(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
05 June 2025 (05.06.2025)

WIPO | PCT

(10) International Publication Number
WO 2025/113643 A1

(51) International Patent Classification:
A61P 35/00 (2006.01) *C07K 16/40* (2006.01)
C07K 14/52 (2006.01)

(21) International Application Number:
PCT/CN2024/135694

(22) International Filing Date:
29 November 2024 (29.11.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/CN2023/135902
01 December 2023 (01.12.2023) CN
PCT/CN2024/131641
12 November 2024 (12.11.2024) CN

(71) Applicant: **GILEAD SCIENCES INC.** [US/US]; 333 Lakeside Drive, Foster City, 94404 California, United States of America (US).

(72) Inventors: **ZHAI, Qianting**; Floor #5, Building #1, 315 Guiping Road, Xuhui District, Shanghai 200233 (CN). **FEI, Mingjian**; Floor #5, Building #1, 315 Guiping Road, Xuhui District, Shanghai 200233 (CN). **CAO, Jun**; Floor #5, Building #1, 315 Guiping Road, Xuhui District, Shanghai 200233 (CN). **SUN, Runzi**; Floor #5, Building #1, 315 Guiping Road, Xuhui District, Shanghai 200233 (CN). **LI, Lixue**; Floor #5, Building #1, 315 Guiping Road, Xuhui District, Shanghai 200233 (CN). **ZHU, Hui**; Floor #5, Building #1, 315 Guiping Road, Xuhui District, Shanghai 200233 (CN).

(74) Agent: **LIU, SHEN & ASSOCIATES**; 10th Floor, Building 1, 10 Caihefang Road, Haidian District, Beijing 100080 (CN).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: ANTI-FAP-LIGHT FUSION PROTEIN AND USE THEREOF

(57) Abstract: The present disclosure relates to an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, a fusion protein, pharmaceutical compositions, and the uses of such antibodies in preventing, diagnosing, or treating tumor diseases.

WO 2025/113643 A1

ANTI-FAP-LIGHT FUSION PROTEIN AND USE THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of priority to International Patent Application No. PCT/CN2023/135902, filed on December 1, 2023, and to International Patent Application No. PCT/CN2024/131641, filed on November 12, 2024, the entire contents of each of which are incorporated herein by reference.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing, which has been submitted via Patent Center. The Sequence Listing titled G24H06545W-PCT-SL.xml, which was created on November 27, 2024 and is 113,094 bytes in size, is hereby incorporated by refence in its entirety.

TECHNICAL FIELD

[0003] The present disclosure relates to the anti-FAP antibody, fusion protein and uses thereof.

BACKGROUND

[0004] The statements in this section merely provide background information related to the present disclosure and do not necessarily constitute prior art.

[0005] TNFSF14 (TNF superfamily member 14), also known as LIGHT, is a member of the TNF superfamily and is homologous to Lymphotoxin. LIGHT is an inducible inflammatory cytokine that binds to TNFRSF14 (TNF receptor superfamily member 14, also known as HVEM), LTβR (lymphotoxin beta receptor), and the decoy receptor DcR3. Additionally, it competes with Herpes Simplex Virus glycoprotein D for the binding to the HVEM receptor. HVEM is expressed on the surface of various immune cells, such as T cells, B cells, NK cells, and dendritic cells. LIGHT binds to HVEM and subsequently stimulates T cells and promotes inflammation. Another receptor, LTβR is

expressed on the surface of epithelial, stromal, immature DCs, and other myeloid cells but not on lymphocytes. Activation of LTβR signaling is critical for the recruitment and organization of immune cells, leading to the development of lymphoid organs and tertiary lymphoid structures (TLS). TLS consists of B and T cell zones, dendritic cells, and other immune cells, and their presence in tumor tissues has been associated with improved clinical outcomes in a variety of cancer indications, including breast, lung, and colon cancer, suggesting that TLS may play an important role in the immune response against cancer.

SUMMARY

[0006] Provided herein is an isolated anti-FAP antibody or a FAP antigen-binding fragment thereof, comprising:

a)

i) a heavy chain variable region (VH) comprising

(1) a heavy chain complementarity determining region 1 (HCDR1) comprising the amino acid sequence of IYGVN (SEQ ID NO: 26),

(2) heavy chain complementarity determining region 2 (HCDR2) comprising the amino acid sequence of AIWSGGRKDYX_2LSLKS (SEQ ID NO: 27), wherein X_2 is N or S, and

(3) heavy chain complementarity determining region 3 (HCDR3) comprising the amino acid sequence of SQDMPGYFDY (SEQ ID NO: 28), and

ii) a light chain variable region (VL) comprising

(1) a light chain complementarity determining region 1 (LCDR1) comprising the amino acid sequence of KTNQNVDYX_1GNTFMH (SEQ ID NO: 23), wherein X_1 is N or S,

(2) a light chain complementarity determining region 2 (LCDR2) comprising the amino acid sequence of LASNLAS (SEQ ID NO: 24), and

(3) a light chain complementarity determining region 3 (LCDR3) comprising the amino acid sequence of QQSRNLPYT (SEQ ID NO: 25);

or

b)

i) a VH region comprising

(1) a HCDR1 comprising the amino acid sequence of TAGMSVG (SEQ ID NO: 32),

(2) a HCDR2 comprising the amino acid sequence of DIWWDDKKHYNPSLKD (SEQ ID NO: 33),

(3) a HCDR3 comprising the amino acid sequence of DMIFNFYFDV (SEQ ID NO: 34), and

ii) a VL region comprising

(1) LCDR1 comprising the amino acid sequence of SASSRVGYMH (SEQ ID NO: 29),

(2) LCDR2 comprising the amino acid sequence of DTSKLAS (SEQ ID NO: 30), and

(3) LCDR3 comprising the amino acid sequence of FQGSGYPFT (SEQ ID NO: 31).

[0007] In some embodiments, the isolated anti-FAP antibody or a FAP antigen-binding fragment thereof comprises:

a) a VH region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 35, and/or a VL region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 36;

b) a VH region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 37, and/or a VL region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 38; or

c) a VH region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 41, and/or a VL region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 42.

[0008] In some embodiments, the isolated anti-FAP antibody or a FAP antigen-binding fragment thereof comprises:

a) a heavy chain (HC) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 1, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, and 16, and/or

b) a light chain (LC) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 2, 3, and 7.

[0009] In some embodiments, the isolated anti-FAP antibody or a FAP antigen-binding fragment thereof comprises:

a) HC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 1, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 2; or

b) a HC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 4, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

c) a first heavy chain (HC1) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 5, a second heavy chain (HC2) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 6, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

d) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 8, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 9, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 7;

e) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 10, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 11, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

f) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 13, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 12, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

g) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 14, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 13, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3; or

h) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 15, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 16, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3.

[0010] In some embodiments, the VH region and/or VL region further comprises human framework sequences. In some embodiments, the VH region and/or VL region further comprises a framework 1 (FR1), a framework 2 (FR2), a framework 3 (FR3) and/or a framework 4 (FR4) sequence. In some embodiments, isolated anti-FAP antibody or a FAP antigen-binding fragment provided herein comprises a fragment crystallizable (Fc) region derived from immunoglobulin. In some embodiments, the Fc fragment is derived from IgG1, IgG2, IgG3, or IgG4, optionally the Fc fragment is derived from IgG4. In some embodiments, the Fc fragment comprises mutation S228P or LALAPG mutation.

[0011] In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a humanized, human or chimeric antibody. In some embodiments, the anti-FAP antibody or FAP antigen-binding fragment cross-reacts with human, cynomolgus and mouse FAP. In some embodiments, the antibody or fragment thereof is a Fab, Fab', F(ab')2, Fv, scFv, (scFv)2, single chain antibody molecule, dual variable region antibody, single variable region antibody, linear antibody, V region, or a multispecific antibody formed from antibody fragments. In some embodiments, the isolated anti-FAP antibody or a FAP antigen-binding fragment thereof binds to FAP with dissociation constant (KD) no more than 20 nM, 15 nM, 10 nM or 5 nM.

[0012] Also provided herein is a bispecific antibody or antigen-binding fragment thereof comprising a FAP antigen-binding moiety and a second binding moiety, wherein the FAP antigen-binding moiety comprises:

a)

i) a heavy chain variable region (VH) comprising

(1) a heavy chain complementarity determining region 1 (HCDR1) comprising the amino acid sequence of IYGVN (SEQ ID NO: 26),

(2) heavy chain complementarity determining region 2 (HCDR2) comprising the amino acid sequence of AIWSGGRKDYX_2LSLKS (SEQ ID NO: 27), wherein X_2 is N or S, and

(3) heavy chain complementarity determining region 3 (HCDR3) comprising the amino acid sequence of SQDMPGYFDY (SEQ ID NO: 28), and

ii) a light chain variable region (VL) comprising

(1) a light chain complementarity determining region 1 (LCDR1) comprising the amino acid sequence of KTNQNVDYX_1GNTFMH (SEQ ID NO: 23), wherein X_1 is N or S,

(2) a light chain complementarity determining region 2 (LCDR2) comprising the amino acid sequence of LASNLAS (SEQ ID NO: 24), and

(3) a light chain complementarity determining region 3 (LCDR3) comprising the amino acid sequence of QQSRNLPYT (SEQ ID NO: 25);

or

b)

i) a VH region comprising

(1) a HCDR1 comprising the amino acid sequence of TAGMSVG (SEQ ID NO: 32),

(2) a HCDR2 comprising the amino acid sequence of DIWWDDKKHYNPSLKD (SEQ ID NO: 33),

(3) a HCDR3 comprising the amino acid sequence of DMIFNFYFDV (SEQ ID NO: 34), and

ii) a VL region comprising

(1) LCDR1 comprising the amino acid sequence of SASSRVGYMH (SEQ ID NO: 29),

(2) LCDR2 comprising the amino acid sequence of DTSKLAS (SEQ ID NO: 30), and

(3) LCDR3 comprising the amino acid sequence of FQGSGYPFT (SEQ ID NO: 31).

[0013] In some embodiments, the FAP antigen-binding moiety comprises:

a) a VH region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 35, and/or a VL region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 36;

b) a VH region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 37, and/or a VL region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 38; or

c) a VH region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 41, and/or a VL region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 42.

[0014] In some embodiments, the FAP antigen-binding moiety comprises:

c) a heavy chain (HC) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 1, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, and 16, and/or

d) a light chain (LC) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 2, 3, and 7.

[0015] In some embodiments, the FAP antigen-binding moiety comprises:

a) HC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 1, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,

or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 2; or

b) a HC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 4, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

c) a first heavy chain (HC1) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 5, a second heavy chain (HC2) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 6, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

d) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 8, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 9, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 7;

e) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 10, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 11, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

f) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 13, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 12, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

g) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 14, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 13, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3; or

h) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 15, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 16, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3.

[0016] In some embodiments, the bispecific antibody or antigen-binding fragment thereof comprises a Fc fragment derived from immunoglobulin at a N-terminus of the FAP antigen-binding moiety. In some embodiments, the Fc fragment is derived from IgG1, IgG2, IgG3, or IgG4, optionally the Fc fragment is derived from IgG4. In some embodiments, the Fc fragment comprises mutation S228P or LALAPG mutation. In some embodiments, the bispecific antibody or antigen-binding fragment thereof comprises an Fc fragment and wherein the Fc fragment comprises one or more modifications

selected from the group consisting of knobs-into-holes, DDKK, electrostatic steering of CH3, DuoBody, SEEDbodies, cFAE, XmAb, Azymetric, and BEAT®, optionally, the Fc fragment comprises modifications knobs-into-holes and/or DDKK.

[0017] In some embodiments, the second binding moiety is at a C-terminus of the FAP antigen-binding moiety. In some embodiments, the second binding moiety is operably linked to the C-terminus of the Fc fragment. In some embodiments, the second binding moiety links to the Fc fragment by a linker. In some embodiments, the second binding moiety binds and/or activates a second target. In some embodiments, the second binding moiety binds and/or activates a tumor associated cell receptor. In some embodiments, the second binding moiety comprises a first portion and a second portion, wherein each portion comprises one or more units. In some embodiments, the one or more units comprise a first unit, a second unit, and/or a third unit. In some embodiments, each of the one or more units are identical. In some embodiments, the one or more units are not identical. In some embodiments, the second binding moiety is a tumor necrosis factor, interleukin, lymphokine, interferon, colony stimulating factor, chemokine or growth factor. In some embodiments, each of the one or more units independently is a tumor necrosis factor, interleukin, lymphokine, interferon, colony stimulating factor, chemokine or growth factor. In some embodiments, each of the one or more units independently comprise an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to any one of the amino acid sequences set forth in TABLE 10.

[0018] In some embodiments, the second binding moiety comprises a second antigen-binding moiety or a cytokine moiety. In some embodiments, the second binding moiety comprises a cytokine moiety. In some embodiments, the cytokine moiety comprises a first cytokine moiety and a second cytokine moiety. In some embodiments, the first cytokine moiety comprises a first cytokine unit. In some embodiments, the first cytokine unit comprises a LIGHT unit or a lymphotoxin-β unit. In some embodiments, the second cytokine moiety comprises a second cytokine unit and a third cytokine unit.

In some embodiments, the second cytokine unit comprises a LIGHT unit, a lymphotoxin-α unit, or a lymphotoxin-β unit. In some embodiments, the third cytokine unit comprises a LIGHT unit, a lymphotoxin-α unit, or a lymphotoxin-β unit. In some embodiments, the first cytokine moiety comprises a first LIGHT unit, the second cytokine unit comprises a second LIGHT unit, and the third cytokine unit comprises a third LIGHT unit. In some embodiments, the first, second, and/or third LIGHT unit each independently comprises an amino acid sequence set forth in SEQ ID NO: 17 or 18. In some embodiments, the first cytokine moiety comprises a lymphotoxin-β unit, the second cytokine unit comprises a lymphotoxin-α unit, and the third cytokine unit comprises a lymphotoxin-β unit. In some embodiments, the lymphotoxin-β unit comprises an amino acid sequence set forth in SEQ ID NO: 39, the lymphotoxin-α unit comprises an amino acid sequence set forth in SEQ ID NO: 40. In some embodiments, the bispecific antibody or antigen-binding fragment thereof binds to FAP with dissociation constant (KD) no more than 20 nM, 15 nM, 10 nM or 5 nM. In some embodiments, the bispecific antibody or antigen-binding fragment thereof binds to LTßR with dissociation constant (KD) no more than 20 nM, 15 nM, 10 nM or 5 nM. In some embodiments, the bispecific antibody or antigen-binding fragment thereof barely binds to human or cynomolgus HVEM. In some embodiments, the second binding moiety in the bispecific antibody or antigen-binding fragment thereof is capable of reduced binding affinity to DcR3. In some embodiments, the bispecific antibody or antigen-binding fragment thereof specifically binds to human FAP, and/or does not bind to DPPIV. In some embodiments, the second binding moiety binds and/or activates LTβR, HER2, PDL-1, PD-1, EGFR, VEGFR, VEGF, CCR8, OX-40, 418B, Angiopoietin-2, IL-4Ra, BCMA, Blys, BTNO2, C5, CD122, CD13, CD133, CD137, CD138, CD16a, CD19, CD20, CD22, CD27, CD28, CD3, CD30, CD33, CD38, CD40, CD47, CD-8, CEA, CGPR/CGRPR, CSPGs, CTLA4, CTLA-4, DLL-4, EpCAM, factor IXa, factor X, GITR, GP130, Her3, HSG, ICOS, IGFl, IGFl/2, IGF-lR, IGF2, IGFR, IL-1, IL- 12, IL-12p40, IL-13, IL-l 7A, IL-1~, IL-23, IL-5, IL-6, IL-6R, Lag-3, LAG3, MAG, Met, NgR, NogoA, OMGp,

OX40, PDGFR, PSMA, RGMA, RGMB, SARS-CoV-2, Te38, TIM-3, TNF, TNFa, TROP-2, TWEAK, or TRAIL. In some embodiments, the second binding moiety is a second antigen-binding moiety. In some embodiments, the second antigen-binding moiety comprises anti-LTβR binding moiety, anti-HER2 binding moiety, anti-PDL-1 binding moiety, anti-PD-1 binding moiety, anti-EGFR binding moiety, anti-VEGFR binding moiety, anti-VEGF binding moiety, anti-CCR8 binding moiety, anti-OX-40 binding moiety, anti-418B binding moiety, anti-Angiopoietin-2 binding moiety, anti-IL-4Ra binding moiety, anti-BCMA binding moiety, anti-Blys binding moiety, anti-BTNO2 binding moiety, anti-C5 binding moiety, anti-CD122 binding moiety, anti-CD13 binding moiety, anti-CD133 binding moiety, anti-CD137 binding moiety, anti-CD138 binding moiety, anti-CD16a binding moiety, anti-CD19 binding moiety, anti-CD20 binding moiety, anti-CD22 binding moiety, anti-CD27 binding moiety, anti-CD28 binding moiety, anti-CD3 binding moiety, anti-CD30 binding moiety, anti-CD33 binding moiety, anti-CD38 binding moiety, anti-CD40 binding moiety, anti-CD47 binding moiety, anti-CD-8 binding moiety, anti-CEA binding moiety, anti-CGPR/CGRPR binding moiety, anti-CSPGs binding moiety, anti-CTLA4 binding moiety, anti-CTLA-4 binding moiety, anti-DLL-4 binding moiety, anti-EpCAM binding moiety, anti-factor IXa binding moiety, anti-factor X binding moiety, anti-GITR binding moiety, anti-GP130 binding moiety, anti-Her3 binding moiety, anti-HSG binding moiety, anti-ICOS binding moiety, anti-IGFl binding moiety, anti-IGFl/2 binding moiety, anti-IGF-lR binding moiety, anti-IGF2 binding moiety, anti-IGFR binding moiety, anti-IL-1 binding moiety, anti-IL-12 binding moiety, anti-IL-12p40 binding moiety, anti-IL-13 binding moiety, anti-IL-l 7A binding moiety, anti-IL-1~ binding moiety, anti-IL-23 binding moiety, anti-IL-5 binding moiety, anti-IL-6 binding moiety, anti-IL-6R binding moiety, anti-Lag-3 binding moiety, anti-LAG3 binding moiety, anti-MAG binding moiety, anti-Met binding moiety, anti-NgR binding moiety, anti-NogoA binding moiety, anti-OMGp binding moiety, anti-OX40 binding moiety, anti-PDGFR binding moiety, anti-PSMA binding moiety, anti-RGMA binding moiety, anti-RGMB binding moiety, anti-SARS-CoV-2

binding moiety, anti-Te38 binding moiety, anti-TIM-3 binding moiety, anti-TNF binding moiety, anti-TNFa binding moiety, anti-TROP-2 binding moiety, anti-TWEAK binding moiety, or anti-TRAIL binding moiety.

[0019] In some embodiments, the bispecific antibody or antigen-binding fragment thereof exhibits a reduced binding affinity to HVEM relative to a comparator bispecific antibody or antigen-binding fragment thereof. In some embodiments, the bispecific antibody or antigen-binding fragment thereof exhibits a reduced binding affinity to DcR3 relative to a comparator bispecific antibody or antigen-binding fragment thereof. In some embodiments, the bispecific antibody or antigen-binding fragment thereof exhibits a reduced binding affinity to DPPIV relative to a comparator bispecific antibody or antigen-binding fragment thereof. In some embodiments, upon contacting the bispecific antibody or antigen-binding fragment thereof to a FAP expressing cell, and the bispecific antibody or antigen-binding fragment thereof induces: the formation of secondary lymphoid organs (SLOs), the formation of tertiary lymphoid structures (TLSs), the stimulation of immune cells, the apoptosis of tumor cells, the treatment of cancer, or any combination thereof.

[0020] Also provided herein is a fusion protein, wherein the fusion protein is comprised in any one of the bispecific antibodies or antigen-binding fragments thereof described herein.

[0021] In some embodiments, the fusion protein comprises a heavy chain 1 (HC1) region comprising a VH, a heavy chain constant domain 1 (CH1), and a Fc fragment comprising a heavy chain constant domain 2 (CH2) and a heavy chain constant domain 3 (CH3). In some embodiments, the HC1 region comprises an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 1, 4, 5, 8, 10, 13, 14, and 15. In some embodiments, the HC1 region comprises an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown

in SEQ ID NO: 5, 8, 10, 13, 14, and 15. In some embodiments, the Fc fragment of the HC1 region comprises one or more units of the second binding moiety fused to a C-terminus of the Fc fragment. In some embodiments, the Fc fragment of the HC1 region comprises a first unit of the second binding moiety fused to the C-terminus of the HC1 Fc fragment. In some embodiments, the first unit of the second binding moiety is fused to the Fc unit by a first linker. In some embodiments, the second binding moiety is a cytokine moiety, and wherein the cytokine moiety comprises a first cytokine unit. In some embodiments, the first cytokine unit comprises a LIGHT unit or a lymphotoxin β unit. In some embodiments, the first unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to any one of the amino acid sequences set forth in TABLE 10. In some embodiments, the unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to SEQ ID NO: 17 or SEQ ID NO: 39.

[0022] In some embodiments, the fusion protein comprises a heavy chain 2 (HC2) region comprising a VH, a CH1, and a Fc fragment comprising a CH1 and a CH3. In some embodiments, the HC2 region comprises an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 1, 4, 6, 9, 11, 12, 13, and 16. In some embodiments, the HC2 region comprises an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 6, 9, 11, 12, 13, and 16. In some embodiments, the Fc fragment of the HC2 region comprises one or more units of the second binding moiety fused to a C-terminus of the HC2 Fc fragment. In some embodiments, the Fc fragment of the HC2 region comprises a second unit and a third unit of the second binding moiety. In some embodiments, the second unit of the second binding moiety is fused to the Fc fragment of the HC2 region. In some embodiments, the third unit of the

second binding moiety is fused to the second unit of the second binding moiety. In some embodiments, the second unit of the second binding moiety is fused to the Fc fragment of the HC2 region by a second linker and the third unit is fused to the second unit of the second binding moiety by a third linker. In some embodiments, the second unit and third unit of the second binding moiety unit are tandem linked. In some embodiments, the second binding moiety is a cytokine moiety, and wherein the cytokine moiety comprises a second cytokine unit and a third cytokine unit. In some embodiments, the second cytokine unit comprises a LIGHT unit, a lymphotoxin α unit, or a lymphotoxin β unit. In some embodiments, the third cytokine unit comprises a LIGHT unit, a lymphotoxin α unit, or a lymphotoxin β unit. In some embodiments, the second cytokine unit comprises a LIGHT unit, and the third cytokine unit comprises a LIGHT unit. In some embodiments, the second cytokine unit comprises a lymphotoxin α unit, and the third cytokine unit comprises a lymphotoxin β unit. In some embodiments, the second unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to any one of the amino acid sequences set forth in TABLE 10. In some embodiments, the second unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to SEQ ID NO: 17 or SEQ ID NO: 40. In some embodiments, the third unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to any one of the amino acid sequences set forth in TABLE 10. In some embodiments, the third unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to SEQ ID NO: 17 or SEQ ID NO: 39.

[0023] In some embodiments, the fusion protein comprises one or more linkers. In some embodiments, each of the one or more linker independently comprises a VH-CHl linker ASTKGPSVFPLAPS; VL-CL linker RTVAAPSVFIFPPS (SEQ ID NO: 91); CH2-CH3 linker ISKAKGQPREPQ (SEQ ID NO: 92); IgM tail linker KSTGKPTLYNVSLVMSDTAGTCY (SEQ ID

NO: 93); GGGGSGGGGSGGGGSGGGGT (SEQ ID NO: 94); G; or (GGGGS)n (SEQ ID NO: 95), wherein n=l, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the HC1 region comprises one or more heterodimerization modification. In some embodiments, the one or more heterodimerization modification is a knob modification or a hole modification. In some embodiments, the one or more heterodimerization modification is a hole modification. In some embodiments, the HC2 region comprises one or more heterodimerization modification. In some embodiments, the one or more heterodimerization modification is a knob modification or a hole modification. In some embodiments, the one or more heterodimerization modification is a knob modification.

