

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 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).
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 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),

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 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), 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 EINPANGDHNHFEKFEIK (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 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).
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. The method of any one of claims 22-24, wherein the subject is administered the adoptive cell therapy after the subject is identified as a suitable subject.
26. The method of claim 25, wherein:
- (a) the adoptive cell therapy comprises a modified immune cell comprising a chimeric antigen receptor (CAR); and/or
 - (b) 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
 - (c) 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
 - (d) 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 22-26, 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. A method for treating a cancer in a subject in need thereof, comprising administering to the subject an isolated binding polypeptide comprising:
- (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.
29. The method of claim 28, 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.
30. The method of claim 28 or 29 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.

31. A method for treating cancer in a subject in need thereof, 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).