressing cell is a cancer-associated cell, wherein the FAP-expressing cancer-associated cell is a FAP-expressing adipocyte; and/or
- (e) the FAP-expressing cell is a cancer-associated cell, wherein the FAP-expressing cancer-associated cell is a tumor-associated macrophage (TAM); and/or
- (f) the FAP-expressing cell is a cancer-associated cell, wherein the FAP-expressing cancer-associated cell is a tumor-associated neutrophil (TAN); and/or
- (g) the FAP-expressing cell is a cancer-associated cell, wherein the FAP-expressing cancer-associated cell is a myeloid-derived suppressor cell (MDSC); and/or
- (h) the FAP-expressing cell is a cancer-associated cell, wherein the FAP-expressing cancer-associated cell is a cancer-initiating cell.

3032. The ~~method-use~~ of any one of claims ~~28 or 29~~ to 31, wherein:

- (a) the binding polypeptide specifically binds to fibroblast activation protein (FAP); and/or
- (b) the binding polypeptide comprises an antibody or an antigen-binding fragment thereof; and/or
- (c) the antigen-binding fragment is selected from the group consisting of a Fab, a single-chain variable fragment (scFv), or a single-domain antibody, and optionally wherein the antibody is a full-length antibody; and/or
- (d) the antibody or antigen-binding fragment is a humanized antibody or an antigen-binding fragment thereof.

33. An isolated binding polypeptide for use in therapy, wherein the isolated binding polypeptide 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
- (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 wherein an effective amount of the isolated binding polypeptide is to be administered to the subject.

34. An isolated binding polypeptide for use in therapy, wherein the antigen-binding domain comprises:

(a) 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 EINPANGDHNHSEKFEIK (SEQ ID NO: 2), and HCDR3 comprises the amino acid sequence LDDSRFWYFDV (SEQ ID NO: 3); and

(b) 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), and wherein an effective amount of the isolated binding polypeptide is to be administered to the subject.

3435. An adoptive cell therapy comprising a modified T cell comprising a chimeric antigen receptor (CAR) that specifically binds to Fibroblast Activation Protein (FAP) for use in therapy ~~A method for treating cancer in a subject in need thereof, wherein the adoptive cell therapy is to be administered to a subject in need thereof after the subject is identified as a suitable subject in a method~~ comprising:

(a) identifying the subject as a suitable subject, wherein the identifying comprises:

(i) isolating a diseased tissue from the subject;

(ii) contacting the isolated tissue with a binding polypeptide that specifically binds FAP; and

(iii) detecting FAP-expressing cells in the isolated tissue; ~~and~~

~~(b) administering to the suitable subject adoptive cell therapy comprising a modified T cell comprising a chimeric antigen receptor (CAR) that specifically binds to Fibroblast Activation Protein (FAP).~~

36. Use of an adoptive cell therapy comprising a modified T cell comprising a chimeric antigen receptor (CAR) that specifically binds to Fibroblast Activation Protein (FAP) in the manufacture of a medicament for the treatment of cancer in a subject in need thereof, wherein the adoptive cell therapy is to be administered to the subject after the subject is identified as a suitable subject in a method comprising:

- _____ (a) identifying the subject as a suitable subject, wherein the identifying comprises:
- _____ (i) isolating a diseased tissue from the subject;
- _____ (ii) contacting the isolated tissue with a binding polypeptide that specifically binds FAP; and
- _____ (iii) detecting FAP-expressing cells in the isolated tissue.