[0024] Also provided herein is an isolated polynucleotide comprising one or more nucleotide sequences encoding: any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment thereof described herein, any one of the bispecific antibodies described herein, or any one of the fusion proteins described herein.

[0025] Also provided herein is a construct comprising any one of the polynucleotides described herein.

[0026] Also provided herein is an antibody expressing system, comprising any one of the constructs described herein or a vector comprising any one of the polynucleotides described herein. In some embodiments, the antibody expressing system is a cell expression system.

[0027] Also provided herein is a method for producing the anti-FAP antibody or FAP antigen-binding fragment thereof, bispecific antibody or antigen-binding fragment thereof or fusion protein, the method comprising: under conditions suitable for expressing the anti-FAP antibody or FAP antigen-binding fragment thereof, bispecific antibody or antigen-binding fragment thereof or fusion protein using the any one of the antibody expressing systems described herein.

[0028] Also provided herein is a pharmaceutical composition, wherein the pharmaceutical composition comprises: any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment

thereof described herein, any one of the bispecific antibodies described herein, or any one of the fusion proteins described herein; and a pharmaceutically acceptable carrier.

[0029] Also provided herein is a kit, wherein the kit comprises: any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment thereof described herein, any one of the bispecific antibodies described herein, or any one of the fusion proteins described herein, comprising any one of the polynucleotides described herein; or any one of the constructs described herein.

[0030] Also provided herein is a use of the anti-FAP antibody or FAP antigen-binding fragment thereof, bispecific antibody or antigen-binding fragment thereof, or fusion protein in the manufacture of a therapeutic agent for preventing, diagnosing, or treating a disease, disorder, or condition, wherein: the anti-FAP antibody or FAP antigen-binding fragment thereof is any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment thereof described herein, the bispecific antibody or antigen-binding fragment thereof is any one of the any one of the bispecific antibodies or antigen-binding fragment thereof described herein, or the fusion protein is any one of the fusion proteins described herein. In some embodiments, the disease, disorder, or condition comprises tumor diseases. In some embodiments, at least a tumor cell expresses FAP. In some embodiments, the tumor diseases are solid tumors. In some embodiments, the tumor diseases comprises gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, skin cancer, bone cancer, multiple myeloma, glioma, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer and lymphoma.

[0031] Also provided herein is a method for treating a subject in need, comprising administrating to the subject a therapeutically effective amount of: any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment thereof described herein, any one of the bispecific antibodies described

herein, or any one of the fusion proteins described herein, or any one of the pharmaceutical compositions described herein.

[0032] Also provided herein is a method for decreasing the rate of tumor growth or the number of tumor cells, comprising contacting a tumor cell with an effective amount of: any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment thereof described herein, any one of the bispecific antibodies described herein, or any one of the fusion proteins described herein, or any one of the pharmaceutical compositions described herein.

[0033] Also provided herein is a method of killing a tumor cell, comprising contacting a tumor cell with an effective amount of: any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment thereof described herein, any one of the bispecific antibodies described herein, or any one of the fusion proteins described herein, or any one of the pharmaceutical compositions described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] The following is a brief description of the drawings, which are presented for the purposes of illustrating the exemplary embodiments disclosed herein and not for the purposes of limiting the same.

[0035] Figures 1A-1C show the cell binding curves of 9E3 chimera and ABC1931, as detected by FACS.

[0036] Figure 2 shows the schematic structure of anti-FAP-LIGHT fusion protein ABC1930.

[0037] Figure 3A-3B show biolayer interferometry (BLI) sensorgrams of immobilized FAP or LTßR ECD proteins with serial dilutions of ABC1930. Figure 3C illustrates BLI sensorgrams of immobilized HVEM ECD proteins with serial dilutions of ABC1947 or ABC1930.

[0038] Figure 4 shows ABC1930 or ABC1947 binding to coated DcR3 protein, as detected by ELISA.

[0039] Figure 5 shows ABC1930 binding to coated human FAP or DPPIV protein, as detected by ELISA.

[0040] Figure 6 shows the schematic formats A and D of the fusion protein generated in Example 2.1. The SDS-PAGE results indicate that format D with LIGHTmu1 or LIGHTwt is correctly assembled, whereas the chain encoding three tandem hmLIGHT units in format A exhibits low expression, resulting in poorly assembly of both constructs.

[0041] Figure 7A shows the yield comparison of ABC2097, ABC1930, and ABC1947 after protein A purification. Figure 7B displays the SDS-PAGE gel image of these proteins.

[0042] Figures 8A-8C show the CCL2 production induced by ABC1931, ABC1773 and ABC1930 from both 3T3 and 3T3-FAP cell lines, respectively.

[0043] Figures 9A-9C show the trans-activation of the endogenous LTβR pathway in 3T3 cells by CT26-FAP cells using CCL2 as a surrogate readout, following treatment of ABC1931, ABC1773 or ABC1930, respectively.

[0044] Figure 10A shows the administration schedule for *in vivo* evaluation in the KPC mouse model. Figures 10B-10C show the tumor volume-time curves in the KPC tumor model.

[0045] Figure 11A shows the administration schedule for *in vivo* evaluation in the LL2 tumor model. Figures 11B-11C show the tumor volume-time curves in the LL2 tumor model.

[0046] Figure 12A shows the administration schedule for *in vivo* evaluation in the LL2-OVA tumor model. Figures 12B-12C show the tumor volume-time curves in the LL2-OVA tumor model.

[0047] Figure 13A shows the administration schedule for *in vivo* evaluation in the subcutaneous EMT6 tumor model. Figures 13B-13C show the tumor volume-time curves in the EMT6 tumor model.

DETAILED DESCRIPTION

[0048] The present disclosure is explained in greater detail below. This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure which do not depart from the instant invention. Hence, the following description is intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

[0049] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains. Although any methods and materials similar or equivalent to those described herein may be used in the practice for testing of the present disclosure, the preferred materials and methods are described herein. In describing and claiming the present disclosure, the following terminology will be used.

[0050] As used herein, the term “about” when preceding a numerical value indicates the value plus or minus a range of 20%,15%, 10%, or 5%.

[0051] The term “antibody” (used interchangeably in the plural) is an immunoglobulin molecule capable of specifically binding to a target, such as carbohydrate, polynucleotides, lipids, polypeptides, etc., through at least one antigen recognition site located in the variable region of the immunoglobulin molecule. As used herein, the term “antibody” includes not only intact (*i.e.*, full-length) polyclonal or monoclonal antibodies, but also antigen-binding fragments thereof (*e.g.*, Fab, Fab', F(ab')2, Fv), single-chain (scFv), mutants thereof, fusion proteins comprising antibody moieties, humanized antibodies, chimeric antibodies, diabodies, nanobodies, linear antibodies, single chain antibodies, multispecific

antibodies (eg, bispecific antibodies), and any other modified constructs of immunoglobulin molecules comprising antigen recognition sites with the desired specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. Antibodies include antibodies of any class, such as IgD, IgE, IgG, IgA, or IgM (or subclasses thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of their heavy chain constant domains, immunoglobulins can be divided into different classes. There are five main classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these can be further divided into subclasses (isotypes), such as IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0052] In the present disclosure, "amino acid" refers to an organic compound containing amine (-NH_2) and carboxyl (-COOH) functional groups, along with a side chain specific to each amino acid. the names of amino acids are represented as standard single-letter or three-letter codes, which are shown as below:

Name of Amino Acid	Three-letter Code	Single-letter Code
Alanine	Ala	A
Cysteine	Cys	C
Aspartic Acid	Asp	D
Glutamic Acid	Glu	E
Phenylalanine	Phe	F
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Lysine	Lys	K
Leucine	Leu	L
Methionine	Met	M
Asparagine	Asn	N
Proline	Pro	P
Glutamine	Gln	Q
Arginine	Arg	R
Serine	Ser	S
Threonine	Thr	T

Valine	Val	V
Tryptophan	Trp	W
Tyrosine	Tyr	Y

[0053] The term “bispecific antibody” or “bispecific molecule” as used herein, refers to an antibody or a molecule that displays a double binding specificity and affinity for two particular epitopes or a composition of antibodies in which all antibodies display a double binding specificity and affinity for two particular epitopes.

[0054] The term “comparator binding agent” as used herein refers to an appropriate corresponding control or competitive binding agent useful to provide a reference level in activities and/or assays described herein. For example, a comparator binding agent of an anti-FAP antibody described herein can be a distinct antibody that binds to FAP.

[0055] An “Fc region” (fragment crystallizable region) or “Fc domain” or “Fc fragment” refers to the C-terminal region of the heavy chain of an antibody that mediates the binding of the immunoglobulin to host tissues or factors, including binding to Fc receptors located on various cells of the immune system (*e.g.*, effector cells) or to the first component (Clq) of the classical complement system. Thus, an Fc region comprises the constant region of an antibody excluding the first constant region immunoglobulin domain (*e.g.*, CH1 or CL). In IgG, IgA and IgD antibody isotypes, the Fc region comprises two identical protein fragments, derived from the second (CH2) and third (CH3) constant domains of the antibody's two heavy chains; IgM and IgE Fc regions comprise three heavy chain constant domains (CH domains 2-4) in each polypeptide chain. For IgG, the Fc region comprises immunoglobulin domains Cγ2 and Cγ3 and the hinge between Cγ1 and Cγ2. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position C226 or P230 (or amino acid between these two amino acids) to the carboxy terminus of the heavy chain, wherein the numbering is

according to the EU index as in Kabat. The CH2 domain of a human IgG Fc region extends from about amino acid 231 to about amino acid 340, whereas the CH3 domain is positioned on C-terminal side of a CH2 domain in an Fc region, *i.e.*, it extends from about amino acid 341 to about amino acid 447 of an IgG. As used herein, the Fc region may be a native sequence Fc, including any allotypic variant, or a variant Fc (*e.g.*, a non-naturally occurring Fc). Fc may also refer to this region in isolation or in the context of an Fc-comprising protein polypeptide.

[0056] The term "fusion" when used with respect to amino acid sequences of a peptide, polypeptide or protein refers to a combination of two or more amino acid sequences from two or more different sources into a single amino acid sequence by, for example, chemical bonding or recombinant means. A fusion amino acid sequence may be produced by genetic recombination of two encoding polynucleotide sequences, and can be expressed by a method of introducing a construct containing the recombinant polynucleotides into a host cell. Accordingly, a "fusion protein" as used herein can refer to a hybrid polypeptide which comprises protein domains from at least two different proteins. While a "fusion protein" and "immunocytokine" are used interchangeably herein, it is understood that not all fusion proteins provided herein are immunocytokines. In some instances, a fusion protein can also refer to a complex of two or more peptides, polypeptides or proteins (*e.g.*, a binding agent), wherein at least one of those peptides, polypeptides or proteins has an amino acid sequence that is a fusion of two or more amino acid sequences from two or more different sources into a single amino acid sequence.

[0057] The term "pharmaceutically acceptable carrier" refers to an ingredient other than the active ingredient in a pharmaceutical composition that is not toxic to the subject.

[0058] The term "treating/preventing" (and its grammatical variants) refers to an attempt to alter the natural progress of a disease in a treated individual and can be prevention or clinical intervention implemented during the course of clinical pathology. The desired effect of treatment includes, but is

not limited to, preventing the occurrence or recurrence of diseases, alleviating the symptoms, relieving any direct or indirect pathological consequences of the disease, preventing the metastasis, slowing the rate of disease progression, improving or lessening the disease states, and relieving or improving the prognosis. In some embodiments, the antibody of the present disclosure is useful for delaying the development of a disease or delaying the progression of a disorder.

[0059] The percent (%) sequence identity is defined as the percentage of amino acid residues or nucleic acids in a candidate sequence that are identical to the amino acid residues or nucleic acids in a reference polypeptide sequence or polynucleotide sequence, respectively, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity when referring to a polypeptide sequence. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. For example, the identity can be determined using the BLAST program of the NCBI database.

[0060] Other features and advantages of the present disclosure will be apparent from the following detailed description and figures, and from the claims.

FAP Binding Agent

[0061] The present disclosure provides fibroblast activation protein (FAP) binding agents (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody thereof, or a fusion protein thereof) that bind to FAP, and in some embodiments, specifically to a FAP polypeptide, FAP polypeptide fragment, a FAP peptide or a FAP epitope comprised in FAP. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), such as a human FAP binding agent, can bind to FAP expressed on the surface of a mammalian (*e.g.*, human) cell, including a FAP expressing cell, such as a FAP

expressing tumor cell. In some embodiments, described herein is a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) that binds to FAP, such as human FAP or portions thereof. In some embodiments, FAP is a human FAP. In some embodiments, a FAP binding agent is a human FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof).

[0062] In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) binds a FAP epitope of a FAP expressing cell (*e.g.*, a target FAP epitope). In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) selectively binds a FAP epitope of a FAP expressing cell over an epitope of any one or combinations of competitive molecules, such as a pro-tumor cell surface molecule (*e.g.*, DPPIV).

[0063] FAP as described herein can be human FAP having an amino acid sequence as set forth in **SEQ ID NO: 43** (UniProt Identifier P51685). However, FAP from any species corresponding to the species of any subject in compositions and methods described herein. For example, FAP as described herein can be a Cynomolgus FAP (Macaca mulatta) having an amino acid sequence as set forth in **SEQ ID NO: 44** (NCBI XP_005573377.1); or a mouse FAP (Mus musculus) having an amino acid sequence as set forth in **SEQ ID NO: 45** (UniProt Identifier Uniprot P97321).

[0064] Specifically, FAP encompassed by the present disclosure has been shown to be highly expressed on the cell surface of cancer-associated stroma cells, and on fibroblastic reticular cells in secondary lymphoid organs, but has otherwise very limited expression in normal tissues. Therefore, targeting FAP specifically targets tumor stromal cells in a tumor microenvironment, such as the tumor endothelium and cancer associated fibroblasts.

[0065] Accordingly, a FAP-related disease, disorder, or condition as encompassed by the present disclosure refers to refer to any disease, disorder or condition that is characterized by aberrant,

upregulated, or selective expression of FAP, and/or alternatively any disease, disorder, or condition in which it is desirable to deplete the expression of FAP and/or FAP expressing cells. Exemplary FAP-related diseases, disorders, or conditions are further described herein.

[0066] In some embodiments, FAP binding agents providing herein bind, or specifically bind to a target FAP polypeptide, FAP polypeptide fragment, a FAP peptide or a FAP epitope comprised in FAP. In some embodiments, FAP binding agents providing herein specifically bind to a target FAP epitope. The target epitope may be continuous or non-continuous, and may be determined by a method known to a person of ordinary skill, including flow cytometry of bound antibody to peptides, hydrogen-deuterium exchange, alanine scanning, and/or x-ray crystallography. The target epitope can comprise or consist of amino acid residues that are determined by FAP binding agent binding to FAP as described herein. The target epitope can be an epitope comprising or consisting of amino acid residues that are determined by epitope binning. The target epitope can be an epitope comprising or consisting of amino acid residues that are determined by FAP binding agent binding to FAP peptide-nanobody complexes. The target epitope can be an epitope comprising or consisting of amino acid residues that are determined by screening FAP binding agent binding to FAP by phage display. The target epitope can be an epitope comprising or consisting of amino acid residues that are determined by in silico screening FAP binding agent binding to FAP by computer learning models. The target epitope can be an epitope comprising or consisting of amino acid residues that are determined by FAP binding agent binding to FAP in a competitive assay or a non-competitive assay.

[0067] In some embodiments, the target epitope comprises an amino acid sequence that is about 60% about 65% about 70% about 75% about 80% about 85% about 90% about 95%, about 97%, about 98%, about 99% identical or is identical to any one of the sequences set forth in **TABLE 5**. In some embodiments, the target epitope comprises an amino acid sequence that is 1 amino acid, 2 amino acids, 3 amino acids, 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino

acids, 10 amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14 amino acids, 15 amino acids, 16 amino acids, 17 amino acids, 18 amino acids, 19 amino acids, or 20 amino acids which are contiguous or non-contiguous and selected from any one of the sequences set forth in **TABLE 5**.

[0068] In some embodiments, FAP binding agents provided herein can have a strong binding affinity and/or specificity for FAP. In some embodiments, FAP binding agents provided herein can have a strong binding affinity and/or specificity for FAP over other competitive molecules such as dipeptidyl peptidase 4 (DPPIV). In some embodiments, FAP binding agents provided herein can have a strong binding affinity and/or specificity for FAP expressing cells. In some embodiments, expression of FAP is on tumor cells, and/or FAP expressing cells are FAP+ stromal cells.

[0069] FAP binding agents comprise an anti-FAP antibody, FAP antigen-binding fragment, or a FAP antigen binding moiety thereof, bispecific antibodies comprising said anti-FAP antibody or FAP antigen-binding moiety, and/or fusion proteins thereof. In some embodiments, a FAP binding agent provided herein is capable of binding two or more targets. In some embodiments, a FAP binding agent provided herein is capable of binding a FAP antigen and binding at least one additional target. In some embodiments, a FAP binding agent provided herein is capable of binding a FAP antigen and cross-linking and/or activating an additional target expressed on a tumor associated cell. In some embodiments, binding of the second target expressed on a tumor associated cell by a FAP binding agent cross-links and/or activates the additional target, thereby effecting anti-tumor activity. Examples of FAP binding agents and activities thereof are described in further detail herein.

[0070] In some embodiments, the FAP binding agents (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) described herein compete for the binding to FAP, such as human FAP, with a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) that comprises a VH region, VL region, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 of any one of

the antibodies described herein, such as an amino acid sequence of a VH region, VL region, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 depicted in **TABLES 6-8**. Accordingly, in some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) described herein competes for the binding to FAP, such as human FAP, with a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) that comprises one, two, and/or three VH CDRs and/or one, two, and/or three VL CDRs from: (a) the antibody designated 9E3 CHIMERA (ABC1139); (b) the antibody designated ABC1931; (c) the antibody designated ABC1930; (d) the antibody designated ABC1773; (e) the antibody designated ABC1947; (f) the antibody designated ABC2066; (g) the antibody designated ABC2067; or (h) the antibody designated ABC2097 as shown in **TABLE 6 or TABLE 7**. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) described herein competes for the binding to FAP, such as human FAP, with a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) that comprises one, two, and/or three VH CDRs and one, two, and/or three VL CDRs from: (a) the antibody designated 9E3 CHIMERA (ABC1139); (b) the antibody designated ABC1931 ; (c) the antibody designated ABC1930; (d) the antibody designated ABC1773; (e) the antibody designated ABC1947; (f) the antibody designated ABC2066; (g) the antibody designated ABC2067; or (h) the antibody designated ABC2097 as shown in **TABLE 6 or TABLE 7**. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) described herein competes for the binding to FAP, such as human FAP, with a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) that comprises a VH region and VL region from: (a) the antibody designated 9E3 CHIMERA (ABC1139); (b) the antibody designated ABC1931 ;

(c) the antibody designated ABC1930; (d) the antibody designated ABC1773; (e) the antibody designated ABC1947; (f) the antibody designated ABC2066; (g) the antibody designated ABC2067; or (h) the antibody designated ABC2097 as shown in **TABLE 8**.

[0071] In some embodiments, the FAP binding agents (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) described herein comprise a VH region, VL region, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 of any one of the antibodies described herein, such as an amino acid sequence of a VH region, VL region, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 depicted in **TABLES 6-8**. Accordingly, in some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) described herein comprises one, two, and/or three heavy chain CDRs and/or one, two, and/or three light chain CDRs from: (a) the antibody designated 9E3 CHIMERA (ABC1139); (b) the antibody designated ABC1931 ; (c) the antibody designated ABC1930; (d) the antibody designated ABC1773; (e) the antibody designated ABC1947; (f) the antibody designated ABC2066; (g) the antibody designated ABC2067; or (h) the antibody designated ABC2097, as shown in **TABLES 6-7**. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) described herein comprises one, two, and/or three heavy chain CDRs and one, two, and/or three light chain CDRs from: (a) the antibody designated 9E3 CHIMERA (ABC1139); (b) the antibody designated ABC1931 ; (c) the antibody designated ABC1930; (d) the antibody designated ABC1773; (e) the antibody designated ABC1947; (f) the antibody designated ABC2066; (g) the antibody designated ABC2067; or (h) the antibody designated ABC2097, as shown in **TABLES 6-7**.

[0072] In some embodiments, a FAP binding agent comprises a heavy chain variable region and a light chain variable region. In some embodiments, a FAP binding agent comprises at least one heavy chain comprising a heavy chain variable region and at least a portion of a heavy chain constant region,

and at least one light chain comprising a light chain variable region and at least a portion of a light chain constant region. In some embodiments, a FAP binding agent comprises two heavy chains, wherein each heavy chain comprises a heavy chain variable region and at least a portion of a heavy chain constant region, and two light chains, wherein each light chain comprises a light chain variable region and at least a portion of a light chain constant region. As used herein, a single-chain Fv (scFv), or any other binding agent (*e.g.*, antibody) that comprises, for example, a single polypeptide chain comprising all six CDRs (three heavy chain CDRs and three light chain CDRs) is considered to have a heavy chain and a light chain. In some embodiments, the heavy chain is the region of the FAP binding agent that comprises the three heavy chain CDRs. In some embodiments, the light chain is the region of the FAP binding agent that comprises the three light chain CDRs.

[0073] Accordingly, in some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) comprises a VH region, which comprises VH CDR1, VH CDR2, and/or VH CDR3, and a VL region, which comprises VL CDR1, VL CDR2, and/or VL CDR3, of any one of the binding agents described herein (see, *e.g.*, **TABLE 6-7**). Accordingly, in some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) described herein comprises one, two, and/or three heavy chain CDRs (*e.g.*, VH CDR1, VH CDR2, and VH CDR3) and/or one, two, and/or three light chain CDRs (*e.g.*, VL CDR1, VL CDR2, and VL CDR3) from **TABLE 6**. In some embodiments, a FAP binding agent described herein comprises one, two, and/or three heavy chain CDRs (*e.g.*, VH CDR1, VH CDR2, and VH CDR3) and/or one, two, and/or three light chain CDRs (*e.g.*, VL CDR1, VL CDR2, and VL CDR3) from **TABLE 7**. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) described herein is multispecific (*e.g.*, bispecific) and comprises a first binding moiety that comprises one, two, and/or three heavy chain CDRs (*e.g.*, VH CDR1, VH

CDR2, and VH CDR3) and/or one, two, and/or three light chain CDRs (*e.g.*, VL CDR1, VL CDR2, and VL CDR3) from **TABLE 6 or TABLE 7** and a second binding moiety that comprises one, two, and/or three heavy chain CDRs (*e.g.*, VH CDR1, VH CDR2, and VH CDR3) and/or one, two, and/or three light chain CDRs (*e.g.*, VL CDR1, VL CDR2, and VL CDR3) from a binding agent that binds to a second target antigen that is not FAP.

[0074] In some embodiments, the CDRs disclosed herein include consensus sequences derived from groups of related antibodies (see, **TABLE 6**). A consensus sequence refers to amino acid sequences having conserved amino acids common among a number of sequences and variable amino acids that vary within a given amino acid sequences. The CDR consensus sequences provided include CDRs corresponding to CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and/or CDRL3. Consensus sequences of CDRs of FAP binding agents (*e.g.*, 9E3 CHIMERA (ABC1139) to ABC2097) are shown in **TABLE 6**. Unless explicitly indicated otherwise, consensus CDRs in the present disclosure have been identified by the Kabat numbering scheme.

[0075] Accordingly, in some embodiments, a FAP binding agent comprises at least one, two, three, four, five, or six CDRs selected from (a) HCDR1 comprising the amino acid sequence of IYGVN (SEQ ID NO: 26), TAGMSVG (SEQ ID NO: 32), GFSLSIY (SEQ ID NO: 52), VSGFSLSIYG (SEQ ID NO: 53), GFSLSIYG (SEQ ID NO: 54), GFSLSTAGM (SEQ ID NO: 55), FSGFSLSTAGMS (SEQ ID NO: 56), or GFSLSTAGMS (SEQ ID NO: 57); (b) HCDR2 comprising the amino acid sequence of DIWWDDKKHYNPSLKD (SEQ ID NO: 33), SGG (SEQ ID NO: 58), IWSGGRKDYNLSLKSR (SEQ ID NO: 59), IWSGGRK (SEQ ID NO: 60), AIWSGGRKDYNLSLKS (SEQ ID NO: 61), IWSGGRKDYSLSLKSR (SEQ ID NO: 62), or AIWSGGRKDYSLSLKS (SEQ ID NO: 63); (c) HCDR3 comprising the amino acid sequence of SQDMPGYFDY (SEQ ID NO: 28), DMIFNFYFDV (SEQ ID NO: 34), QDMPGYFD (SEQ ID NO: 67), SQDMPGYFD (SEQ ID NO: 68), ARSQDMPGYFDY (SEQ ID NO: 69), MIFNFYFD (SEQ ID

NO: 70), DMIFNFYFD (SEQ ID NO: 71), or ARDMIFNFYFDV (SEQ ID NO: 72); (d) LCDR1 comprising the amino acid sequence of SASSRVGYMH (SEQ ID NO: 29), NQNVDYNGNTF (SEQ ID NO: 73), TNQNVDYNGNTF (SEQ ID NO: 74), QNVDYNGNTF (SEQ ID NO: 75), KTNQNVDYNGNTFMH (SEQ ID NO: 76), TNQNVDYSGNTF (SEQ ID NO: 77), or NQNVDYSGNTF (SEQ ID NO: 78); (e) LCDR2 comprising the amino acid sequence of LASNLAS (SEQ ID NO: 24), DTSKLAS (SEQ ID NO: 30), LAS (SEQ ID NO: 83), LASNLASGIPDR (SEQ ID NO: 84), LASNLASGIPER (SEQ ID NO: 85), or DTS (SEQ ID NO: 86); and (f) LCDR3 comprising the amino acid sequence of QQSRNLPYT (SEQ ID NO: 25), FQGSGYPFT (SEQ ID NO: 31), SRNLPY (SEQ ID NO: 88), GSGYPF (SEQ ID NO: 89).

[0076] In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) described herein comprises one, two, and/or three heavy chain CDRs and/or one, two, and/or three light chain CDRs from: (a) the antibody designated 9E3 CHIMERA (ABC1139); (b) the antibody designated ABC1931 ; (c) the antibody designated ABC1930; (d) the antibody designated ABC1773; (e) the antibody designated ABC1947; (f) the antibody designated ABC2066; (g) the antibody designated ABC2067; or (h) the antibody designated ABC2097, as shown in **TABLE 3**.

[0077] In some embodiments, any of the six CDRs provided herein can be combined as subparts with any of the other CDRs provided herein, for a total of six CDRs in a construct. Thus, in some embodiments, two CDRs from a first antibody (for example, HCDR1 and HCDR2) can be combined with four CDRs from a second antibody (HCDR3, LCDR1, LCDR2, and LCDR3). In some embodiments, two or fewer amino acid residues in one or more of the CDRs can be modified to obtain a variant or derivative thereof. In some embodiments, two or fewer amino acid residues can be modified in 1, 2, 3, 4, 5, or 6 of the CDRs.

[0078] In some embodiments, the CDRs of FAP binding agents including a human FAP binding agent, can be determined according to any suitable numbering system. Examples of numbering systems used herein are Kabat, IMGT, Honegger, and Chothia, however, a person of ordinary skill in the art would readily understand that the position of one or more CDRs along the VH (*e.g.*, CDR1, CDR2, or CDR3) and/or VL (*e.g.*, CDR1, CDR2, or CDR3) region of a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), including a human FAP binding agent, described herein may vary by one, two, three, four, five, or six amino acid positions so long as binding to FAP (*e.g.*, human FAP) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). For example, in some embodiments, the position defining a CDR as described in **TABLE 3** may vary by shifting the N-terminal and/or C-terminal boundary of the CDR by one, two, three, four, five, or six amino acids, relative to the current CDR position, so long as binding to FAP (*e.g.*, human FAP) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In other embodiments, the length of one or more CDRs along the VH (*e.g.*, CDR1, CDR2, or CDR3) and/or VL (*e.g.*, CDR1, CDR2, or CDR3) region of a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), including a human FAP binding agent, described herein may vary (*e.g.*, be shorter or longer) by one, two, three, four, five, or more amino acids, so long as binding to FAP (*e.g.*, human FAP) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). For example, in some embodiments, a VH and/or VL CDR1, CDR2, and/or CDR3 described herein may be one, two, three, four, five or more amino acids shorter than one or more of the CDRs described by any one of the sequences set forth in **TABLES 6 and 7**, so long as binding to FAP (*e.g.*, human FAP) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least

90%, at least 95%). In other embodiments, a VH and/or VL CDR1, CDR2, and/or CDR3 described herein may be one, two, three, four, five or more amino acids longer than one or more of the CDRs described by any one of the sequences set forth in **TABLES 6 and 7**, so long as binding to FAP (*e.g.*, human FAP) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In other embodiments, the amino terminus of a VH and/or VL CDR1, CDR2, and/or CDR3 described herein may be extended by one, two, three, four, five or more amino acids compared to one or more of the CDRs described by any one of the sequences set forth in **TABLES 6 and 7**, so long as binding to FAP (*e.g.*, human FAP) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In other embodiments, the carboxy terminus of a VH and/or VL CDR1, CDR2, and/or CDR3 described herein may be extended by one, two, three, four, five or more amino acids compared to one or more of the CDRs described by any one of the sequences set forth in **TABLES 6 and 7**, so long as binding to FAP (*e.g.*, human FAP) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In other embodiments, the amino terminus of a VH and/or VL CDR1, CDR2, and/or CDR3 described herein may be shortened by one, two, three, four, five or more amino acids compared to one or more of the CDRs described by any one of the sequences set forth in **TABLES 6 and 7**, so long as binding to FAP (*e.g.*, human FAP) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In some embodiments, the carboxy terminus of a VH and/or VL CDR1, CDR2, and/or CDR3 described herein may be shortened by one, two, three, four, five or more amino acids compared to one or more of the CDRs described by any one of the sequences set forth in **TABLES 6 and 7**, so long as binding to FAP (*e.g.*, human FAP) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). Any method known in the art can be used to ascertain whether

binding to FAP (*e.g.*, human FAP) is maintained, for example, the binding assays and conditions described in the "Examples" section described herein. For example, **EXAMPLE 2** described herein describes an assay for measuring binding to FAP (*e.g.*, human FAP).

[0079] In some embodiments, FAP binding agents (*e.g.*, antibodies, such as bispecific antibodies), including human FAP binding agents, described herein comprise a VH region or VH domain. In other embodiments, FAP binding agents (*e.g.*, antibodies, such as bispecific antibodies), including human FAP binding agents, described herein comprise a VL region or VL domain. In some embodiments, FAP binding agents (*e.g.*, antibodies, such as bispecific antibodies), including human FAP binding agents, described herein have a combination of (i) a VH domain or VH region; and/or (ii) a VL domain or VL region.

[0080] In some embodiments, a FAP binding agent comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 35, 37, or 41. In some embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains amino acid modifications, such as substitutions (for example, conservative substitutions), insertions, or deletions relative to the reference sequence, but a FAP binding agent comprising that sequence retains the ability to bind to FAP. In some embodiments, a total of 1 to 10 amino acids (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) have been modified, *e.g.*, substituted, inserted and/or deleted in SEQ ID NO: 35, 37, or 41. In some embodiments, modifications, *e.g.*, substitutions, insertions, or deletions, occur in regions outside the CDRs (that is, in the FRs). Optionally, the FAP binding agent comprises the VH sequence in SEQ ID NO: 35, 37, or 41, including post-translational modifications of that sequence. In some embodiments, such a FAP binding agent comprises (a) HCDR1 comprising the amino acid sequence of IYGVN (SEQ ID NO: 26), TAGMSVG (SEQ ID NO: 32), GFSLSIY (SEQ ID NO: 52), VSGFSLSIYG (SEQ ID NO: 53), GFSLSIYG (SEQ ID NO: 54), GFSLSTAGM (SEQ

ID NO: 55), FSGFSLSTAGMS (SEQ ID NO: 56), or GFSLSTAGMS (SEQ ID NO: 57); (b) HCDR2 comprising the amino acid sequence of DIWWDDKKHYNPSLKD (SEQ ID NO: 33), SGG (SEQ ID NO: 58), IWSGGRKDYNLSLKSR (SEQ ID NO: 59), IWSGGRK (SEQ ID NO: 60), AIWSGGRKDYNLSLKS (SEQ ID NO: 61), IWSGGRKDYSLSLKSR (SEQ ID NO: 62), or AIWSGGRKDYSLSLKS (SEQ ID NO: 63); (c) HCDR3 comprising the amino acid sequence of SQDMPGYFDY (SEQ ID NO: 28), DMIFNFYFDV (SEQ ID NO: 34), QDMPGYFD (SEQ ID NO: 67), SQDMPGYFD (SEQ ID NO: 68), ARSQDMPGYFDY (SEQ ID NO: 69), MIFNFYFD (SEQ ID NO: 70), DMIFNFYFD (SEQ ID NO: 71), or ARDMIFNFYFDV (SEQ ID NO: 72);.

[0081] In some embodiments, a FAP binding agent is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 36, 38, or 42. In some embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains amino acid modifications, such as substitutions (for example, conservative substitutions), insertions, or deletions relative to the reference sequence, but a FAP binding agent comprising that sequence retains the ability to bind to FAP. In some embodiments, a total of 1 to 10 amino acids (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) have been modified, *e.g.*, substituted, inserted and/or deleted in SEQ ID NO: 36, 38, or 42. In some embodiments, the modifications, *e.g.*, substitutions, insertions, or deletions, occur in regions outside the CDRs (that is, in the FRs). Optionally, the FAP binding agent comprises the VL sequence in SEQ ID NO: 36, 38, or 42, including post-translational modifications of that sequence. In some embodiments, such a FAP binding agent comprises (d) LCDR1 comprising the amino acid sequence of SASSRVGYMH (SEQ ID NO: 29), NQNVDYNGNTF (SEQ ID NO: 73), TNQNVDYNGNTF (SEQ ID NO: 74), QNVDYNGNTF (SEQ ID NO: 75), KTNQNVDYNGNTFMH (SEQ ID NO: 76), TNQNVDYSGNTF (SEQ ID NO: 77), or NQNVDYSGNTF (SEQ ID NO: 78); (e) LCDR2 comprising the amino acid sequence of LASNLAS

(SEQ ID NO: 24), DTSKLAS (SEQ ID NO: 30), LAS (SEQ ID NO: 83), LASNLASGIPDR (SEQ ID NO: 84), LASNLASGIPER (SEQ ID NO: 85), or DTS (SEQ ID NO: 86); and (f) LCDR3 comprising the amino acid sequence of QQSRNLPYT (SEQ ID NO: 25), FQGSGYPFT (SEQ ID NO: 31), SRNLPY (SEQ ID NO: 88), GSGYPF (SEQ ID NO: 89).

[0082] In some embodiments, a VH as provided herein can be combined as a subpart of a FAP binding agent with any of one VL provided herein, for a total of one VH and one VL in a subpart of a construct, and 2 VHs and 2 VLs in a construct. Thus, in some embodiments, a VH from a first antibody can be combined with a VL from a second antibody.

[0083] In some embodiments, a FAP binding agent comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 35, 37, or 41 and a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 36, 38, or 42. In some embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains modifications, such as substitutions (for example, conservative substitutions), insertions, or deletions relative to the reference sequence, and a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains modifications, such as substitutions (for example, conservative substitutions), insertions, or deletions relative to the reference sequence, but a FAP binding agent comprising that sequence retains the ability to bind to FAP. In some embodiments, a total of 1 to 10 amino acids (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) have been modified, *e.g.*, substituted, inserted and/or deleted in SEQ ID NO: 35, 37, or 41; and a total of 1 to 10 amino acids (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) have been modified, *e.g.*, substituted, inserted and/or deleted in SEQ ID NO: 36, 38, or 42. In some embodiments, modifications, *e.g.*, substitutions, insertions, or deletions occur in regions outside the CDRs (that is, in the FRs). In some embodiments,

such a FAP binding agent comprises (a) HCDR1 comprising the amino acid sequence of IYGVN (SEQ ID NO: 26), TAGMSVG (SEQ ID NO: 32), GFSLSIY (SEQ ID NO: 52), VSGFSLSIYG (SEQ ID NO: 53), GFSLSIYG (SEQ ID NO: 54), GFSLSTAGM (SEQ ID NO: 55), FSGFSLSTAGMS (SEQ ID NO: 56), or GFSLSTAGMS (SEQ ID NO: 57); (b) HCDR2 comprising the amino acid sequence of DIWWDDKKHYNPSLKD (SEQ ID NO: 33), SGG (SEQ ID NO: 58), IWSGGRKDYNLSLKSR (SEQ ID NO: 59), IWSGGRK (SEQ ID NO: 60), AIWSGGRKDYNLSLKS (SEQ ID NO: 61), IWSGGRKDYSLSLKSR (SEQ ID NO: 62), or AIWSGGRKDYSLSLKS (SEQ ID NO: 63); (c) HCDR3 comprising the amino acid sequence of SQDMPGYFDY (SEQ ID NO: 28), DMIFNFYFDV (SEQ ID NO: 34), QDMPGYFD (SEQ ID NO: 67), SQDMPGYFD (SEQ ID NO: 68), ARSQDMPGYFDY (SEQ ID NO: 69), MIFNFYFD (SEQ ID NO: 70), DMIFNFYFD (SEQ ID NO: 71), or ARDMIFNFYFDV (SEQ ID NO: 72); (d) LCDR1 comprising the amino acid sequence of SASSRVGYMH (SEQ ID NO: 29), NQNVDYNGNTF (SEQ ID NO: 73), TNQNVDYNGNTF (SEQ ID NO: 74), QNVDYNGNTF (SEQ ID NO: 75), KTNQNVDYNGNTFMH (SEQ ID NO: 76), TNQNVDYSGNTF (SEQ ID NO: 77), or NQNVDYSGNTF (SEQ ID NO: 78); (e) LCDR2 comprising the amino acid sequence of LASNLAS (SEQ ID NO: 24), DTSKLAS (SEQ ID NO: 30), LAS (SEQ ID NO: 83), LASNLASGIPDR (SEQ ID NO: 84), LASNLASGIPER (SEQ ID NO: 85), or DTS (SEQ ID NO: 86); and (f) LCDR3 comprising the amino acid sequence of QQSRNLPYT (SEQ ID NO: 25), FQGSGYPFT (SEQ ID NO: 31), SRNLPY (SEQ ID NO: 88), GSGYPF (SEQ ID NO: 89).

[0084] In some embodiments, a FAP binding agent is provided, wherein the antibody comprises a heavy chain (HC) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 1, 4-6, or 8-16. Optionally, the FAP binding agent comprises the HC sequence in SEQ ID NO: 1, 4-6, or 8-16, including post-translational modifications.

[0085] In some embodiments, a FAP binding agent is provided, wherein the antibody comprises a heavy chain (HC) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 5 or 6. Optionally, the FAP binding agent comprises the HC sequence in SEQ ID NO: 5 or 6, including post-translational modifications.

[0086] In some embodiments, a FAP binding agent is provided, wherein the antibody comprises a LC having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 2, 3, or 7. Optionally, the FAP binding agent comprises the LC sequence in SEQ ID NO: 2, 3, or 7, including post-translational modifications.

[0087] In some embodiments, a FAP binding agent is provided, wherein the antibody comprises a LC having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 3. Optionally, the FAP binding agent comprises the LC sequence in SEQ ID NO: 3, including post-translational modifications.

[0088] In some embodiments, a FAP binding agent comprises a HC as in any of the embodiments provided herein, and a LC as in any of the embodiments provided herein.

[0089] In some embodiments, a FAP binding agent is provided, wherein the antibody comprises a heavy chain (HC) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 1, 4-6, or 8-16 and a LC having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 2, 3, or 7, including post-translational modifications of those sequences.

[0090] In some embodiments, a FAP binding agent is provided, wherein the antibody comprises a heavy chain (HC) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 5 or 6 and a LC having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 3, including post-translational modifications of those sequences.

[0091] In some embodiments, a FAP binding agent is provided, wherein the antibody comprises a first heavy chain (HC1) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 5, a second heavy chain heavy chain (HC2) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 6, and a LC having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 3, including post-translational modifications of those sequences.

[0092] FAP binding agents provided herein can be an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody thereof, or a fusion protein thereof. It is understood that referring to an antibody FAP binding agent, reference is also made to an antibody targeting the same or antigen-binding fragment thereof, a bispecific antibody thereof, or a fusion protein comprising the same, and vice versa. Accordingly, provided herein are anti-FAP antibodies and fragments thereof.

Anti-FAP Antibody or antigen-binding fragment thereof

[0093] In some embodiments, a FAP binding agent provided herein is an anti-FAP antibody or a functional fragment thereof. Accordingly, provided herein are anti-FAP antibodies, fragments thereof, or uses thereof. In some embodiments, provided herein is an isolated anti-FAP antibody. In some embodiments, provided herein is an isolated anti-FAP antibody fragment. In some embodiments, an anti-FAP antibody provided herein can be used in therapeutic and/or diagnostic methods, such as methods of detection of FAP in a sample, methods of treatment, methods of diagnosis, and/or methods of prognosis. Diagnostic and therapeutic methods are further described herein. It is understood that when referencing an antibody herein, reference is also being made to a functional fragment thereof (*e.g.*, a single-chain antibody, an isolated antibody hypervariable domain, a binding fragment thereof)

and modified variants thereof, including a derivative thereof (*e.g.*, an antibody conjugated with a substrate or with the protein or ligand of a protein-ligand pair).

[0094] In some embodiments, antibodies described herein include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, including molecules that contain one or more antigen-binding sites that bind to a FAP antigen.

[0095] Antibodies can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA or IgY), any class, (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2), or any subclass (*e.g.*, IgG2a or IgG2b) of immunoglobulin molecule. In some embodiments, antibodies described herein are IgG antibodies (*e.g.*, human IgG), or a class (*e.g.*, human IgG1, IgG2, IgG3 or IgG4) or subclass thereof.

[0096] In some embodiments, an antibody is a 4-chain antibody unit comprising two heavy (H) chain / light (L) chain pairs, wherein the amino acid sequences of the H chains are identical or non-identical and the amino acid sequences of the L chains are identical. In some embodiments, the amino acid sequences of the H chains are not identical. Modifications of heavy chain amino acid sequences to generate non-identical heavy chain amino acid sequences, for example, knob in hole mutations, are further described herein.

[0097] In some embodiments, the H and L chains comprise constant regions, for example, human constant regions. In some embodiments, the L chain constant region of such antibodies is a kappa or lambda light chain constant region, for example, a human kappa or lambda light chain constant region. In some embodiments, the H chain constant region of such antibodies comprise a gamma heavy chain constant region, for example, a human gamma heavy chain constant region. In some embodiments, such antibodies comprise IgG constant regions, for example, human IgG constant regions (*e.g.*, IgG1, IgG2, IgG3, and/or IgG4 constant regions).

[0098] Antibodies and fragments thereof described herein include, but are not limited to, synthetic antibodies, monoclonal antibodies, recombinantly produced antibodies, multispecific antibodies (*e.g.*,

including bispecific antibodies), human antibodies, humanized antibodies, chimeric antibodies, intrabodies, single-chain Fvs (scFv) (*e.g.*, including monospecific, bispecific, *etc.*), camelized antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), anti-idiotypic (anti-Id) antibodies, Fabs-in-tandem-lg (FIT-lg); DVD-lg; hybrid hybridoma (quadroma or tetradoma); anticalin platform (Pieris); diabodies; single chain diabodies; tandem single chain Fv fragments; TandAbs, Trispecific Abs (Affimed); Darts dual affinity retargeting (Macrogenics); Bispecific Xmabs (Xencor); Bispecific T cell engagers (BiTE; Amgen; 55kDa); Triplebodies; Tribody = Fab-scFv Fusion Protein multifunctional recombinant antibody derivates (CreativeBiolabs); Duobody platform (Genmab); dock and lock platform; knobs-into-holes (KIH) platform; humanized bispecific IgG antibody (REGN1979) (Regeneron); Mab2 bispecific antibodies (F-Star); DVD-lg = dual variable domain immunoglobulin (AbbVie); kappa-lambda bodies; TBTI = tetravalent bispecific tandem Ig; and CrossMab (Roche), and epitope-binding fragments of any of the above.

[0099] A typical antibody molecule includes a heavy chain variable region (VH) and a light chain variable region (VL). The variable region is a region with large changes in amino acid composition and arrangement at the N-terminal of the antibody molecule. The site of specific binding, that is, the antigen-binding site, is used to determine the specificity of antibody recognition. The VH and VL regions can be further subdivided into hypervariable regions, also known as "complementarity determining regions" (CDRs), interspersed with more conserved regions known as "framework regions" (FR). Each VH and VL is generally composed of three CDRs and four FRs arranged in the following order from amino terminus to carboxy terminus: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

Exemplary Anti-FAP Antibodies or a FAP antigen-binding fragment thereof

[0100] In some embodiments, the present disclosure provides examples of novel anti-FAP antibody or an antigen-binding fragment thereof, including heavy chain complementarity determining regions

(CDR) HCDR1, HCDR2 and HCDR3, and light chain complementarity determining regions LCDR1, LCDR2 and LCDR3. The extent of framework regions and CDRs can be precisely identified using methods known in the art, *e.g.*, by Kabat definitions, Chothia definitions, AbM definitions, IMGT definitions, Honegger definitions, and/or Contact definitions, all of which are well known in the art. *See, e.g.*, Kabat, E.A. et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Chothia et al., (1989) Nature 342:877; Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917; Al-lazikani et al. (1997) J. Molec. Biol. 273:927-948; IMGT®, the international ImMunoGeneTics information system® http://www.imgt.org, Lefranc, M.-P. et al., Nucleic Acids Res., 27:209-212 (1999); Ruiz, M. et al., Nucleic Acids Res., 28:219-221 (2000); Lefranc, M.-P., Nucleic Acids Res., 29:207-209 (2001); Lefranc, M.-P., Nucleic Acids Res., 31:307-310 (2003); Lefranc, M.-P. et al., In Silico Biol., 5, 0006 (2004) [Epub], 5:45-60 (2005); Lefranc, M.-P. et al., Nucleic Acids Res., 33:D593-597 (2005); Lefranc, M.-P. et al., Nucleic Acids Res., 37:D1006-1012 (2009); Lefranc, M.-P. et al., Nucleic Acids Res., 43:D413-422 (2015); Honegger and Plückthun, *J. Mol. Biol.* 309: 657-670 (2001); and Almagro, J. Mol. Recognit. 17:132-143 (2004). *See also* hgmp.mrc.ac.uk and bioinf.org.uk/abs.

[0101] In some embodiments, the CDRs of anti-FAP antibody or a fragment thereof including a human anti-FAP antibody or a fragment thereof, can be determined according to any suitable numbering system. In some embodiments, the CDR is defined by Kabat system, which is well-known to the skilled in the art. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., (1971) Ann. NY Acad. Sci. 190:382-391 and Kabat, et al., (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Chothia refers instead to the location of the structural loops (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). Numbering systems used herein are Kabat, IMGT, Honegger, and Chothia (*see e.g.*, **TABLE 7**).

[0102] In some embodiments, LCDR1 includes an amino acid sequence as shown in KTNQNVDYX$_1$GNTFMH (SEQ ID NO: 23), wherein X$_1$ is N or S; LCDR2 includes an amino acid sequence as shown in LASNLAS (SEQ ID NO: 24), LCDR3 includes an amino acid sequence as shown in QQSRNLPYT (SEQ ID NO: 25); HCDR1 includes an amino acid sequence as shown in IYGVN (SEQ ID NO: 26), HCDR2 includes an amino acid sequence as shown in AIWSGGRKDYX$_2$LSLKS (SEQ ID NO: 27), wherein X$_2$ is N or S, HCDR3 includes an amino acid sequence as shown in SQDMPGYFDY (SEQ ID NO: 28). In some embodiments, LCDR1 includes an amino acid sequence as shown in SASSRVGYMH (SEQ ID NO: 29), LCDR2 includes an amino acid sequence as shown in DTSKLAS (SEQ ID NO: 30), LCDR3 includes an amino acid sequence as shown in FQGSGYPFT (SEQ ID NO: 31); HCDR1 includes an amino acid sequence as shown in TAGMSVG (SEQ ID NO: 32), HCDR2 includes an amino acid sequence as shown in DIWWDDKKHYNPSLKD (SEQ ID NO: 33), HCDR3 includes an amino acid sequence as shown in DMIFNFYFDV (SEQ ID NO: 34).

[0103] In some embodiments, the anti-FAP antibody or antigen-binding fragment thereof includes a heavy chain variable region (VH) and a light chain (VL), wherein the VH and/or VL includes the CDRs described above. In some embodiments, the anti-FAP antibody or antigen-binding fragment thereof includes a VH having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to the amino acid sequence as shown in SEQ ID NO: 35, and a VL having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to the amino acid sequence as shown in SEQ ID NO: 36. In some embodiments, the anti-FAP antibody or antigen-binding fragment thereof includes a VH having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to the amino acid sequence as shown in SEQ ID NO: 37, and a VL having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to the amino acid sequence as shown in SEQ ID NO: 38.

[0104] In some embodiments, the anti-FAP antibody or antigen-binding fragment thereof includes a fragment crystallizable (Fc) region derived from immunoglobulin. In some embodiments, the Fc fragment is derived from IgG1, IgG2, IgG3, or IgG4. In some embodiments, the Fc fragment is derived from IgG4. In some embodiments, the Fc fragment includes one or more amino acid substitutions as compared to wild type IgG1, IgG2, IgG3, or IgG4. In some embodiments, the Fc fragment includes mutation S228P. In some embodiments, the Fc fragment includes LALAPG mutation, i.e., L234A, L235A, and/or P329G. In some embodiments, the Fc fragment is heterodimeric.

[0105] In some embodiments, the anti-FAP antibody or antigen-binding fragment thereof comprises: heavy chain (HC) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 1, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, and 16, and/or a light chain (LC) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 2, 3, and 7.

[0106] In some embodiments, the anti-FAP antibody or antigen-binding fragment thereof comprises a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 1, 4, 5, 8, 10, 13, 14, and 15.

[0107] In some embodiments, the anti-FAP antibody or antigen-binding fragment thereof comprises an HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 5, 8, 10, 13, 14, and 15.

[0108] In some embodiments, the anti-FAP antibody or antigen-binding fragment thereof comprises an HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 1, 4, 6, 9, 11, 12, 13, and 16.

[0109] In some embodiments, the anti-FAP antibody or antigen-binding fragment thereof comprises an HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 6, 9, 11, 12, 13, and 16.

[0110] In some embodiments, the anti-FAP antibody or antigen-binding fragment thereof includes a heavy chain having an amino acid sequence as shown in SEQ ID NO: 1, and a light chain having an amino acid sequence as shown in SEQ ID NO: 2. In some embodiments, the anti-FAP antibody or antigen-binding fragment thereof includes a heavy chain having an amino acid sequence as shown in SEQ ID NO: 3, and a light chain having an amino acid sequence as shown in SEQ ID NO: 4. In some embodiments, the anti-FAP antibody or antigen-binding fragment thereof includes a heavy chain having an amino acid sequence set forth in SEQ ID NO: 4, and a light chain having an amino acid sequence set forth in SEQ ID NO: 3. Further CDRs, VHs, VLs, HCs, and/or LCs of anti-FAP antibodies are described in detail in the FAP binding agent section herein.

[0111] In some embodiments, the anti-FAP antibody or antigen-binding fragment thereof cross-reacts with human, cynomolgus and mouse FAP.

[0112] The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof is a monoclonoal antibody, humanized antibody, human antibody, chimeric antibody, Fab, Fab', F(ab')2, Fv, scFv, (scFv)2, single chain antibody molecule, dual variable region antibody, single variable region antibody, linear antibody, V region, or a multispecific antibody formed from antibody fragments.

Humanized antibodies

[0113] In some embodiments, an antibody provided herein is a humanized antibody. In some embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is

humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which the CDRs (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the CDR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

[0114] Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008), and are further described, *e.g.*, in Riechmann *et al.*, Nature 332:323-329 (1988); Queen *et al.*, Proc. Nat'l Acad. Sci. USA 86:10029-10033 (1989); U.S. Pat. Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, Methods 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan, Mol. Immunol. 28:489-498 (1991) (describing “resurfacing”); Dall'Acqua *et al.*, Methods 36:43-60 (2005) (describing “FR shuffling”); and Osbourn *et al.*, Methods 36:61-68 (2005) and Klimka *et al.*, Br. J. Cancer, 83:252-260 (2000) (describing the “guided selection” approach to FR shuffling).

[0115] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the “best-fit” method (see, *e.g.*, Sims *et al.* J. Immunol. 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, *e.g.*, Carter *et al.* Proc. Natl. Acad. Sci. USA, 89:4285 (1992); and Presta *et al.* J. Immunol., 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, *e.g.*, Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, *e.g.*,

Baca *et al.*, J. Biol. Chem. 272:10678-10684 (1997) and Rosok *et al.*, J. Biol. Chem. 271:22611-22618 (1996)).

Human antibodies

[0116] In some embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, Curr. Opin. Pharmacol. 5: 368-74 (2001) and Lonberg, Curr. Opin. Immunol. 20:450-459 (2008).

[0117] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, *see* Lonberg, Nat. Biotech. 23:1117-1125 (2005). *See* also, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Pat. No. 5,770,429 describing HUMAB® technology; U.S. Pat. No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, *e.g.*, by combining with a different human constant region.

[0118] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, *e.g.*, Kozbor J. Immunol., 133: 3001 (1984); Brodeur *et al.*, Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (1987); and Boerner *et al.*, J. Immunol., 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li

et al., Proc. Natl. Acad. Sci. USA, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Pat. No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, Xiandai Mianyixue, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, Histology and Histopathology, 20(3):927-937 (2005) and Vollmers and Brandlein, Methods and Findings in Experimental and Clinical Pharmacology, 27(3): 185-91 (2005).

[0119] Human antibodies may also be generated by isolating variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

[0120] Antibodies may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, for example, in Hoogenboom *et al.* in Methods in Molecular Biology 178: 1-37 (O'Brien *et al.*, ed., Human Press, Totowa, NJ, 2001) and further described, for example, in the McCafferty *et al*, Nature 348:552-554 (1990); Clackson *et al*, Nature 352: 624-628 (1991); Marks *et al*, J. Mol. Biol 222: 581-597(1992); Marks and Bradbury, in Methods in Molecular Biology 248: 161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu *et al*, J. Mol. Biol. 338(2) (2004): 299-310; Lee *et al.*, J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, Proc. Natl. Acad. Sci. USA 101(34): 12467-12472 (2004); and Lee *et al*, (2004) J. Immunol. Methods 284(1-2): 119-132 and PCT publication WO 99/10494.

[0121] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter *et al.*, Ann. Rev. Immunol., 12:433-455

(1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (for example, from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths *et al.*, EMBO J 12:725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, J. Mol. Biol, 227:381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0122] In some embodiments, a chimeric human anti-FAP antibody is provided, where the antibody comprises the variable region from a human antibody that binds FAP and the constant region from a different human antibody. In some embodiments, a chimeric human anti-FAP antibody, where the antibody comprises the CDRs from a human antibody that binds FAP and a framework from a different human antibody is provided. In some embodiments, the antibody is not a naturally occurring human antibody.

[0123] In some embodiments, a human anti-FAP antibody comprises one or more human constant regions. In some embodiments, the human heavy chain constant region is of an isotype selected from IgA, IgG, IgD, and IgE. In some embodiments, the human light chain constant region is of an isotype selected from κ and λ. In some embodiments, a human antibody described herein comprises a human IgG constant region. In some embodiments, a human antibody described herein comprises a human

IgG4 heavy chain constant region. In some embodiments, a human antibody described herein comprises a human IgG4 constant region and a human κ light chain.

[0124] In some embodiments, when effector function is desirable, a human anti-FAP antibody comprising a human IgG1 heavy chain constant region or a human IgG3 heavy chain constant region is selected. In some embodiments, when effector function is not desirable, a human anti-FAP antibody comprising a human IgG4 or IgG2 heavy chain constant region is selected.

[0125] When describing a human antibody, references is made to the genus of possible sequences for the antibody construct, rather than a source of the antibody.

Multispecific antibodies

[0126] In some embodiments, an antibody provided herein is a multispecific antibody, *e.g.*, a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites, *e.g.*, different epitopes on different antigens or different epitopes on the same antigen. In certain embodiments, the multispecific antibody has three or more binding specificities. In certain embodiments, one of the binding specificities is for FAP and the other specificity is for any other antigen. In certain embodiments, bispecific antibodies may bind to two (or more) different epitopes of FAP. Multispecific (*e.g.*, bispecific) antibodies may also be used to localize cytotoxic agents or cells to cells which express FAP. Multispecific antibodies may be prepared as full-length antibodies or antibody fragments.

[0127] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (*see* Milstein and Cuello, Nature 305: 537 (1983)) and "knob-in-hole" engineering (see, *e.g.*, U.S. Pat. No. 5,731,168, and Atwell *et al.*, J. Mol. Biol. 270:26 (1997)). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (see, *e.g.*, WO 2009/089004); cross-linking two or more antibodies or fragments (see, *e.g.*, U.S. Pat. No.

4,676,980, and Brennan *et al.*, Science, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, *e.g.*, Kostelny *et al.*, J. Immunol., 148(5):1547-1553 (1992) and WO 2011/034605); using the common light chain technology for circumventing the light chain mis-pairing problem (see, *e.g.*, WO 98/50431); using "diabody" technology for making bispecific antibody fragments (see, *e.g.*, Hollinger *et al.*, Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see, *e.g.*, Gruber *et al.*, J. Immunol., 152:5368 (1994)); and preparing trispecific antibodies as described, *e.g.*, in Tutt *et al.* J. Immunol. 147: 60 (1991).

[0128] Anti-FAP antibodies provided herein can be fused or conjugated to a second molecule. Multispecific anti-FAP antibodies provided herein can be fused or conjugated to an additional molecule. Fusion proteins and antibody conjugates are further described herein.

Bispecific Antibody or antigen-binding fragment thereof

[0129] Provided herein is a bispecific antibody, an antigen-binding fragment thereof, or a use thereof. In some embodiments, a bispecific antibody comprises a first binding moiety and a second binding moiety. In some embodiments, the first binding moiety comprises a FAP antigen-binding moiety described herein. An antigen-binding moiety includes, in some embodiments, the specific region or component of an antibody that directly interacts with and binds to an antigen. This moiety can include one or more variable regions of a heavy chain and/or a light chain, which form an antigen-binding site. In some embodiments, the FAP antigen-binding moiety includes part or full length of the anti-FAP antibody or antigen-binding fragment thereof described herein. A FAP antigen-binding moiety comprises any one or more of the CDRs, VHs, VLs, HC including HC1s or HC2, and/or LCs as described herein.

[0130] In some embodiments, a bispecific antibody described herein comprise two heavy chains, wherein each heavy chain binds nonidentical epitopes. In some embodiments, a bispecific antibody

described herein comprise two heavy chains, wherein each heavy chain binds the same epitope. Each heavy chain can have at one end a variable domain (VH) followed by a number of constant domains (three or four constant domains, CH1, CH2, CH3 and CH4, depending on the antibody class). In some embodiments, bispecific antibodies described herein comprise one or more light chains. Each light chain can have a variable domain (VL) at one end and a constant domain (CL) at its other end; the constant domain of the light chain is aligned with the first constant domain (CH1) of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. In some embodiments, the light chains comprise kappa light chain or lambda light chain. Bispecific antibodies such as kappa or lambda antibodies can be made using any of a variety of art-recognized techniques, including those disclosed in WO 2012/023053, the contents of which are hereby incorporated by reference in their entirety.

[0131] In some embodiments, antibody variable domains with the desired binding specificities can be linked to immunoglobulin constant domain sequences to form bispecific antibodies. In some embodiments, the fusion is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. In some embodiments, the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, may be inserted into separate expression vectors, and may be co-transfected into a suitable host organism.

[0132] In some embodiments, the interface between a pair of antibody molecules in constructs herein is engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains to form a protuberance or knob (*e.g.*, tyrosine or tryptophan). Compensatory cavities or holes of identical or similar size to the large side chain(s) are

created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.*, serine, threonine, valine or alanine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

[0133] Techniques for generating bispecific antibodies from antibody functional fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. The bispecific antibodies can be used as agents for the selective immobilization of enzymes.

[0134] Various techniques for making and isolating bispecific antibody functional fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab′ portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology provides an alternative mechanism for making bispecific antibody functional fragments. The functional fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one functional fragment are forced to pair with the complementary VL and VH domains of another functional fragment, thereby forming two target-binding sites. Another strategy for making bispecific antibody functional fragments includes use of single-chain Fv (sFv) dimers.

[0135] Antibodies with more than two valences are contemplated. For example, trispecific antibodies can be prepared. Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the target described herein. Alternatively, a target arm of an immunoglobulin molecule can be combined with an arm which binds to a TACR, such as a TACR described herein. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular

protein. These antibodies may possess a target-binding arm and an arm which binds a cytotoxic agent, such as those described herein.

[0136] Several strategies have been used to generate such multispecific molecules (*e.g.*, bispecific molecules, trispecific molecules) such as chemical cross-linking of antibody functional fragments, forced heterodimerization, quadroma technology, fusion of antibody functional fragments via polypeptide linkers and use of single domain antibodies. The availability of recombinant DNA technologies has led to the generation of a multitude of bispecific antibody formats. Linkers and mutations have frequently been introduced into different regions of the antibody to force heterodimer formation or to connect different binding moieties into a single molecule.

[0137] In some embodiments, the bispecific antibody or antigen-binding fragment thereof includes a Fc fragment derived from immunoglobulin at N-terminus of the FAP antigen-binding moiety. In some embodiments, the Fc fragment is derived from IgG1, IgG2, IgG3, or IgG4, optionally the Fc fragment is derived from IgG4. In some embodiments, the Fc fragment includes mutation S228P. In some embodiments, the Fc fragment includes one or more modifications selected from the group consisting of knobs-into-holes, DDKK, electrostatic steering of CH3, DuoBody, SEEDbodies, cFAE, XmAb, Azymetric, and BEAT®. In some embodiments, the Fc fragment includes modifications knobs-into-holes and/or DDKK.

[0138] A knob-into-hole mutation can force the pairing of two different IgG heavy chains by introducing mutations into the CH3 domains to modify the contact interface. On one chain amino acids with large side chains are introduced, to create a 'knob'. Conversely, bulky amino acids are replaced by amino acids with short side chains to create a 'hole' into the other CH3 domain. By co-expressing these two heavy chains, more than 90% heterodimer formation can be observed ('knob- hole') versus homodimers formation ('hole -hole' or 'knob-knob'). Engineered strand- exchange engineered domain (SEED) human CH3 domains based on human IgG and human IgA sequences can also lead to the

formation of heterodimeric molecules that can carry two different specificities. Recently an improvement over the 'knob into hole' approach; "CrossMab" has been described in WO 2009/080253 Al. This method involves the exchange of some of the light chain and heavy chain domains in addition to the 'knob into hole' mutations. DDKK is a modification mediating electrostatic steering effect that is used to enhance antibody Fc heterodimer formation as inter alia described by Gunasekaran et al., (J. Biol. Chem. 2010,19637-19646). Accordingly, in some embodiments, provided herein are one or more heterodimerization modifications.

[0139] In some embodiments, the second binding moiety binds and/or activates a second target. In some embodiments, the second target is a tumor associated cell receptor (TACR). A TACR can be expressed on a tumor cell or on an immune cell associated with a tumor cell or a tumor microenvironment. In some embodiments, the second binding moiety binds a TACR. In some embodiments, the second binding moiety activates a TACR. In some embodiments, a TACR comprises LTβR, HER2, PDL-1, PD-1, EGFR, VEGFR, VEGF, CCR8, OX-40, 418B, Angiopoietin-2, IL-4Ra, BCMA, Blys, BTNO2, C5, CD122, CD13, CD133, CD137, CD138, CD16a, CD19, CD20, CD22, CD27, CD28, CD3, CD30, CD33, CD38, CD40, CD47, CD-8, CEA, CGPR/CGRPR, CSPGs, CTLA4, CTLA-4, DLL-4, EpCAM, factor IXa, factor X, GITR, GP130, Her3, HSG, ICOS, IGFl, IGFl/2, IGF-lR, IGF2, IGFR, IL-1, IL- 12, IL-12p40, IL-13, IL-l 7A, IL-1~, IL-23, IL-5, IL-6, IL-6R, Lag-3, LAG3, MAG, Met, NgR, NogoA, OMGp, OX40, PDGFR, PSMA, RGMA, RGMB, SARS-CoV-2, Te38, TIM-3, TNF, TNFa, TROP-2, TWEAK, or TRAIL.

[0140] The second binding moiety can be a second antigen-binding moiety, such as an additional antibody fragment, or can be a binding polypeptide or molecule that effects an activity, such as a cytokine moiety that can stimulate immune cells. In some embodiments, the second binding moiety is a cytokine moiety that can bind and/or activate a TACR (*e.g.*, LTβR).

[0141] In some embodiments, the second binding moiety comprises a first portion and a second portion, wherein each first portion and second portion comprises one or more units. In some embodiments, the one or more units comprise a first unit, a second unit, and/or a third unit. In some embodiments, each of the one or more units are identical. In some embodiments, the one or more units are not identical. In some embodiments, the second binding moiety comprises one or more units. wherein each of the one or more units independently is a tumor necrosis factor, interleukin, lymphokine, interferon, colony stimulating factor, chemokine or growth factor. In some embodiments, the one or more units form a complex which acts as the second binding moiety, alternatively, the one or more units are each individually act as the second binding moiety depending on the second binding moiety selected. Each of the first, second, or third units can individually comprise any one of the proteins described in **TABLE 10**. For example, each of the first, second, or third units individually comprises a LIGHT unit, a lymphotoxin-α unit, or a lymphotoxin-β unit. In some embodiments, each of the one or more units independently comprise an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to any one of the amino acid sequences set forth in **TABLE 10**.

[0142] In some embodiments, the second binding moiety is located at a C-terminus of the FAP antigen-binding moiety. In some embodiments, the second binding moiety is operably linked to the C-terminus of the Fc fragment. In some embodiments, the second binding moiety links to the Fc fragment by a linker. In some embodiments, the second binding moiety binds and/or activates a second target. In some embodiments, the second binding moiety binds and/or activates a tumor associated cell receptor. In some embodiments, the second binding moiety is a tumor necrosis factor, interleukin, lymphokine, interferon, colony stimulating factor, chemokine or growth factor.

Exemplary Anti-FAP antigen-binding moiety

[0143] In some embodiments, the FAP antigen-binding moiety includes part or full length of the anti-FAP antibody or antigen-binding fragment thereof described above. In some embodiments, the FAP antigen-binding moiety includes LCDR1 having an amino acid sequence as shown in KTNQNVDYX$_1$GNTFMH (SEQ ID NO: 23), wherein X$_1$ is N or S, LCDR2 having an amino acid sequence as shown in LASNLAS (SEQ ID NO: 24), LCDR3 having an amino acid sequence as shown in QQSRNLPYT (SEQ ID NO: 25); HCDR1 having an amino acid sequence as shown in IYGVN (SEQ ID NO: 26), HCDR2 having an amino acid sequence as shown in AIWSGGRKDYX$_2$LSLKS (SEQ ID NO: 27), wherein X$_2$ is N or S, HCDR3 having an amino acid sequence as shown in SQDMPGYFDY (SEQ ID NO: 28). In some embodiments, the FAP antigen-binding moiety includes LCDR1 having an amino acid sequence as shown in SASSRVGYMH (SEQ ID NO: 29), LCDR2 having an amino acid sequence as shown in DTSKLAS (SEQ ID NO: 30), LCDR3 having an amino acid sequence as shown in FQGSGYPFT (SEQ ID NO: 31); HCDR1 having an amino acid sequence as shown in TAGMSVG (SEQ ID NO: 32), HCDR2 having an amino acid sequence as shown in DIWWDDKKHYNPSLKD (SEQ ID NO: 33), HCDR3 having an amino acid sequence as shown in DMIFNFYFDV (SEQ ID NO: 34). In some embodiments, the FAP antigen-binding moiety includes a VH and/or a VL as the same as the VH and/or a VL of anti-FAP antibody or antigen-binding fragment thereof described above.

[0144] In some embodiments, the bispecific antibody or antigen-binding fragment thereof includes a first heavy chain, a second heavy chain, and two light chains paired with the first heavy chain and the second heavy chain, respectively, wherein (1) the first heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 5; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity

to an amino acid sequence set forth in SEQ ID NO: 6; the light chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 3; (2) the first heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 8; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 9; the light chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 7; (3) the first heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 10; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 11; the light chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 3.

[0145] In some embodiments, the bispecific antibody or antigen-binding fragment thereof includes the first heavy chain having an amino acid sequence set forth in SEQ ID NO: 5, the second heavy chain having an amino acid sequence set forth in SEQ ID NO: 6, and the light chain having an amino acid sequence set forth in SEQ ID NO: 3. In some embodiments, the bispecific antibody or antigen-binding fragment thereof includes the first heavy chain having an amino acid sequence set forth in SEQ ID NO: 8, the second heavy chain having an amino acid sequence set forth in SEQ ID NO: 9, and the light chain having an amino acid sequence set forth in SEQ ID NO: 7. In some embodiments, the bispecific antibody or antigen-binding fragment thereof includes the first heavy chain having an amino acid

sequence set forth in SEQ ID NO: 10, the second heavy chain having an amino acid sequence set forth in SEQ ID NO: 11, and the light chain having an amino acid sequence set forth in SEQ ID NO: 3.

[0146] Further CDRs, VHs, VLs, HCs, and/or LCs of FAP antigen-binding moiety are described in detail in the FAP binding agent section herein.

[0147] In the present disclosure, the bispecific antibody or monoclonal antibody could include conservatively modified variants, such as FR region. The conservatively modified variants include individual substitutions, deletions or additions to the polypeptide sequence which result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art and are further described herein. Other amino acid modifications are further described herein.

Exemplary Structure

[0148] In some embodiments, a bispecific antibody provided herein comprises a structure and/or configuration of one or more components that enable targeting and binding of two or more targets. For example, the bispecific antibody can comprise any one of structures A-E as described in WO2024/193705, which is incorporated herein by reference in its entirety.

[0149] In some embodiments, a bispecific antibody provided herein comprises a 4 chain antibody unit comprising two H chain pairs and two L chain pairs, and one or more pairs of a polypeptide linker and second binding moiety are attached to the Fc region of each H chain, wherein the amino acid sequences of the H chains are non-identical and the amino acid sequences of the L chains are identical. In some embodiments, the bispecific antibody described herein comprises a first polypeptide linker and second binding moiety pair attached to the FC region of a first heavy chain, and comprises a second polypeptide linker and second binding moiety pair attached to the FC region of a second heavy chain and a third polypeptide linker and second binding moiety pair attached to the second linker and binding moiety pair. In some embodiments, the bispecific antibody described herein comprises a first

polypeptide linker and second binding moiety pair attached to the FC region of a second heavy chain, and comprises a second polypeptide linker and second binding moiety pair attached to the FC region of a first heavy chain and a third polypeptide linker and second binding moiety pair attached to the second linker and binding moiety pair.

[0150] The bispecific antibodies of the present disclosure can be a dual-variable domain immunoglobulin (DVD-Ig™) as described in Jakob, C. G., Edalji, R., Judge, R. A., DiGiammarino, E., Li, Y., Gu, J., & Ghayur, T. (2013). Structure reveals function of the dual variable domain immunoglobulin (DVD-IgTM) molecule. mAbs, 5(3), 358–363, which combines the target binding domains of two monoclonal antibodies via flexible naturally occurring linkers, which yields a tetravalent IgG - like molecule.

[0151] In some embodiments, the bispecific antibody or antigen-binding fragment thereof has the format D as shown in Figure 2 or 6. The bispecific antibody or antigen-binding fragment thereof has the format D as shown in Figure 2 or 6.

Cytokine Moiety

[0152] In some embodiments, the present disclosure relates to a bispecific antibody or antigen-binding fragment thereof, including a FAP antigen-binding moiety, and a cytokine moiety capable of stimulating immune cells. A cytokine moiety can be any molecule capable of stimulating immune cells. The cytokine moiety can be at the C-terminus of the bispecific antibody or antigen-binding fragment thereof. In some embodiments, the cytokine moiety is operably linked to the C-terminus of the Fc fragment of the bispecific antibody or antigen-binding fragment thereof. In some embodiments, the cytokine moiety links to the Fc fragment directly. In some embodiments, the cytokine moiety links to the Fc fragment via a linker. Exemplary linkers are further described herein.

[0153] In some embodiments, the cytokine moiety includes a first cytokine moiety and a second cytokine moiety. Each of a first cytokine moiety and a second cytokine moiety can comprise a first,

second and/or third cytokine unit. In some embodiments, the first cytokine moiety comprises a first cytokine unit. In some embodiments, the first cytokine unit comprises a LIGHT unit or a lymphotoxin-β unit. In some embodiments, the second cytokine moiety comprises a second cytokine unit and a third cytokine unit. In some embodiments, the second cytokine unit comprises a LIGHT unit, a lymphotoxin-α unit, or a lymphotoxin-β unit. In some embodiments, the third cytokine unit comprises a LIGHT unit, a lymphotoxin-α unit, or a lymphotoxin-β unit. In some embodiments, the first cytokine moiety comprises a first LIGHT unit, the second cytokine unit comprises a second LIGHT unit, and the third cytokine unit comprises a third LIGHT unit. In some embodiments, the first, second, and/or third LIGHT unit each independently comprises an amino acid sequence set forth in SEQ ID NO: 17 or 18. In some embodiments, the first cytokine moiety comprises a lymphotoxin-β unit, the second cytokine unit comprises a lymphotoxin-α unit, and the third cytokine unit comprises a lymphotoxin-β unit. In some embodiments, the lymphotoxin-β unit comprises an amino acid sequence set forth in SEQ ID NO: 39, the lymphotoxin-α unit comprises an amino acid sequence set forth in SEQ ID NO: 40.

[0154] In some embodiments, the cytokine moiety includes a first cytokine moiety and a second cytokine moiety, the first cytokine moiety contains one LIGHT mutant and the second cytokine moiety contains tandem-linked two LIGHT mutants.

[0155] In some embodiments, the cytokine moiety is linked to the Fc fragment by one or more linkers. In some embodiments, the first cytokine moiety is linked to the Fc fragment by a linker. In some embodiments, the second cytokine moiety is linked to the Fc fragment by a linker. In some embodiments, each of the cytokine units of the first and/or second cytokine moieties are linked by one or more linkers. In some embodiments, the first cytokine moiety links to the Fc fragment via linker A, and the second cytokine moiety links to the Fc fragment via linker B. In some embodiments, tandem-linked two LIGHT mutants link to each other directly or via a linker C. In some embodiments, the

linker A, linker B and linker C independently are peptide linker having the formula $(Gly4Ser)_n$, where n is 1, 2, 3, 4, or 5. In some embodiments, n is 2 or 3, *i.e.*, the linker is $(Gly4Ser)_2$, or $(Gly4Ser)_3$. In some embodiments, the linker A, linker B and linker C independently are amino acid G. Exemplary linkers are further described herein.

[0156] In some embodiments, the cytokine moiety binds and/or activates a TACR. In some embodiments, the cytokine moiety binds and/or activates a LTβR. In some embodiments, the cytokine moiety binds and/or activates a TACR that comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to any one of the amino acid sequences set forth in **TABLE 5.1**. In some embodiments, the first cytokine moiety binds and/or activates a TACR that comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to any one of the amino acid sequences set forth in **TABLE 5.1.** In some embodiments, the second cytokine moiety binds and/or activates a TACR that comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to any one of the amino acid sequences set forth in **TABLE 5.1.** In some embodiments, the first and second cytokine moiety comprises one or more units each individually or collectively binds and/or activates a TACR that comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to any one of the amino acid sequences set forth in **TABLE 5.1.**

[0157] In some embodiments, the cytokine moiety comprises a tumor necrosis factor, an interleukin, lymphokine, an interferon, a colony stimulating factor, a chemokine or a growth factor. In some embodiments, the tumor necrosis factor comprises LIGHT, lymphotoxin α, lymphotoxin β, or 4-1 BBL, or a combination thereof. In some embodiments, the cytokine moiety comprises one or more LIGHT, lymphotoxin α, lymphotoxin β, or 4-1 BBL, or a combination thereof.

[0158] In some embodiments, the cytokine moiety comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical, to any one of the amino acid sequences set forth in **TABLE 10**. In some embodiments, the first cytokine moiety comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical, to any one of the amino acid sequences set forth in **TABLE 10.** In some embodiments, the second cytokine moiety comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical, to any one of the amino acid sequences set forth in **TABLE 10.** In some embodiments, the first and second cytokine moiety comprises one or more units wherein each unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical, to any one of the amino acid sequences set forth in **TABLE 10.**

[0159] In some embodiments, the cytokine moiety is a tumor associated cell receptor ligand. In some embodiments, the cytokine moiety is a LIGHT unit. In some embodiments, the cytokine moiety is a LIGHT mutant. In some embodiments, the first cytokine moiety is a LIGHT mutant. In some embodiments, the second cytokine moiety is a LIGHT mutant. In some embodiments, the first and second cytokine moiety comprises one or more units wherein each unit is a LIGHT mutant or a portion of a LIGHT mutant. In some embodiments, the LIGHT mutant includes an amino acid sequence set forth in SEQ ID NO: 17 or 18. In some embodiments, the first cytokine moiety comprises one Lymphotoxin-β mutant, and the second cytokine moiety comprises tandem-linked Lymphotoxin-αβ mutant, the Lymphotoxin-β mutant includes an amino acid sequence as shown in SEQ ID NO: 39, the Lymphotoxin-α mutant includes an amino acid sequence as shown in SEQ ID NO: 40.

[0160] In some embodiments, the cytokine moiety comprises at least one amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity or is identical, to any one of the cytokine moiety amino acid sequences described in WO2024/193705, which is

incorporated herein by reference in its entirety. In some embodiments, each unit of the cytokine moiety comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity or is identical to any one of the cytokine moiety amino acid sequences described in WO2024/193705, which is incorporated herein by reference in its entirety.

[0161] As described, the bispecific antibody or antigen-binding fragment thereof could trigger LTβR signaling for cancer-associated fibroblasts (CAF) reprogramming. Without being bound by theory, it is believed that FAP engagement in the bispecific antibody or antigen-binding fragment thereof in cis or trans fashion would enhance the efficiency of LTβR pathway activation.

[0162] LTβR signaling is critical for driving the formation of 2nd lymphoid organs (SLOs) and, in some cases, tertiary lymphoid structures (TLSs). The present disclosure aims to trigger LTβR signaling in FAP+ CAFs (Cancer-associated fibroblasts), and to reprogram them into a TLS-promoting phenotype, consequently enhancing anti-tumor immunity. It is also believed that cross-linking FAP expressed on tumor stromal cells with highly specific LTβR targeting selectively targets LTβR and restricts LTβR agonism exclusively to the tumor microenvironment (tumor endothelium and cancer associated fibroblasts), thereby reducing potential side effects.

[0163] In some embodiments, the bispecific antibody or antigen-binding fragment thereof, for example, produced using platform D, is capable of generating immunocytokine robustly, demonstrating promising anti-tumor efficacy.

[0164] In some embodiments, the bispecific antibody or antigen-binding fragment thereof binds to FAP with dissociation constant (KD, KD=koff/kon, or KD= Kd/Ka) no more than 20 nM, 15 nM, 10 nM or 5 nM. In some embodiments, the bispecific antibody or antigen-binding fragment thereof binds to LTßR with dissociation constant (KD) no more than 20 nM, 15 nM, 10 nM or 5 nM. In some embodiments, the bispecific antibody or antigen-binding fragment thereof barely binds to human or cynomolgus HVEM. In some embodiments, the LIGHT mutant is capable to reduce binding affinity

to DcR3. In some embodiments, the bispecific antibody or antigen-binding fragment thereof specifically binds to human FAP, and/or does not bind to DPPIV. Activities of bispecific antibodies are further described herein.

[0165] In some embodiments, the bispecific antibody or antigen-binding fragment thereof comprises a fusion protein. In some embodiments, the bispecific antibody or antigen-binding fragment thereof comprises an anti-FAP fusion protein. In some embodiments, the bispecific antibody or antigen-binding fragment thereof comprises an anti-FAP cytokine fusion protein. In some embodiments, the bispecific antibody or antigen-binding fragment thereof comprises an anti-FAP-LIGHT fusion protein. In some embodiments, the bispecific antibody or antigen-binding fragment comprises an anti-FAP-Lymphotoxin-αββ fusion protein. In some embodiments, the bispecific antibody or antigen-binding fragment thereof is an anti-FAP-LIGHT fusion protein. In some embodiments, the bispecific antibody or antigen-binding fragment thereof is an anti-FAP-Lymphotoxin-αββ fusion protein. In some embodiments, the fusion protein includes bispecific antibody. Fusion proteins are further described herein.

Second antigen-binding moieties

[0166] In some embodiments, FAP binding portion of the FAP binding agents provided herein are linked, conjugated or fused to a second antibody and/or antigen-binding fragments thereof, a second antigen-binding moiety, or constructs of the same.

[0167] In some embodiments, FAP binding portion of the FAP binding agents provided herein are linked, conjugated or fused to a second antibody to form an antibody heteroconjugate. In some embodiments, FAP binding portion of the FAP binding agents provided herein are linked, conjugated or fused to a second binding moiety generating multispecific antibodies. Multispecific antibodies, such as bispecific antibodies, are monoclonal antibodies that have binding specificities for at least two different targets (*e.g.*, antigens) or two different epitopes on the same target (*e.g.*, a bispecific antibody

directed to FAP with a first binding moiety for a first epitope of a FAP, and a second binding moiety for a second epitope of FAP). In some embodiments, the multispecific (*e.g.*, bispecific) antibodies can be constructed based on the sequences of the antibodies described herein, for example, the CDR sequences in **TABLE 6** and **7**. In some embodiments, the multispecific antibodies described herein are bispecific antibodies. In some embodiments, bispecific antibodies are mouse, chimeric, human or humanized antibodies. In some embodiments, one of the binding specificities of the multispecific antibody is for FAP and the other is for any other target (*e.g.*, antigen). In some embodiments, a multispecific (*e.g.*, bispecific) antibody can comprise more than one target (*e.g.*, antigen) binding moiety, in which different binding moieties are specific for different targets (*e.g.*, a first binding moiety that binds to FAP and a second binding moiety that binds another target (*e.g.*, antigen), such as an immune checkpoint regulator (*e.g.*, a negative checkpoint regulator). In some embodiments, multispecific (*e.g.*, bispecific) antibody molecules can bind than one (*e.g.*, two or more) epitopes on the same target (*e.g.*, antigen). For example, a second binding moiety can be a moiety derived from any one of the additional therapeutic antibodies as further described herein.

[0168] In some embodiments, one of the binding specificities is FAP and the other is for one or more of a TACR. A TACR can be a chemokine receptor, a cell-surface protein such as cell surface protein expressed on immune cells or a tissue- or cell type-specific antigen, or a cell surface molecule associated with T-cell activation.

[0169] For example, a TACR can be a tumor necrosis factor receptor, such as LTβR. In some embodiments, the second antibody fragment is an anti- LTβR antibody described in U.S. 6,312,691, WO 96/22788, WO2018119118, WO9622788, WO2006/114284, WO2004/058191, WO02/30986, WO2022117572 and WO2007146414, the contents of each of which are hereby incorporated herein by reference in their entirety. In some embodiments, the second antibody fragment is BKA11, CDH10, BCG6, AGH1, BDA8, CBE11 or BHA10.

[0170] In another example, a TACR can be a chemokine receptor. Exemplary different chemokine receptors include CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CX3CR1 or CXCR1. For example, the second binding moiety of the bispecific antibody is Mogamulizumab or an antigen-binding fragment thereof.

[0171] Exemplary binding moieties which target a TACR include cell-surface proteins such as cell surface protein expressed on immune cells or a tissue- or cell type-specific antigen. Such binding moieties include an antibody or antigen-binding fragment targeting a checkpoint protein, such as an anti-PD1 antibody, an anti-PD-L1 antibody, or a CTLA-4 antibody. Suitable checkpoint protein targeting antibodies include Nivolumab, Pembrolizumab, Atezolizumab, Avelumab, Durvalumab, Cemiplimab, Dostarlimab, or Ipilimumab. In some embodiments, the second binding moiety of the bispecific antibody is a HER2 targeting antibody, such as Trastuzumab, Pertuzumab and/or Margetuximab. Exemplary cell surface molecules associated with T-cell activation include CD25, CTLA-4, PD-1, LAG3, TIGIT, ICOS, and TNF receptor super family members, 4-1BB, OX-40, and GITR.

[0172] Other second antigen-binding fragments suitable for use with a FAP antigen-binding moiety provided herein are described in WO 2020208049, WO 2018178074, WO 2017060144, WO 2018127473, WO 2020007817, WO 2017055398, WO 2023073225, WO 2023025194, WO 2024175105, WO 2023025194, WO 2024175105, WO 2024179567, WO 2019222449, WO 2019086500, WO 2021236658, CN 113307879, WO 2020230899, WO 2020230899, WO 2020230899, WO 2019086497, WO 2022262496, WO 2022262496, WO 2018178074, WO 2017055398, WO 2018185045, WO 2020127628, WO 2020070041, WO 2020070035, WO 2023117834, WO 2021198335, WO 2021198333, WO 2021198335, WO 2023117834, WO 2021257808, WO 2024133330, WO 2023050826, WO 2024199269, WO 2023050826, WO 2024199269, WO 2019195623, N/A, WO-2018178074, WO-2024179567, N/A, N/A, WO-

2020245173, WO 2016075278, WO 2023110788, WO 2024184287, WO 2024188966, WO-2014161845, WO 2016055432, WO-2022101458, and WO202413330, the contents of each of which are incorporated by reference herein in its entirety. Such second antigen-binding fragments target CD40 molecule (CD40), Fc gamma receptor IIIa (FCGR3A), cytotoxic T-lymphocyte associated protein 4 (CTLA4), CD28 molecule (CD28), CD276 molecule (CD276), CD3 Complex (T Cell Receptor Complex), transforming growth factor beta receptor 2 (TGFbR2) and therefore each of which are suitable secondary targets as described herein.

[0173] In some embodiments, the second antigen-binding moiety is an anti-LTβR binding moiety, anti-HER2 binding moiety, anti-PDL-1 binding moiety, anti-PD-1 binding moiety, anti-EGFR binding moiety, anti-VEGFR binding moiety, anti-VEGF binding moiety, anti-CCR8 binding moiety, anti-OX-40 binding moiety, anti-418B binding moiety, anti-Angiopoietin-2 binding moiety, anti-IL-4Ra binding moiety, anti-BCMA binding moiety, anti-Blys binding moiety, anti-BTNO2 binding moiety, anti-C5 binding moiety, anti-CD122 binding moiety, anti-CD13 binding moiety, anti-CD133 binding moiety, anti-CD137 binding moiety, anti-CD138 binding moiety, anti-CD16a binding moiety, anti-CD19 binding moiety, anti-CD20 binding moiety, anti-CD22 binding moiety, anti-CD27 binding moiety, anti-CD28 binding moiety, anti-CD3 binding moiety, anti-CD30 binding moiety, anti-CD33 binding moiety, anti-CD38 binding moiety, anti-CD40 binding moiety, anti-CD47 binding moiety, anti-CD-8 binding moiety, anti-CEA binding moiety, anti-CGPR/CGRPR binding moiety, anti-CSPGs binding moiety, anti-CTLA4 binding moiety, anti-CTLA-4 binding moiety, anti-DLL-4 binding moiety, anti-EpCAM binding moiety, anti-factor IXa binding moiety, anti-factor X binding moiety, anti-GITR binding moiety, anti-GP130 binding moiety, anti-Her3 binding moiety, anti-HSG binding moiety, anti-ICOS binding moiety, anti-IGFl binding moiety, anti-IGFl/2 binding moiety, anti-IGF-lR binding moiety, anti-IGF2 binding moiety, anti-IGFR binding moiety, anti-IL-1 binding moiety, anti-IL-12 binding moiety, anti-IL-12p40 binding moiety, anti-IL-13 binding moiety, anti-IL-l 7A binding

moiety, anti-IL-1~ binding moiety, anti-IL-23 binding moiety, anti-IL-5 binding moiety, anti-IL-6 binding moiety, anti-IL-6R binding moiety, anti-Lag-3 binding moiety, anti-LAG3 binding moiety, anti-MAG binding moiety, anti-Met binding moiety, anti-NgR binding moiety, anti-NogoA binding moiety, anti-OMGp binding moiety, anti-OX40 binding moiety, anti-PDGFR binding moiety, anti-PSMA binding moiety, anti-RGMA binding moiety, anti-RGMB binding moiety, anti-SARS-CoV-2 binding moiety, anti-Te38 binding moiety, anti-TIM-3 binding moiety, anti-TNF binding moiety, anti-TNFa binding moiety, anti-TROP-2 binding moiety, anti-TWEAK binding moiety, or anti-TRAIL binding moiety.

[0174] In some embodiments, a bispecific antibody provided herein comprises a FAP antigen-binding moiety and a second antigen-binding moiety targeting a second target, and is attached to a drug moiety. In some embodiments, the second antigen-binding moiety is sacituzumab, the drug moiety is govitecan, and the second target is TROP2. In some embodiments, the second antigen-binding moiety is tisotumab, the drug moiety is vedotin, and the second target is Tissue Factor. In some embodiments, the second antigen-binding moiety is enfortumab, the drug moiety is vedotin, and the second target is Nectin4. In some embodiments, the second antigen-binding moiety is brentuximab, the drug moiety is vedotin, and the second target is CD30. In some embodiments, the second antigen-binding moiety is trastuzumab, the drug moiety is deruxtecan, and the second target is HER2. In some embodiments, the second antigen-binding moiety is trastuzumab, the drug moiety is emtansine, and the second target is HER2. In some embodiments, the second antigen-binding moiety is polatuzumab, the drug moiety is vedotin, and the second target is CD79. In some embodiments, the second antigen-binding moiety is inotuzumab, the drug moiety is ozogamicin, and the second target is CD22. In some embodiments, the second antigen-binding moiety is gemtuzumab, the drug moiety is ozogamicin, and the second target is CD33. In some embodiments, the second antigen-binding moiety is loncastuximab, the drug moiety is tesirine, and the second target is CD19. In some embodiments, the second antigen-

binding moiety is belantamab, the drug moiety is mafodotin, and the second target is BCMA. In some embodiments, the second antigen-binding moiety is mirvetuximab, the drug moiety is soravtansine, and the second target is FRα. In some embodiments, the second antigen-binding moiety is moxetumomab, the drug moiety is pasudotox, and the second target is CD22.

[0175] In some embodiments, a bispecific antibody described herein comprises a fusion protein.

Fusion Protein

[0176] Provided herein is a fusion protein or a use thereof. In some embodiments, the present disclosure relates to a fusion protein comprised in a bispecific antibody. In some embodiments, a bispecific antibody described herein comprises a fusion protein comprising a FAP antigen-binding moiety or a portion thereof (*e.g.* anti-FAP VH comprising CDRH1, CDRH2, and CDRH3) fused to one or more additional binding moiety, and optionally one or additional moieties. In some embodiments, an additional binding moiety is a second binding moiety as described herein. In some embodiments, a second binding moiety can be a moiety which targets a TACR. In some embodiments, a second binding moiety can be a cytokine moiety or a second antibody moiety which targets a TACR. In some embodiments, the additional moiety can be any one or more of a masking/cleavable moiety, detectable/diagnostic agents, an effector cell or a portion thereof, a heterologous protein or a moiety thereof, a drug moiety such as a cytolytic agent, or a linker.

[0177] In some embodiments, a bispecific antibody described herein comprises a fusion protein comprising a FAP antigen-binding moiety or a portion thereof (*e.g.*, anti-FAP VH comprising CDRH1, CDRH2, and CDRH3) fused to an additional binding moiety. A binding moiety includes, in some embodiments, the specific region or component of a molecule (*e.g.,* a peptide, polypeptide, or protein) or a complex of molecules (*e.g.,* two or more peptides, polypeptides, or proteins) that directly interacts with and binds to a target molecule. This moiety can include the functional groups or structural

elements that facilitate the recognition and binding specificity to the target's binding site. In some embodiments, a bispecific antibody described herein comprises a fusion protein comprising a FAP antigen-binding moiety or a portion thereof (*e.g.*, anti-FAP VH comprising CDRH1, CDRH2, and CDRH3) fused to a linker fused to one or more additional binding moiety. In some embodiments, a bispecific antibody described herein comprises a FAP antigen-binding moiety or a portion thereof (*e.g.*, anti-FAP VH comprising CDRH1, CDRH2, and CDRH3) fused to a linker fused to an additional binding moiety, fused to a linker, fused to tandem linked additional binding moieties. In some embodiments, the fusion protein has the format D as shown in Figure 2 or 6.

[0178] In some embodiments, the present disclosure relates to a fusion protein, including a FAP antigen-binding moiety or a portion thereof (*e.g.*, anti-FAP VH comprising CDRH1, CDRH2, and CDRH3), and a cytokine moiety capable of stimulating immune cells. In some embodiments, the fusion protein provided herein is an anti-FAP cytokine fusion protein.

[0179] In some embodiments, the FAP antigen-binding moiety includes part or full length of the anti-FAP antibody or antigen-binding fragment thereof described above.

[0180] In some embodiments, the bispecific antibody comprises two or more fusion proteins. In some embodiments, the bispecific antibody comprises two or more fusion proteins wherein the first fusion protein comprises a heavy chain 1 (HC1) region and the second fusion protein comprises a heavy chain 2 (HC2) region.

[0181] In some embodiments, the fusion protein comprises a heavy chain 1 (HC1) region comprising a VH, a heavy chain constant domain 1 (CH1), and a Fc fragment comprising a heavy chain constant domain 2 (CH2) and a heavy chain constant domain 3 (CH3). In some embodiments, the HC1 region comprises an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 1, 4, 5, 8, 10, 13, 14, and 15. In some embodiments, the HC1 region comprises

an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 5, 8, 10, 13, 14, and 15. In some embodiments, the Fc fragment of the HC1 region comprises one or more units of the second binding moiety fused to a C-terminus of the Fc fragment. In some embodiments, the Fc fragment of the HC1 region comprises a first unit of the second binding moiety fused to the C-terminus of the HC1 Fc fragment. In some embodiments, the first unit of the second binding moiety is fused to the Fc unit by a first linker. In some embodiments, the second binding moiety is a cytokine moiety, and wherein the cytokine moiety comprises a first cytokine unit. In some embodiments, the first cytokine unit comprises a LIGHT unit or a lymphotoxin β unit. In some embodiments, the first unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to any one of the amino acid sequences set forth in **TABLE 10**. In some embodiments, the unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to SEQ ID NO: 17 or SEQ ID NO: 39.

[0182] In some embodiments, the fusion protein comprises a heavy chain 2 (HC2) region comprising a VH, a CH1, and a Fc fragment comprising a CH1 and a CH3. In some embodiments, the HC2 region comprises an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 1, 4, 6, 9, 11, 12, 13, and 16. In some embodiments, the HC2 region comprises an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 6, 9, 11, 12, 13, and 16. In some embodiments, the Fc fragment of the HC2 region comprises one or more units of the second binding moiety fused to a C-terminus of the HC2 Fc fragment. In some embodiments, the Fc fragment of the HC2 region comprises a second unit and a

third unit of the second binding moiety. In some embodiments, the second unit of the second binding moiety is fused to the Fc fragment of the HC2 region. In some embodiments, third unit of the second binding moiety is fused to the second unit of the second binding moiety. In some embodiments, the second binding moiety is fused to the Fc fragment of the HC2 region by a second linker and the third unit is fused to the second unit of the second binding moiety by a third linker. In some embodiments, the second unit and third unit of the second binding moiety unit are tandem linked. In some embodiments, the second binding moiety is a cytokine moiety, and wherein the cytokine moiety comprises a second cytokine unit and a third cytokine unit. In some embodiments, the second cytokine unit comprises a LIGHT unit, a lymphotoxin α unit, or a lymphotoxin β unit. In some embodiments, the third cytokine unit comprises a LIGHT unit, a lymphotoxin α unit, or a lymphotoxin β unit. In some embodiments, the second cytokine unit comprises a LIGHT unit, and the third cytokine unit comprises a LIGHT unit. In some embodiments, the second cytokine unit comprises a lymphotoxin α unit, and the third cytokine unit comprises a lymphotoxin β unit. In some embodiments, the second unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to any one of the amino acid sequences set forth in **TABLE 10**. In some embodiments, the second unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to SEQ ID NO: 17 or SEQ ID NO: 40. In some embodiments, the third unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to any one of the amino acid sequences set forth in **TABLE 10**. In some embodiments, the third unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to SEQ ID NO: 17 or SEQ ID NO: 39.

[0183] In some embodiments, the fusion protein comprises one or more linkers. Linkers are further described herein.

[0184] In some embodiments, the fusion protein comprises one or more heterodimerization modifications. In some embodiments, the HC1 region comprises one or more heterodimerization modification. In some embodiments, the one or more heterodimerization modification of the HC1 region is a knob modification or a hole modification. In some embodiments, the one or more heterodimerization modification of the HC1 region is a hole modification. In some embodiments, the HC2 comprises one or more heterodimerization modification. In some embodiments, the one or more heterodimerization modification of the HC2 region is a knob modification or a hole modification. In some embodiments, the one or more heterodimerization modification of the HC2 region is a knob modification.

Exemplary Anti-FAP antigen-binding moiety

[0185] In some embodiments, the FAP antigen-binding moiety includes LCDR1 having an amino acid sequence as shown in KTNQNVDYX$_1$GNTFMH (SEQ ID NO: 23), wherein X$_1$ is N or S, LCDR2 having an amino acid sequence as shown in LASNLAS (SEQ ID NO: 24), LCDR3 having an amino acid sequence as shown in QQSRNLPYT (SEQ ID NO: 25), HCDR1 having an amino acid sequence as shown in IYGVN (SEQ ID NO: 26), HCDR2 having an amino acid sequence as shown in AIWSGGRKDYX$_2$LSLKS (SEQ ID NO: 27) , wherein X$_2$ is N or S, and HCDR3 having an amino acid sequence as shown in SQDMPGYFDY (SEQ ID NO: 28).

[0186] In some embodiments, the FAP antigen-binding moiety includes LCDR1 having an amino acid sequence as shown in SASSRVGYMH (SEQ ID NO: 29), LCDR2 having an amino acid sequence as shown in DTSKLAS (SEQ ID NO: 30), LCDR3 having an amino acid sequence as shown in FQGSGYPFT (SEQ ID NO: 31), HCDR1 having an amino acid sequence as shown in TAGMSVG (SEQ ID NO: 32), HCDR2 having an amino acid sequence as shown in DIWWDDKKHYNPSLKD (SEQ ID NO: 33), and HCDR3 having an amino acid sequence as shown in DMIFNFYFDV (SEQ ID NO: 34).

[0187] In some embodiments, the FAP antigen-binding moiety includes a VH and/or a VL as the same as the VH and/or a VL of anti-FAP antibody or antigen-binding fragment thereof described above.

[0188] In some embodiments, the fusion protein includes a first heavy chain, a second heavy chain, and two light chains paired with the first heavy chain and the second heavy chain, respectively, wherein: (1) the first heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence as shown in SEQ ID NO: 3; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence as shown in SEQ ID NO: 5; the light chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence as shown in SEQ ID NO: 6; . (2) the first heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence as shown in SEQ ID NO: 7; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence as shown in SEQ ID NO: 8; the light chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence as shown in SEQ ID NO: 9; (3) the first heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence as shown in SEQ ID NO: 3; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence as shown in SEQ ID NO: 10; the light chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence as shown in SEQ ID NO: 11; (4) the first heavy chain comprising an amino acid sequence having at least 80%, 85%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence as shown in SEQ ID NO: 3; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence as shown in SEQ ID NO: 15; the light chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence as shown in SEQ ID NO: 16.

[0189] In some embodiments, the fusion protein includes the first heavy chain having an amino acid sequence as shown in SEQ ID NO: 3, the second heavy chain having an amino acid sequence as shown in SEQ ID NO: 5, and the light chain having an amino acid sequence as shown in SEQ ID NO: 6. In some embodiments, the fusion protein includes the first heavy chain having an amino acid sequence as shown in SEQ ID NO: 7, the second heavy chain having an amino acid sequence as shown in SEQ ID NO: 8, and the light chain having an amino acid sequence as shown in SEQ ID NO: 9. In some embodiments, the fusion protein includes the first heavy chain having an amino acid sequence as shown in SEQ ID NO: 3, the second heavy chain having an amino acid sequence as shown in SEQ ID NO: 10, and the light chain having an amino acid sequence as shown in SEQ ID NO: 11. In some embodiments, the fusion protein includes the first heavy chain having an amino acid sequence as shown in SEQ ID NO: 3, the second heavy chain having an amino acid sequence as shown in SEQ ID NO: 15, and the light chain having an amino acid sequence as shown in SEQ ID NO: 16.

[0190] Further CDRs, VHs, VLs, HCs, and/or LCs of FAP antigen-binding moiety are described in detail in the FAP binding agent section herein.

[0191] In some embodiments, the fusion protein includes a Fc fragment derived from immunoglobulin at N-terminus of the FAP antigen-binding moiety. In some embodiments, the Fc fragment is derived from IgG1, IgG2, IgG3, or IgG4, optionally the Fc fragment is derived from IgG4. In some embodiments, the Fc fragment includes mutation S228P. In some embodiments, the Fc

fragment includes LALAPG mutation. In some embodiments, the Fc fragment includes one or more modifications selected from the group consisting of knobs-into-holes, DDKK, electrostatic steering of CH3, DuoBody, SEEDbodies, cFAE, XmAb, Azymetric, and BEAT®. In some embodiments, the Fc fragment includes modifications knobs-into-holes and/or DDKK.

[0192] In the present disclosure, the fusion protein could include conservatively modified variants, such as FR region or Fc region. The conservatively modified variants include individual substitutions, deletions or additions to the polypeptide sequence which result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art and are further described herein. Other amino acid modifications are further described herein.

Exemplary Cytokine Moieties

[0193] Exemplary cytokine moieties are described in detail herein, for example in the bispecific antibody section. The cytokine moiety can be at the C-terminus of the fusion protein. In some embodiments, the cytokine moiety is operably linked to the C-terminus of the Fc fragment. In some embodiments, the cytokine moiety links to the Fc fragment directly. In some embodiments, the cytokine moiety links to the Fc fragment via a linker. In some embodiments, the cytokine moiety comprises a first cytokine moiety and a second cytokine moiety, the first cytokine moiety comprises one cytokine mutant and the second cytokine moiety comprises tandem-linked two cytokine mutants, the first cytokine moiety and the second cytokine moiety link to different C-terminus of the protein fragments within the Fc fragment. In some embodiments, the first cytokine moiety links to the C-terminus of the Fc fragment with hole mutations, and the second cytokine moiety links to the C-terminus of the Fc fragment with knob mutations.

[0194] In some embodiments, the cytokine mutant is a LIGHT mutant, the first cytokine moiety contains one LIGHT mutant, and the second cytokine moiety contains tandem-linked two LIGHT

mutants. In some embodiments, the first cytokine moiety comprises one Lymphotoxin-β mutant, and the second cytokine moiety comprises tandem-linked Lymphotoxin-αβ mutant, the Lymphotoxin-β mutant includes an amino acid sequence as shown in SEQ ID NO:39, the Lymphotoxin-α mutant includes an amino acid sequence as shown in SEQ ID NO:40. In some embodiments, the first cytokine moiety links to the Fc fragment via linker A, and the second cytokine moiety links to the Fc fragment via linker B. In some embodiments, tandem-linked two LIGHT mutants link to each other directly or via a linker C. In some embodiments, the LIGHT mutant includes an amino acid sequence as shown in SEQ ID NO: 17 or 18.

[0195] In some embodiments, the fusion protein binds to FAP with dissociation constant (KD, KD=koff/kon, or KD= Kd/Ka) no more than 20 nM, 15 nM, 10 nM or 5 nM. In some embodiments, the fusion protein binds to LTßR with dissociation constant (KD) no more than 20 nM, 15 nM, 10 nM or 5 nM. In some embodiments, the fusion protein barely binds to human or cynomolgus HVEM. In some embodiments, the LIGHT mutant is capable to reduce binding affinity to DcR3. In some embodiments, the fusion protein specifically binds to human FAP, and/or does not bind to DPPIV. Activity of fusion proteins are further described herein.

[0196] The fusion protein, produced using platform D, is capable of generating immunocytokine robustly, demonstrating promising anti-tumor efficacy.

Masking/Cleavable moieties

[0197] In some embodiments, FAP binding portion of the fusion protein (*e.g.*, the anti-FAP antibody, the FAP antigen-binding moiety, or the bispecific antibody comprising the FAP antigen-binding moiety) is linked, fused, or conjugated (directly or indirectly), to a masking moiety and/or cleavable moiety in which one or more of the FAP binding moieties of the FAP binding portion of the fusion protein (*e.g.*, the anti-FAP antibody, the FAP antigen-binding moiety, or the bispecific antibody comprising the FAP antigen-binding moiety) are masked (*e.g.*, via a masking moiety) and/or

activatable (*e.g.*, via a cleavable moiety). Technologies for masking of a FAP binding portion of the fusion protein (*e.g.*, the anti-FAP antibody, the FAP antigen-binding moiety, or the bispecific antibody comprising the FAP antigen-binding moiety) are well known in the art, including SAFE body masking technology (see, *e.g.*, US Patent Application Publication No. 2019/0241886) and Probody masking technology (see, *e.g.*, US Patent Application Publication No. 2015/0079088). Such technologies can be used to generate a FAP binding portion of the fusion protein (*e.g.*, the anti-FAP antibody, the FAP antigen-binding moiety, or the bispecific antibody comprising the FAP antigen-binding moiety) that is masked and/or activatable. Such masked and/or activatable FAP binding portions of the fusion protein (*e.g.*, the anti-FAP antibody, the FAP antigen-binding moiety, or the bispecific antibody comprising the FAP antigen-binding moiety) are useful for the preparation of conjugates, including immunoconjugates, antibody-drug conjugates (ADCs), masked ADCs and activatable antibody-drug conjugates (AADCs), comprising any one of the FAP binding portions of the fusion protein (*e.g.*, the anti-FAP antibody, the FAP antigen-binding moiety, or the bispecific antibody comprising the FAP antigen-binding moiety), such as human FAP binding portions of the fusion protein, of the present disclosure, including those directly or indirectly linked to a second agent with effector function.

[0198] In some embodiments, FAP binding portions of the fusion protein is linked, fused, or conjugated (directly or indirectly), to a second agent. In some embodiments, a second agent comprises a diagnostic agent, a detectable agent, or a therapeutic agent, such as a cytolytic agent, an effector cell, or a heterologous protein.

Detectable/Diagnostic agents

[0199] In some embodiments, FAP binding portions of the fusion protein provided herein can be linked, fused or conjugated to a detectable agent or a diagnostic agent. Examples of detectable agents include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals, nonradioactive

paramagnetic metal ions and reactive moieties. The detectable agent can be coupled or conjugated either directly to the antibody or fragment thereof or indirectly, *e.g.* through a linker known in the art or another moiety, using techniques known in the art. Examples of enzymatic labels include luciferases (*e.g.*, firefly luciferase and bacterial luciferase; U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, β-galactosidase, acetylcholinesterase, glucoamylase, lysozyme, saccharide oxidases (*e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like.

[0200] Examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include 125I, 131I, 111In or 99mTc.

[0201] Detection of expression of a FAP generally involves contacting a biological sample (tumor, cells, tissue, or body fluid of an individual) with one or more FAP binding portion of a fusion protein provided herein (optionally conjugated to a detectable moiety), and detecting whether or not the sample is positive for FAP, or whether the sample has altered (*e.g.*, reduced or increased) expression as compared to a control sample. Further detectable agents and diagnostic agents are described further herein, for example as detectable labels and/or diagnostic labels.

Effector cells

[0202] In some embodiments, a FAP binding portion of a fusion protein provided herein is linked, conjugated or fused to an effector cell. In some embodiments, an effector cell comprises an immune

cell as described herein (*e.g.*, NK cell, dendrite, B cell, macrophage, and the like) or a T cell expressing a chimeric antigen receptor (CAR T cell) engineered for chemokine receptor or FAP targeting. Recently, CAR T cells have gained attention from their clinical successes and expedited FDA approvals, cf. WO2020102240, incorporated herein in its entirety. In the CAR T cell approach, T cells are collected from patient blood and are then genetically engineered to express CARs that are specific for an antigen present on tumor cells. These engineered T cells are then re-administered to the same patient. Upon injection, CAR T cells recognize the targeted antigen on target cells to induce target cell death. CAR T cells thus constitute a novel modality for medical uses such as tumor treatment. The chimeric antigen receptor (CAR) is a genetically engineered receptor that is designed to target a specific antigen, for example, a tumor antigen. This targeting can result in cytotoxicity against the tumor, for example, such that CAR T cells expressing CARs can target and kill tumors via the specific tumor antigens. According to the present disclosure, the FAP binding portion of a fusion protein as described herein provided for FAP recognition can be used to engineer CAR T cells for specific recognition of FAP expressing cells. CARs encompassed herein can comprise a) a recognition region, *e.g.*, a single chain fragment variable (scFv) region derived from a provided anti-FAP or anti-chemokine receptor antibody for recognition and binding to the FAP expressed by the target cell, and b) an activation signaling domain, *e.g.*, the CD3 chain of T cells, which can serve as a T cell activation signal in CARs.

[0203] In some embodiments, CARs provided herein comprise a co-stimulation domain (*e.g.*, CD137, CD28 or CD134) to achieve prolonged activation of T cells *in vivo*. Addition of a co-stimulation domain enhances the *in vivo* proliferation and survival of T cells containing CARs, and initial clinical data have shown that such constructs are promising therapeutic agents in the treatment of diseases, such as cancer. In some embodiments, CAR T cells provided herein are useful in methods

as described herein. For example, such CAR T cells can be used to treat any disease with local or systemic aberrant presence of cells expressing FAP, such as tumor associated T cells.

Heterologous proteins

[0204] In some embodiments, a FAP binding portion of a fusion protein provided herein is linked, conjugated or fused to a heterologous protein or polypeptide (or fragment thereof, for example, to a polypeptide (*e.g.*, of about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 80, about 90, or about 100 amino acids). In some embodiments, the linking, conjugation and/or fusion of heterologous proteins to a FAP binding portion of a fusion protein of the present disclosure generates fusion proteins, accordingly, provided herein are fusion proteins as well as uses thereof. In some embodiments, described herein are fusion proteins comprising an antigen-binding fragment of a FAP binding portion of the fusion protein (*e.g.*, the anti-FAP antibody, the FAP antigen-binding moiety, or the bispecific antibody comprising the FAP antigen-binding moiety) , including a human FAP binding agent, described herein (*e.g.*, comprising CDR1, CDR2, and/or CDR3 of VH and/or VL) and a heterologous protein, polypeptide, or peptide. In some embodiments, the heterologous protein, polypeptide, or peptide that a FAP binding portion of the fusion protein (*e.g.*, the anti-FAP antibody, the FAP antigen-binding moiety, or the bispecific antibody comprising the FAP antigen-binding moiety) is linked to is useful for targeting the FAP binding agent to a particular cell (*e.g.*, a FAP-expressing cell, including a tumor cell). In some embodiments, the heterologous protein is a signal peptide. In some embodiments, the heterologous protein is a cell penetrating peptide. In some embodiments, the heterologous protein is a subcellular localization signal.

Cytolytic agents

[0205] In some embodiments, a FAP binding portion of a fusion protein provided herein is linked, conjugated or fused to one or more cytolytic agents. As used herein, a cytolytic agent is a moiety that reduces the proliferative capacity of one or more cells. A cell has reduced proliferative capacity when the cell becomes less able to proliferate, for example, because the cell undergoes apoptosis or otherwise

dies, the cell fails to proceed through the cell cycle and/or fails to divide, the cell differentiates, *etc*. Nonlimiting exemplary cytolytic agents include, but are not limited to, radioisotopes, photosensitizers (PS), cytotoxins, and chemotherapeutic agents.

[0206] In some embodiments, a FAP binding portion of a fusion protein provided herein can be linked, fused or conjugated to one or more radioisotopes, also referred to herein as radionuclides. Exemplary radionuclides include: a beta particle, an alpha particle, or an Auger electron emitter. Suitable beta emitters are for example yttrium-90, iodine-131, strontium-89-chloride, lutetium-177, holmium-166, rhenium-186, rhenium-188, copper-67, promethium-149, gold-199, and rhodium-105. Suitable Auger electron emitters are for example bromine-77, indium-111, iodine-123, and iodine-125. Suitable alpha emitters are for example thorium-227, bismuth-213, radium-223, actinium-225 and astatine-211.

[0207] For example, thorium-227 (227Th) can be efficiently complexed with octadentate 3,2-hydroxypyridinone (3,2-HOPO) chelators that are conjugated to a FAP binding portion of a fusion protein according to the current disclosure, resulting in highly stable targeted thorium-227 conjugates (TTCs). Targeted thorium conjugates (TTCs) comprise three main building blocks. Following the β-particle decay of actinium-227, the first building block, α-particle-emitting radionuclide 227Th is purified by ion exchange chromatography. The second building block is a chelator, such as a siderophore-derived chelator containing HOPO groups bearing four 3-hydroxy-N-methyl-2-pyridinone moieties on a symmetrical polyamine scaffold functionalized with a carboxylic acid linker for bioconjugation. Conjugation to a targeting moiety can be achieved through the amide bond formation with the ε-amino groups of lysine residues. These octadentate 3,2-HOPO chelators can be efficiently labeled with 227Th, with high yield, purity, and stability at ambient conditions. Compared with the tetra-azacyclododecane-1,4,7,10-tetraacetic acid (DOTA) chelator, which often requires heating, the HOPO chelators are superior due to efficient radiolabeling at ambient temperatures and

high stability of formed complexes. The third building block is the targeting moiety, that is the FAP binding portion of a fusion protein provided herein.

[0208] In some embodiments, a FAP binding portion of a fusion protein provided herein can be linked, fused or conjugated to one or more photosensitizers (PS). Photodynamic therapy (PDT) is a non-invasive treatment that involves the accumulation of a PS in solid tumors followed by the localized delivery of light of the correct wavelength to cause activation of the PS, which, in the presence of oxygen, leads to the in situ generation of reactive oxygen species (ROS) that cause damage to cellular components and, ultimately, necrosis or apoptosis. PDT is a promising tool in oncology but is frequently limited by side-effects caused by inadequate targeting of the photosensitizer. Accordingly, encompassed by the present disclosure is the conjugation of PS's to tumor-specific binding agents (*e.g.*, antibodies). Also provided herein is the use of antigen-binding Ab fragments, *e.g.*, Fab or scFv fragments, as, in some embodiments, antigen-binding fragments retain the same binding specificity as full-size antibodies, but are more efficient at penetrating tumor masses due to their smaller size and are more effectively cleared from the circulation because of the lack of an Fc domain. In some embodiments, provided are porphyrins for use in the field of photodynamic therapy and photodiagnosis, and are one of the most prominent classes of photosensitizer in these areas of biomedical science (Sandland J, Boyle RW. Bioconjug Chem. 30(4):975-993 (2019)). In some embodiments, the photosensitizer is a tetrapyrrolic macrocycle. In some embodiments, the tetrapyrrolic macrocycle is a porphyrin, a chlorin, a bacteriochlorin, or a phthalocyanine.

[0209] In some embodiments, a FAP binding portion of a fusion protein provided herein can be linked, fused or conjugated to one or more cytotoxic agents, which can in some embodiments from antibody drug conjugate (ADC), referred to an anti-FAP ADC herein. One skilled in the art can select a suitable cytotoxin according to the intended application. In some embodiments, the cytolytic agent

is at least one of an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, or an apoptotic agent.

[0210] In some embodiments, the cytotoxic agent is an auristatin, a maytansinoid, a kinesin-spindle protein (KSP) inhibitor, a nicotinamide phosphoribosyltransferase (NAMPT) inhibitor or a pyrrolobenzodiazepine derivative. Generation of conjugates comprising maytansinoid may occur as described in Chari, Ravi V J, *et al.* Cancer research 52.1 (1992): 127-131, or EP2424569 B1, both incorporated herein in their entirety. Generation of conjugates comprising kinesin-spindle protein (KSP) inhibitors may occur as described in WO2019243159 A1, incorporated herein in its entirety. Generation of conjugates comprising a nicotinamide phosphoribosyltransferase (NAMPT) inhibitor may occur as described in WO2019149637 A1, incorporated herein in its entirety. Generation of conjugates comprising a pyrrolobenzodiazepine may be obtained as described in EP3355935 A1, incorporated herein in its entirety.

[0211] The cytotoxic and/or cytostatic agent of the anti-FAP ADC may be any agent known to inhibit the growth and/or replication of, and/or kill cells. Numerous agents having cytotoxic and/or cytostatic properties are known in the literature. Non-limiting examples of classes of cytotoxic and/or cytostatic agents include, by way of example and not limitation, cell cycle modulators, apoptosis regulators, kinase inhibitors, protein synthesis inhibitors, alkylating agents, DNA cross-linking agents, intercalating agents, mitochondria inhibitors, nuclear export inhibitors, topoisomerase I inhibitors, topoisomerase II inhibitors, RNA/DNA antimetabolites and antimitotic agents.

[0212] The linkers linking the cytotoxic and/or cytostatic agent(s) to the antigen-binding moiety of an anti-FAP ADC may be long, short, flexible, rigid, hydrophilic or hydrophobic in nature, or may comprise segments that have different characteristics, such as segments of flexibility, segments of rigidity, *etc.* The linker may be chemically stable to extracellular environments, for example, chemically stable in the blood stream, or may include linkages that are not stable and release the

cytotoxic and/or cytostatic agents in the extracellular milieu. In some embodiments, the linkers include linkages that are designed to release the cytotoxic and/or cytostatic agents upon internalization of the anti-FAP ADC, within the cell. In some specific embodiments, the linkers include linkages designed to cleave and/or immolate or otherwise breakdown specifically or non-specifically inside cells. A wide variety of linkers useful for linking drugs to antigen-binding moieties such as antibodies in the context of ADCs are known in the art. Any of these linkers, as well as other linkers, may be used to link the cytotoxic and/or cytostatic agents to the antigen-binding moiety of the anti-FAP ADCs, described herein.

[0213] The number of cytotoxic and/or cytostatic agents linked to the antigen-binding moiety of an anti-FAP ADC (drug-to-antibody ratio: DAR) can vary and will be limited only by the number of available attachments sites on the antigen-binding moiety and the number of agents linked to a single linker. Typically, a linker will link a single cytotoxic and/or cytostatic agent to the antigen-binding moiety of anti-FAP ADC. In embodiments of anti-FAP ADC, which include more than a single cytotoxic and/or cytostatic agent, each agent may be the same or different. As long as the anti-chemokine receptor or anti-FAP ADC, does not exhibit unacceptable levels of aggregation under the conditions of use and/or storage, anti-FAP ADCs, with DARs of twenty, or even higher, are contemplated. In some embodiments, the anti-FAP ADC s, described herein may have a DAR in the range of about 1-10, 1-8, 1-6, or 1-4. In certain specific embodiments, the anti-FAP ADC may have a DAR of 2, 3 or 4. In some embodiments, the anti-FAP ADCs, are compounds according to structural formula (1):

$$[\text{D-L-XY}]n\text{-Ab}$$

Formula 1

or salts thereof, where each “D” represents, independently of the others, a cytotoxic and/or cytostatic agent; each “L” represents, independently of the others, a linker; “Ab” represents an anti-FAP receptor

binding moiety, *e.g.* an anti-FAP antibody provided herein; each "XY" represents a linkage formed between a functional group Rx on the linker and a "complementary" functional group Ry on the anti-chemokine receptor binding moiety; and n represents the DAR of the anti-chemokine receptor ADC.

[0214] In a specific exemplary embodiment, the anti-FAP ADCs are compounds according to structural formula (1) in which each "D" is the same and is either a cell-permeating auristatin (for example, dolastatin-10 or MMAE) or a cell-permeating minor groove-binding DNA cross-linking agent; each "L" is the same and is a linker cleavable by a lysosomal enzyme; each "XY" is a linkage formed between a maleimide and a sulfhydryl group; "Ab" is an antibody or fragment thereof comprising six CDRs corresponding to the six CDRs of an anti-chemokine receptor or FAP antibody according to the current disclosure; and n is 2, 3 or 4. In a specific embodiment "Ab" is a fully human antibody comprising human derived CDRs.

[0215] Cytotoxic and cytostatic agents are agents known to inhibit the growth and/or replication of and/or kill cells and in particular tumor cells or intra-tumoral Treg cells. These compounds may be used in a combination therapy with an anti-chemokine receptor antibody such as a FAP antibody, or as part of an anti-chemokine receptor ADC as described herein: In some embodiments, the drug moiety of the anti-chemokine receptor or anti-FAP ADC is a cytostatic agent selected from radionuclides, alkylating agents, DNA cross-linking agents, DNA intercalating agents (*e.g.*, groove binding agents such as minor groove binders), cell cycle modulators, apoptosis regulators, kinase inhibitors, protein synthesis inhibitors, mitochondria inhibitors, nuclear export inhibitors, topoisomerase I inhibitors, topoisomerase II inhibitors, RNA/DNA antimetabolites and antimitotic agents. In some embodiments, the drug moiety of the anti-chemokine receptor or anti-FAP ADC is an alkylating agent selected from asaley (L-Leucine, N—[N-acetyl-4-[bis-(2-chloroethyl)amino]-DL-phenylalanyl]-, ethylester); AZQ (1,4-cyclohexadiene-1,4-dicarbamic acid, 2, 5-bis(1-aziridinyl)-3,6-dioxo-, diethyl ester); BCNU (N,N′-Bis(2-chloroethyl)-N-nitrosourea); busulfan (1,4-butanediol dimethanesulfonate);

(carboxyphthalato)platinum; CBDCA (cis-(1,1-cyclobutanedicarboxylato)diammineplatinum(II))); CCNU (N-(2-chloroethyl)-N′-cyclohexyl-N-nitrosourea); CHIP (iproplatin; NSC 256927); chlorambucil; chlorozotocin (2-[[[(2-chloroethyl) nitrosoamino]carbonyl]amino]-2-deoxy-D-glucopyranose); cis-platinum (cisplatin); clomesone; cyanomorpholinodoxorubicin; cyclodisone; dianhydrogalactitol (5,6-diepoxydulcitol); fluorodopan ((5-[(2-chloroethyl)-(2-fluoroethy)amino]-6-methyl-uracil); hepsulfam; hycanthone; indolinobenzodiazepine dimer DGN462; melphalan; methyl CCNU ((1-(2-chloroethyl)-3-(trans-4-methylcyclohexane)-1-nitrosourea); mitomycin C; mitozolamide; nitrogen mustard ((bis(2-chloroethyl) methylamine hydrochloride); PCNU ((1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea)); piperazine alkylator ((1-(2-chloroethyl)-4-(3-chloropropyl)-piperazine dihydrochloride)); piperazinedione; pipobroman (N,N′-bis(3-bromopropionyl) piperazine); porfiromycin (N-methylmitomycin C); spirohydantoin mustard; teroxirone (triglycidylisocyanurate); tetraplatin; thio-tepa (N,N′,N″-tri-1,2-ethanediylthio phosphoramide); triethylenemelamine; uracil nitrogen mustard (desmethyldopan); Yoshi-864 ((bis(3-mesyloxy propyl)amine hydrochloride).

[0216] In some embodiments, the drug moiety of anti-FAP ADC is a DNA alkylating-like agent selected from Cisplatin; Carboplatin; Nedaplatin; Oxaliplatin; Satraplatin; Triplatin tetranitrate; Procarbazine; altretamine; dacarbazine; mitozolomide; temozolomide.

[0217] In some embodiments, the drug moiety of the anti-chemokine receptor or anti-FAP ADC is an alkylating antineoplastic agents selected from Carboquone; Carmustine; Chlornaphazine; Chlorozotocin; Duocarmycin; Evofosfamide; Fotemustine; Glufosfamide; Lomustine; Mannosulfan; Nimustine; Phenanthriplatin; Pipobroman; Ranimustine; Semustine; Streptozotocin; ThioTEPA; Treosulfan; Triaziquone; Triethylenemelamine; Triplatin tetranitrate.

[0218] In some embodiments, the drug moiety of anti-FAP ADC is a DNA replication and repair inhibitor selected from Altretamine; Bleomycin; Dacarbazine; Dactinomycin; Mitobronitol;

Mitomycin; Pingyangmycin; Plicamycin; Procarbazine; Temozolomide; ABT-888 (veliparib); olaparib; KU-59436; AZD-2281; AG-014699; BSI-201; BGP-15; INO-1001; ONO-2231.

[0219] In some embodiments, the drug moiety of the anti-FAP ADC is a cell cycle modulator, such as Paclitaxel; Nab-Paclitaxel; Docetaxel; Vincristine; Vinblastine; ABT-348; AZD-1152; MLN-8054; VX-680; Aurora A-specific kinase inhibitors; Aurora B-specific kinase inhibitors and pan-Aurora kinase inhibitors; AZD-5438; BMI-1040; BMS-032; BMS-387; CVT-2584; flavopyridol; GPC-286199; MCS-5A; PD0332991; PHA-690509; seliciclib (CYC-202, R-roscovitine); ZK-304709; AZD4877, ARRY-520: GSK923295A.

[0220] In some embodiments, the drug moiety of the anti-FAP ADC is an apoptosis regulator such as AT-101 ((−)gossypol); G3139 or oblimersen (Bcl-2-targeting antisense oligonucleotide); IPI-194; IPI-565; N-(4-(4-((4′-chloro(1,1′-biphenyl)-2-yl)methyl)piperazin-1-ylbenzoyl)-4-(((1R)-3-(dimethylamino)-1-((phenylsulfanyl)methyl)propyl)amino)-3-nitrobenzenesulfonamide); N-(4-(4-((2-(4-chlorophenyl)-5,5-dimethyl-1-cyclohex-1-en-1-yl)methyl)piperazin-1-yl)benzoyl)-4-(((1R)-3-(morpholin-4-yl)-1-((phenylsulfanyl)methyl)propyl)amino)-3-((trifluoromethyl)sulfonyl)benzenesulfonamide; GX-070 (Obatoclax®; 1H-Indole, 2-(2-((3,5-dimethyl-1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl)-)); HGS1029; GDC-0145; GDC-0152; LCL-161; LBW-242; venetoclax; agents that target TRAIL or death receptors (*e.g.*, DR4 and DR5) such as ETR2-ST01, GDC0145, HGS-1029, LBY-135, PRO-1762; drugs that target caspases, caspase-regulators, BCL-2 family members, death domain proteins, TNF family members, Toll family members, and/or NF-kappa-B proteins.

[0221] In some embodiments, the drug moiety of the anti-FAP ADC is an angiogenesis inhibitor such as ABT-869; AEE-788; axitinib (AG-13736); AZD-2171; CP-547,632; IM-862; pegaptamib; sorafenib; BAY43-9006; pazopanib (GW-786034); vatalanib (PTK-787, ZK-222584); sunitinib; SU-11248; VEGF trap; vandetanib; ABT-165; ZD-6474; DLL4 inhibitors.

[0222] In some embodiments, the drug moiety of the anti-FAP ADC is a proteasome inhibitor such as Bortezomib; Carfilzomib; Epoxomicin; Ixazomib; Salinosporamide A.

[0223] In some embodiments, the drug moiety of the anti-chemokine receptor or anti-FAP ADC is a kinase inhibitor such as Afatinib; Axitinib; Bosutinib; Crizotinib; Dasatinib; Erlotinib; Fostamatinib; Gefitinib; Ibrutinib; Imatinib; Lapatinib; Lenvatinib; Mubritinib; Nilotinib; Pazopanib; Pegaptanib; Sorafenib; Sunitinib; SU6656; Vandetanib; Vemurafenib; CEP-701 (lesaurtinib); XL019; INCB018424 (ruxolitinib); ARRY-142886 (selemetinib); ARRY-438162 (binimetinib); PD-325901; PD-98059; AP-23573; CCI-779; everolimus; RAD-001; rapamycin; temsirolimus; ATP-competitive TORC1/TORC2 inhibitors including PI-103, PP242, PP30, Torin 1; LY294002; XL-147; CAL-120; ONC-21; AEZS-127; ETP-45658; PX-866; GDC-0941; BGT226; BEZ235; XL765.

[0224] In some embodiments, the drug moiety of the anti-FAP ADC is a protein synthesis inhibitor such as Streptomycin; Dihydrostreptomycin; Neomycin; Framycetin; Paromomycin; Ribostamycin; Kanamycin; Amikacin; Arbekacin; Bekanamycin; Dibekacin; Tobramycin; Spectinomycin; Hygromycin B; Paromomycin; Gentamicin; Netilmicin; Sisomicin; Isepamicin; Verdamicin; Astromicin; Tetracycline; Doxycycline; Chlortetracycline; Clomocycline; Demeclocycline; Lymecycline; Meclocycline; Metacycline; Minocycline; Oxytetracycline; Penimepicycline; Rolitetracycline; Tetracycline; Glycylcyclines; Tigecycline; Oxazolidinone; Eperezolid; Linezolid; Posizolid; Radezolid; Ranbezolid; Sutezolid; Tedizolid; Peptidyl transferase inhibitors; Chloramphenicol; Azidamfenicol; Thiamphenicol; Florfenicol; Pleuromutilins; Retapamulin; Tiamulin; Valnemulin; Azithromycin; Clarithromycin; Dirithromycin; Erythromycin; Flurithromycin; Josamycin; Midecamycin; Miocamycin; Oleandomycin; Rokitamycin; Roxithromycin; Spiramycin; Troleandomycin; Tylosin; Ketolides; Telithromycin; Cethromycin; Solithromycin; Clindamycin; Lincomycin; Pirlimycin; Streptogramins; Pristinamycin; Quinupristin/dalfopristin; Virginiamycin.

[0225] In some embodiments, the drug moiety of the anti-FAP ADC is a histone deacetylase inhibitor such as Vorinostat; Romidepsin; Chidamide; Panobinostat; Valproic acid; Belinostat; Mocetinostat; Abexinostat; Entinostat; SB939 (pracinostat); Resminostat; Givinostat; Quisinostat; thioureidobutyronitrile (Kevetrin™); CUDC-10; CHR-2845 (tefinostat); CHR-3996; 4SC-202; CG200745; ACY-1215 (rocilinostat); ME-344; sulforaphane.

[0226] In some embodiments, the drug moiety of the anti-FAP ADC is a topoisomerase I inhibitor such as camptothecin; various camptothecin derivatives and analogs (for example, NSC 100880, NSC 603071, NSC 107124, NSC 643833, NSC 629971, NSC 295500, NSC 249910, NSC 606985, NSC 74028, NSC 176323, NSC 295501, NSC 606172, NSC 606173, NSC 610458, NSC 618939, NSC 610457, NSC 610459, NSC 606499, NSC 610456, NSC 364830, and NSC 606497); morpholinisoxorubicin; SN-38.

[0227] In some embodiments, the drug moiety of the anti-FAP ADC is a topoisomerase II inhibitor such as doxorubicin; amonafide (benzisoquinolinedione); m-AMSA (4′-(9-acridinylamino)-3′-methoxymethanesulfonanilide); anthrapyrazole derivative ((NSC 355644); etoposide (VP-16); pyrazoloacridine ((pyrazolo[3,4,5-kl]acridine-2(6H)-propanamine, 9-methoxy-N, N-dimethyl-5-nitro-, monomethanesulfonate); bisantrene hydrochloride; daunorubicin; deoxydoxorubicin; mitoxantrone; menogaril; N,N-dibenzyl daunomycin; oxanthrazole; rubidazone; teniposide.

[0228] In some embodiments, the drug moiety of the anti-FAP ADC is a DNA intercalating agent such as anthramycin; chicamycin A; tomaymycin; DC-81; sibiromycin; pyrrolobenzodiazepine derivative; SGD-1882 ((S)-2-(4-aminophenyl)-7-methoxy-8-(3 S)-7-methoxy-2-(4-methoxyphenyl)-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)propoxy)-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one); SG2000 (SJG-136; (11aS,11a'S)-8,8′-(propane-1,3-diylbis(oxy))bis(7-methoxy-2-methylene-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one)).

[0229] In some embodiments, the drug moiety of the anti-FAP ADC is a RNA/DNA antimetabolite such as L-alanosine; 5-azacytidine; 5-fluorouracil; acivicin; aminopterin derivative N-[2-chloro-5[[(2, 4-diamino-5-methyl-6-quinazolinyl)methyl]amino]benzoyl]L-aspartic acid (NSC 132483); aminopterin derivative N-[4-[[(2, 4-diamino-5-ethyl-6-quinazolinyl)methyl]amino]benzoyl]L-aspartic acid; aminopterin derivative N-[2-chloro-4-[[(2, 4-diamino-6-pteridinyl)methyl]amino]benzoyl]L-aspartic acid monohydrate; antifolate PT523 ((Nα-(4-amino-4-deoxypteroyl)-Nγ-hemiphthaloyl-L-ornithine)); Baker's soluble antifol (NSC 139105); dichlorallyl lawsone ((2-(3, 3-dichloroallyl)-3-hydroxy-1,4-naphthoquinone); brequinar; ftorafur ((pro-drug; 5-fluoro-1-(tetrahydro-2-furyl)-uracil); 5,6-dihydro-5-azacytidine; methotrexate; methotrexate derivative (N-[[4-[[(2, 4-diamino-6-pteridinyl)methyl]methylamino]-1-naphthalenyl]carbonyl]L-glutamic acid); PALA ((N-(phosphonoacetyl)-L-aspartate); pyrazofurin; trimetrexate.

[0230] In some embodiments, the drug moiety of the anti-FAP ADC is a DNA antimetabolite such as 3-HP; 2′-deoxy-5-fluorouridine; 5-HP; α-TGDR (α-2′-deoxy-6-thioguanosine); aphidicolin glycinate; ara C (cytosine arabinoside); 5-aza-2′-deoxycytidine; β-TGDR (β-2′-deoxy-6-thioguanosine); cyclocytidine; guanazole; hydroxyurea; inosine glycodialdehyde; macbecin II; pyrazoloimidazole; thioguanine; thiopurine.

[0231] In some embodiments, the drug moiety of the anti-FAP ADC, is a mitochondria inhibitor such as pancratistatin; phenpanstatin; rhodamine-123; edelfosine; d-alpha-tocopherol succinate; compound 11β; aspirin; ellipticine; berberine; cerulenin; GX015-070 (Obatoclax®; 1H-Indole, 2-(2-((3,5-dimethyl-1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl)-); celastrol (tripterine); metformin; Brilliant green; ME-344.

[0232] In some embodiments, the drug moiety of the anti-FAP ADC, is an antimitotic agent such as allocolchicine; auristatins, such as MMAE (monomethyl auristatin E) and MMAF (monomethyl auristatin F); halichondrin B; cemadotin; colchicine; cholchicine derivative (N-benzoyl-deacetyl

benzamide); dolastatin-10; dolastatin-15; maytansine; maytansinoids, such as DM1 (N2′-deacetyl-N2′-(3-mercapto-1-oxopropyl)-maytansine); rhozoxin; paclitaxel; paclitaxel derivative ((2′-N-[3-(dimethylamino)propyl]glutaramate paclitaxel); docetaxel; thiocolchicine; trityl cysteine; vinblastine sulfate; vincristine sulfate.

[0233] In some embodiments, the drug moiety of the anti-FAP ADC is a nuclear export inhibitor such as callystatin A; delactonmycin; KPT-185 (propan-2-yl (Z)-3-[3-[3-methoxy-5-(trifluoromethyl)phenyl]-1,2,4-triazol-1-yl]prop-2-enoate); kazusamycin A; leptolstatin; leptofuranin A; leptomycin B; ratjadone; Verdinexor ((Z)-3-[3-[3,5-bis(trifluoromethyl)phenyl]-1,2,4-triazol-1-yl]-N-pyridin-2-ylprop-2-enehydrazide).

[0234] In some embodiments, the drug moiety of the anti-FAP ADC is a hormonal therapeutics such as anastrozole; exemestane; arzoxifene; bicalutamide; cetrorelix; degarelix; deslorelin; trilostane; dexamethasone; flutamide; raloxifene; fadrozole; toremifene; fulvestrant; letrozole; formestane; glucocorticoids; doxercalciferol; sevelamer carbonate; lasofoxifene; leuprolide acetate; megesterol; mifepristone; nilutamide; tamoxifen citrate; abarelix; prednisone; finasteride; rilostane; buserelin; luteinizing hormone releasing hormone (LHRH); Histrelin; trilostane or modrastane; fosrelin; goserelin.

[0235] In some embodiments, an anti-FAP ADC comprises an additional binding moiety targeting a second target, conjugated to a drug moiety. In some embodiments, an anti-FAP ADC comprises a bispecific antibody comprising a FAP antigen-binding moiety and a second antigen-binding moiety targeting a second target, conjugated to a drug moiety. In some embodiments, the second antigen-binding moiety is sacituzumab, the drug moiety is govitecan, and the second target is TROP2. In some embodiments, the second antigen-binding moiety is tisotumab, the drug moiety is vedotin, and the second target is Tissue Factor. In some embodiments, the second antigen-binding moiety is enfortumab, the drug moiety is vedotin, and the second target is Nectin4. In some embodiments, the second antigen-

binding moiety is brentuximab, the drug moiety is vedotin, and the second target is CD30. In some embodiments, the second antigen-binding moiety is trastuzumab, the drug moiety is deruxtecan, and the second target is HER2. In some embodiments, the second antigen-binding moiety is trastuzumab, the drug moiety is emtansine, and the second target is HER2. In some embodiments, the second antigen-binding moiety is polatuzumab, the drug moiety is vedotin, and the second target is CD79. In some embodiments, the second antigen-binding moiety is inotuzumab, the drug moiety is ozogamicin, and the second target is CD22. In some embodiments, the second antigen-binding moiety is gemtuzumab, the drug moiety is ozogamicin, and the second target is CD33. In some embodiments, the second antigen-binding moiety is loncastuximab, the drug moiety is tesirine, and the second target is CD19. In some embodiments, the second antigen-binding moiety is belantamab, the drug moiety is mafodotin, and the second target is BCMA. In some embodiments, the second antigen-binding moiety is mirvetuximab, the drug moiety is soravtansine, and the second target is FRα. In some embodiments, the second antigen-binding moiety is moxetumomab, the drug moiety is pasudotox, and the second target is CD22.

[0236] Any of these agents that include, or that may be modified to include, a site of attachment to an antibody and/or binding fragment can be included in an anti-FAP ADC.

[0237] Other post-translational modifications include marker or "tag" sequences, such as a peptide, to facilitate purification. In some embodiments, the marker or tag amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (see, *e.g.*, QIAGEN, Inc.), among others, many of which are commercially available. For example, as described in Gentz *et al.*, Proc. Natl. Acad. Sci. USA 86:821-24, 1989, hexa-histidine provides for convenient purification of a fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin ("HA") tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, Cell 37:767-78, 1984), and the "FLAG" tag.

[0238] Methods for linking or conjugating (directly or indirectly) moieties (including polypeptides) to FAP binding portions of a fusion protein (*e.g.*, anti-FAP antibodies, FAP antigen-binding fragments, or a bispecific antibody comprising the FAP antigen-binding fragment) are well known in the art, any one of which can be used to make a modified FAP binding agent, including ADCs and/or fusion proteins described herein.

Linkers

[0239] Provided herein is one or more linkers, or a use thereof. In some embodiments, fusion proteins provided herein comprise one, 2, 3, 4, 5 or more linkers. In some embodiments, each linker in a fusion protein can be distinct or can be the same, or can be a combination thereof. A linker can be a peptide linker or a synthetic linker. In some embodiments, linkers are a specific length to effect an activity on a fusion protein. A linker can be configured to drive formation of a secondary structure for target binding as described herein, such as heterodimerization of antibody components. For example, a linker is short in length to prevent pairing between two or more components of a fusion protein described herein and/or to drive pairing between two or more components of a fusion protein described herein. In another example, a linker can be flexible.

[0240] Examples of linkers include a VH-CHl linker ASTKGPSVFPLAPS (SEQ ID NO: 90); VL-CL linker RTVAAPSVFIFPPS (SEQ ID NO: 91); CH2-CH3 linker ISKAKGQPREPQ (SEQ ID NO: 92); IgM tail linker KSTGKPTLYNVSLVMSDTAGTCY (SEQ ID NO: 93); GGGGSGGGGSGGGGSGGGGT (SEQ ID NO: 94); G; and (GGGGS)n, n=l, 2, 3, 4, 5, 6, 7, 8, 9, or 10, (SEQ ID NO: 95).

[0241] Formats of fusion proteins comprising linker A, B, and C are described herein. In some embodiments, the linker A, linker B and linker C independently are peptide linker having the formula $(Gly4Ser)_n$, where n is 1, 2, 3, 4, or 5 (SEQ ID NO: 95). In some embodiments, n is 2 or 3, *i.e.*, the

linker is $(Gly4Ser)_2$, or $(Gly4Ser)_3$. In some embodiments, the linker A, linker B and linker C independently are amino acid G.

[0242] Fusion protein of the disclosure can be produced using methods known in the art.

Modifications

[0243] In some embodiments, FAP binding agents provided herein are engineered with one or more modifications. Such modification can include modifications that alter the amino acid sequence of the FAP binding agents resulting in one or more amino acid alterations, and/or post-translational modifications resulting in one or more chemical alterations.

[0244] In some embodiments, one or more modifications comprise one or more amino acid alterations, one or more chemical alterations, one or more conjugation or fusion to one or more second agents, one or more linkers or any combinations thereof.

Amino acid alterations

[0245] In some embodiments, one or more modifications comprise one or more amino acid alterations. In some embodiments, FAP binding agents provided herein are engineered with one, 2, 3, 4, 5 or more amino acid alterations. Amino acid alterations comprise one or more amino acid substitutions, deletions, and/or insertions. In some embodiments, the one or more amino acid substitutions comprises conservative substitutions or non-conservative substitutions. With respect to polypeptides that are FAP binding agents (*e.g.*, antibodies, fragments, and/or binding polypeptides), such as human FAP binding agents, conservative amino acid substitutions that include ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine,

threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles. The following eight groups contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)). In some embodiments, the term "conservative sequence modifications" are used to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence.

[0246] Thus, in some embodiments, a predicted nonessential amino acid residue in a FAP binding agent is replaced with another amino acid residue from the same side chain family. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate antigen-binding are well-known in the art (see, *e.g.*, Brummell *et al.*, Biochem. 32:1180-1187 (1993); Kobayashi *et al.* Protein Eng. 12(10):879-884 (1999); and Burks *et al.* Proc. Natl. Acad. Sci. USA 94:412-417 (1997)). In some embodiments, the conservative amino acid alterations described herein modify the amino acid sequences of the FAP binding agents (*e.g.*, antibodies), including human FAP binding agents, by 50%, or 55%, or 60%, or 65%, or 70%, or 75%, or 80%, or 85%, or 90%, or 95%, or 98%, or 99%. In some embodiments, the nucleotide and amino acid substitutions refer to at most 1, 2, 3, 4, 5, or 6 amino acid substitutions to the CDRs described in **TABLE 6** or **TABLE 7**. Thus, for example, each such CDR may contain up to 5 conservative amino acid substitutions, for example up to (not more than) 4 conservative amino acid substitutions, for example up to (not more than) 3

conservative amino acid substitutions, for example up to (not more than) 2 conservative amino acid substitutions, or no more than 1 conservative amino acid substitution.

[0247] In some embodiments, antibodies provided here are affinity matured wherein such an antibody comprises one or more amino acid alterations in one or more CDRs compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen. Anti-FAP antibodies provided herein can be affinity matured using suitable selection and/or mutagenesis methods. In some embodiments, affinity matured antibodies have an affinity which is 1.5 times or more, 2 times or more, 3 times or more, 4 times or more, 5 times or more, 10 times or more, 20 times or more, or 30 times or more than that of the starting antibody (*e.g.*, a murine or rabbit humanized or human antibody) from which the matured antibody is prepared from.

[0248] In some embodiments, FAP binding agents provided herein comprise one or more modifications which modulate the half-life of antibodies *in vivo*. For example, addressing the interaction of Fc with FcRn allows to modulate the half-life of antibodies *in vivo*. In some embodiments, abrogating the interaction by *e.g.* introduction of mutation H435A leads to an extremely short half-life, since the antibody is no longer protected from lysosomal degradation by FcRn recycling. In some embodiments, FAP binding agents provided herein (*e.g.,* an antibody) comprise a modification comprising a H435A substitution or has otherwise been engineered for a reduced half-life.

[0249] In some embodiments, FAP binding agents described herein comprise one or more modifications which extend the half-life of anti-FAP biding agents provided herein. For example, antibodies comprising "YTE" mutations (M252Y/S254T/T256E) and/or equivalent mutations such as "LS" mutations (M428L/N434S) have been shown to significantly extend the half-life by more efficient recycling from endosomes in both pre-clincal species as well as humans (Dall'Acqua, William F., *et al*. The Journal of Immunology 169.9: 5171-5180 (2002); Zalevsky, Jonathan, *et al* "Enhanced

antibody half-life improves *in vivo* activity." Nature biotechnology 28.2 (2010): 157-159.). Accordingly, in some embodiments, the FAP binding agents provided herein comprise YTE mutations (M252Y/S254T/T256E) and/or equivalent mutations such as LS (M428L/N434S) or has otherwise been engineered for an improved half-life. Suitable Fc engineering approaches for extension of half-life can be found in Haraya, Kenta, Tatsuhiko Tachibana, and Tomoyuki Igawa. Drug metabolism and pharmacokinetics 34.1: 25-41 (2019), and/or Lee, Chang-Han, *et al.* Nature communications 10.1: 1-11 (2019), both incorporated herein by reference.

[0250] In some embodiments, a FAP binding agent provided herein comprise one or more modifications which promote the association of the first and the second subunit of the Fc domain. Such modifications include manipulation of the peptide backbone or the post-translational modifications of an Fc domain subunit that reduces or prevents the association of a polypeptide comprising the Fc domain subunit with an identical polypeptide to form a homodimer. Antibodies comprising an Fc region may or may not comprise a modification promoting the association of the first and the second subunit of the Fc domain. A modification promoting association as used herein includes separate modifications made to each of the two Fc domain subunits desired to associate (*e.g.* the first and the second subunit of the Fc domain), wherein the modifications are complementary to each other so as to promote association of the two Fc domain subunits. For example, a modification promoting association may alter the structure or charge of one or both of the Fc domain subunits so as to make their association sterically or electrostatically favorable. Thus, (hetero)dimerization occurs between a polypeptide comprising the first Fc domain subunit and a polypeptide comprising the second Fc domain subunit, which might be non-identical, *e.g.* in the sense that further components fused to each of the subunits (*e.g.* antigen-binding moieties) are not the same. In some embodiments the modification promoting association comprises an amino acid alteration in the Fc domain, specifically an amino acid substitution. In a particular embodiment, the modification promoting association comprises a separate

amino acid alteration, specifically an amino acid substitution, in each of the two subunits of the Fc domain.

Chemical alterations

[0251] In some embodiments, FAP binding agents provided herein are modified by one or more chemical alterations comprising glycosylation (*e.g.*, afucosylation), acetylation, pegylation, phosphorylation, sulfation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, *etc*. In some embodiments, an antibody modified by one or more chemical alterations are referred to herein as a derivatized antibody, or a derivative. Additionally, the derivative may contain one or more non-natural amino acids, *e.g.*, using ambrx technology, see, *e.g.*, Wolfson, Wendy. "Amber codon flashing ambrx augments proteins with unnatural amino acids." Chemistry & biology 13.10 (2006): 1011-1012.

[0252] In some embodiments, FAP binding agents provided herein comprise one or more modifications which alter at least one constant region-mediated biological effector function. For example, in some embodiments, a FAP binding agent may be modified to reduce or enhance at least one constant region-mediated biological effector function relative to the unmodified FAP binding agent, *e.g.*, reduced or improved binding to the Fc receptor (FcγR). FcγR binding may be reduced, *e.g.* by mutating the immunoglobulin constant region segment of the antibody at particular regions necessary for FcγR interactions (see, *e.g.*, Canfield, Stephen M., and Sherie L. Morrison. The Journal of experimental medicine 173.6: 1483-1491 (1991); and Lund, John, *et al*. The Journal of Immunology 147.8: 2657-2662 (1991)). FcγR binding may be enhanced, *e.g.* by afucosylation. Reducing FcγR binding may also reduce other effector functions which rely on FcγR interactions, such as opsonization (*e.g.*, CDC), phagocytosis (*e.g.*, ADCP) and antigen-dependent cellular cytotoxicity (*e.g.*, ADCC)

[0253] Accordingly, in some embodiments, antibodies and/or binding polypeptides provided herein are modified such that the oligosaccharides in the Fc region of the antibody do not have any or have reduced fucose sugar units (*e.g.*, afucosylated). Removal of the core fucose from the biantennary complex-type oligosaccharides attached to the Fc can greatly increase ADCC effector function without altering antigen-binding or CDC effector function. Several ways are known for reducing or abolishing fucosylation of Fc-containing molecules, *e.g.*, antibodies. These include recombinant expression in certain mammalian cell lines including a FUT8 knockout cell line, variant CHO line Lec13, rat hybridoma cell line YB2/0, a cell line comprising a small interfering RNA specifically against the FUT8 gene, and a cell line co-expressing α-1,4-N-acetylglucosaminyltransferase III and Golgi α-mannosidase II. Alternatively, the Fc-containing molecule may be expressed in a non-mammalian cell such as a plant cell, yeast, or prokaryotic cell, *e.g.,* E. coli. Zinc-finger nucleases are another known method of generating afucosylated antibodies. *See e.g.*, Haryadi *et al.*, Bioengineered 4:2, 90-94 (2013); Ripka *et al.* Arch. Biochem. Biophys. 249:533-545 (1986); Yamane-Ohnuki *et al.* Biotech. Bioeng. 87: 614 (2004); Pereira *et al.* mAbs 10(5): 693-711 (2018).

[0254] In some embodiments, a FAP binding agent provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the FAP binding agent include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in

manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the FAP binding agent to be improved, whether the antibody derivative will be used in a therapy under defined conditions.

Activity

[0255] In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) provided herein can bind FAP. In some embodiments, a FAP binding agent (*e.g.*, bispecific antibody, or a fusion protein thereof) can bind a TACR. Binding affinities for an anti-FAP antibody, a bispecific antibody or a fusion protein thereof provided herein to a target such as FAP or a TACR can be determined by measuring, for example, the half maximal effective concentration (EC_{50}) for binding to FAP-expressing cell lines. EC_{50}, expressed as a molar concentration (*e.g.*, nM), is the concentration of the anti-FAP antibody, bispecific antibody or fusion protein thereof that achieves half of the maximal binding.

[0256] In some embodiments, an anti-FAP antibody, a bispecific antibody or a fusion protein thereof provided herein can bind FAP or bind cells expressing FAP with an EC_{50} of no more than 20 nM, no more than 19 nM, no more than 18 nM, no more than 17 nM, no more than 16 nM, no more than 15 nM, no more than 14 nM, no more than 13 nM, no more than 12 nM, no more than 11 nM, no more than 10 nM, no more than 9 nM, no more than 8 nM, no more than 7 nM, no more than 6 nM, or no more than 5 nM. In some embodiments, an anti-FAP antibody, a bispecific antibody or a fusion protein thereof provided herein can bind FAP or bind cells expressing FAP with an EC_{50} of about 0.001 nM to about 5 nM, about 0.01 nM to about 3 nM, or about 0.1 nM to about 2 nM.

[0257] In some embodiments, a bispecific antibody or a fusion protein thereof provided herein can bind a TACR (*e.g.*, LTßR) or bind cells expressing a TACR (*e.g.*, LTßR) with an EC_{50} of no more than 20 nM, no more than 19 nM, no more than 18 nM, no more than 17 nM, no more than 16 nM, no more than 15 nM, no more than 14 nM, no more than 13 nM, no more than 12 nM, no more than 11 nM, no more than 10 nM, no more than 9 nM, no more than 8 nM, no more than 7 nM, no more than 6 nM, or no more than 5 nM. In some embodiments, a bispecific antibody or a fusion protein thereof provided herein can bind a TACR (*e.g.*, LTßR) or bind cells expressing a TACR (*e.g.*, LTßR) with an EC_{50} of about 0.001 nM to about 5 nM, about 0.01 nM to about 3 nM, or about 0.1 nM to about 2 nM.

[0258] Binding affinities for an anti-FAP antibody, a bispecific antibody or a fusion protein thereof provided herein to a target such as FAP or a TACR (*e.g.*, LTßR) can be determined by measuring, for example, the dissociation constant (K_D, $K_D = k_{off}/k_{on}$, or K_D = Kd/Ka). K_D as used herein has nanomolar units (nM) and corresponds to the concentration of the antibody, bispecific antibody or fusion protein thereof at which half of the target proteins are occupied at equilibrium. The smaller the dissociation constant is, the higher is the affinity between the antibody, bispecific antibody or fusion protein and its target.

[0259] In some embodiments, an anti-FAP antibody, a bispecific antibody or a fusion protein provided herein can specifically bind FAP, or can specifically bind cells expressing FAP with a K_D of no more than 20 nM, no more than 19 nM, no more than 18 nM, no more than 17 nM, no more than 16 nM, no more than 15 nM, no more than 14 nM, no more than 13 nM, no more than 12 nM, no more than 11 nM, no more than 10 nM, no more than 9 nM, no more than 8 nM, no more than 7 nM, no more than 6 nM, or no more than 5 nM. In some embodiments, an anti-FAP antibody, a bispecific antibody or a fusion protein provided herein can specifically bind FAP, or can specifically bind cells expressing FAP with a K_D of about 0.5 nM to about 5 nM, about 1 nM to about 4 nM, or about 2 nM to about 3 nM. In some embodiments, the bispecific antibody or the fusion protein binds to FAP with

dissociation constant (KD, KD=koff/kon, or KD= Kd/Ka) no more than 20 nM, 15 nM, 10 nM or 5 nM.

[0260] In some embodiments, a bispecific antibody or a fusion protein provided herein can specifically bind a TACR (*e.g.*, LTßR), or can specifically bind cells expressing a TACR (*e.g.*, LTßR) with a K_D of no more than 20 nM, no more than 19 nM, no more than 18 nM, no more than 17 nM, no more than 16 nM, no more than 15 nM, no more than 14 nM, no more than 13 nM, no more than 12 nM, no more than 11 nM, no more than 10 nM, no more than 9 nM, no more than 8 nM, no more than 7 nM, no more than 6 nM, or no more than 5 nM. In some embodiments, a bispecific antibody or a fusion protein provided herein can specifically bind a TACR (*e.g.*, LTßR), or can specifically bind cells expressing a TACR (*e.g.*, LTßR) with a K_D of about 0.1 nM to about 10 nM, about 0.2 to about 5 nM, or about 0.3 nM to about 3nM. In some embodiments, the bispecific antibody or the fusion protein binds to a TACR (*e.g.*, LTßR) with dissociation constant (KD) no more than 20 nM, 15 nM, 10 nM or 5 nM.

[0261] The K_D values can be determined by means of surface plasmon resonance (SPR) spectroscopy, biolayer interferometry (BLI), or a radiolabeled antigen-binding assay (RIA). K_{on} or, an on-rate, rate of association, association rate as well as k_{off}, or an off-rate, rate of dissociation, dissociation rate can can also be determined with the same SPR or BLI techniques described above. Where assay conditions were found to influence the determined K_D, the assay setup with the least standard deviation shall be used.

[0262] In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) can specifically bind to FAP and/or to a TACR (*e.g.*, with an affinity that is at least 2, 5, 10, 15, 20, 25, 50, 100, 250, 500, 1000, 10,000 times greater, or more, than the affinity for an unrelated control protein (*e.g.*, hen egg white lysozyme) or a competitor molecule. A competitor molecule can be a non-tumor associated molecule, a non-

tumor cell surface molecule, a pro-tumor cell surface molecule (*e.g.*, HVEM and/or DPPIV), a non-human cell (*e.g.*, cynomolgus HVEM), and/or an inhibitor molecule (*e.g.*, DcR3). In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) may react with FAP sequences other than human FAP sequences (*e.g.*, cynomolgus sequences). Exemplary FAP and TACR sequences are described in **TABLE 5** and **5.1** respectively.

[0263] In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) exhibits reduced binding affinity to a competitive HVEM molecule relative to a comparator binding agent. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) exhibits at least about 1%, at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or 100% reduced binding affinity to a competitive HVEM molecule relative to comparator binding agent. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) barely binds to human or cynomolgus HVEM.

[0264] In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) exhibits reduced binding affinity to a competitive DcR3 relative to a comparator binding agent. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) exhibits at least about 1%, at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or 100% reduced binding affinity to a competitive DcR3

molecule relative to comparator binding agent. In some embodiments, the LIGHT mutant is capable to reduce binding affinity to DcR3.

[0265] In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) exhibits reduced binding affinity to a competitive DPPIV relative to a comparator binding agent. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) exhibits at least about 1%, at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or 100% reduced binding affinity to a competitive DPPIV molecule relative to comparator binding agent. In some embodiments, the fusion protein specifically binds to human FAP, and/or does not bind to DPPIV.

[0266] Exemplary competitive molecules are set forth in the Examples.

[0267] In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) induces the stimulation of one or more immune cells (*e.g.*, dendritic cells, T cells, and/or B cells) in a sample. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) induces the stimulation of one or more immune cells (*e.g.*, dendritic cells, T cells, and/or B cells) in a sample relative to a comparator binding agent. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) induces the stimulation of at least about 1%, at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or 100% of immune cells (*e.g.*, dendritic cells, T cells, and/or B cells) in a sample relative to a comparator binding agent.

[0268] In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) activates one or more cancer associated fibroblast cells (CAFs) in a sample. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) activates one or more CAFs in a sample relative to a comparator binding agent. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) activates at least about 1%, at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or 100% CAFs in a sample relative to a comparator binding agent.

[0269] In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) effects the formation of secondary lymphoid organs (SLOs), tertiary lymphoid structures (TLSs) or both. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) effects the increased formation of secondary lymphoid organs (SLOs), tertiary lymphoid structures (TLSs) or both in a sample relative to a comparator binding agent. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) effects at least about 1%, at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 110%, at least about 120%, at least about 130%, at least about 140%, at least about 150%, at least about 160%, at least about 170%, at least about 180%, at least about 190%, or at least about 200% increased formation of secondary lymphoid organs (SLOs), tertiary lymphoid structures (TLSs) or both in a sample relative to a comparator binding agent.

[0270] In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) effects a decrease in rate of tumor growth or number of tumor cells in a sample relative to a comparator binding agent. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) effects at least about 1%, at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or 100% decrease in rate of tumor growth or number of tumor cells in a sample relative to a comparator binding agent.

[0271] In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) effects an increase in tumor cell death in a sample relative to a comparator binding agent. In some embodiments, a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) effects at least about 1%, at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 110%, at least about 120%, at least about 130%, at least about 140%, at least about 150%, at least about 160%, at least about 170%, at least about 180%, at least about 190%, or at least about 200% increase in tumor cell death in a sample relative to a comparator binding agent.

Generating and Expressing FAP binding agents

Polynucleotide

[0272] The present disclosure provides an isolated polynucleotide or polynucleotides encoding a FAP binding agent (*e.g.*, anti-FAP antibody or antigen-binding fragment thereof, a bispecific comprising the same, or the fusion protein described above) or a portion thereof. The polynucleotide

is polymers of DNA, RNA, DNA/RNA hybrids, or modifications thereof. In some embodiments, the polynucleotide is polymers of DNA. In some embodiments, the polynucleotide is polymers of RNA.

[0273] Also provided herein is a nucleic acid molecule comprising at least one polynucleotide encoding FAP binding agent (*e.g.*, anti-FAP antibody or antigen-binding fragment thereof, a bispecific comprising the same, or the fusion protein described above) or a portion thereof. In some embodiments, a nucleic acid molecule comprises the polynucleotide that encodes a heavy chain or a light chain of a FAP binding agent. In some embodiments, a nucleic acid molecule comprises a polynucleotide that encodes a first heavy chain, a polynucleotide that encodes a second heavy chain, and a polynucleotide that encodes a light chain, of a FAP binding agent. In some embodiments, a first nucleic acid molecule comprises a first polynucleotide that encodes a heavy chain and a second nucleic acid molecule comprises a second polynucleotide that encodes a light chain. In some embodiments, a first nucleic acid molecule comprises a first polynucleotide that encodes a heavy chain, a second nucleic acid molecule comprises a second polynucleotide that encodes a light chain, and a third nucleic acid molecule comprises a third polynucleotide that encodes a second heavy chain. In some embodiments, the heavy chain and the light chain are expressed from one nucleic acid molecule, or from two separate nucleic acid molecules, as two separate polypeptides. In some embodiments, the first heavy chain, second heavy chain and the light chain are expressed from one nucleic acid molecule, from two separate nucleic acid molecules, as two separate polypeptides, or from three separate nucleic acid molecules as three separate polypeptides. In some embodiments, a single polynucleotide encodes a single polypeptide comprising a first heavy chain, a second heavy chain and a light chain linked together.

[0274] In some embodiments, when the antibody is a bispecific antibody, a nucleotide acid molecule comprises both a polynucleotide that encodes a first targeting moiety (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof), and a polynucleotide that encodes a second targeting moiety (*e.g.*,

a second antibody or antigen-binding fragment there of or a TACR). In some embodiments, a first nucleic acid molecule comprises a first polynucleotide that encodes a first targeting moiety (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof) and a second nucleic acid molecule comprises a second polynucleotide that encodes a second targeting moiety (*e.g.*, a second antibody or antigen-binding fragment there of or a TACR). In some embodiments, the first targeting moiety (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof) and the second targeting moiety (*e.g.*, a second antibody or antigen-binding fragment there of or a TACR) are expressed from one nucleic acid molecule, or from two separate nucleic acid molecules, as two separate polypeptides. In some embodiments, a single polynucleotide encodes a single polypeptide comprising both a first targeting moiety (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof) and a second targeting moiety (*e.g.*, a second antibody or antigen-binding fragment there of or a TACR) are linked together.

[0275] A person of ordinary skill in the art would understanding that any single or combination of polynucleotides described herein can be comprised on a single nucleic acid molecule or on more than one nucleic acid molecule, such as on separate nucleic acid molecules.

[0276] In some embodiments, a polynucleotide encoding a heavy chain or light chain of a FAP binding agent comprises a nucleotide sequence that encodes at least one of the CDRs provided herein. In some embodiments, a polynucleotide encoding a heavy chain or light chain of a FAP binding agent comprises a nucleotide sequence that encodes at least 3 of the CDRs provided herein. In some embodiments, a polynucleotide encoding a heavy chain or light chain of a FAP binding agent comprises a nucleotide sequence that encodes at least 6 of the CDRs provided herein. In some embodiments, a polynucleotide encoding a heavy chain or light chain of a FAP binding agent comprises a nucleotide sequence that encodes a leader sequence, which, when translated, is located at the N terminus of the heavy chain or light chain. As discussed above, the leader sequence may be the native heavy or light chain leader sequence, or may be another heterologous leader sequence.

[0277] In some embodiments, the polynucleotide is one that encodes for any of the amino acid sequences for the FAP binding agent provided herein. In some embodiments, the polynucleotide is one that is at least 80% identical to a nucleotide sequence encoding any of the amino acid sequences in **TABLES 6-10** described herein, for example, at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical.

[0278] In some embodiments, a polynucleotide provided herein comprise a nucleotide sequence that encodes a FAP binding agent (*e.g.*, anti-FAP antibody or antigen-binding fragment thereof, a bispecific comprising the same, or the fusion protein described above), or a portion (*e.g.*, a domain, region or moiety) thereof as described herein. In some embodiments, polynucleotides provided herein comprise a nucleotide sequence that encodes any one or more of the amino acid sequences set forth in **TABLES 6-10**. In some embodiments, a polynucleotide provided herein comprises a nucleotide sequence that encodes any one or more of the CDR sequences set forth in **TABLE 6 or 7**. In some embodiments, a polynucleotide provided herein comprises a nucleotide sequence that encodes any one or more of the VH and/or VL sequences set forth in **TABLE 8**. In some embodiments, a polynucleotide provided herein comprises a nucleotide sequence that encodes any one or more of the HC and/or LC sequences set forth in **TABLE 9**. In some embodiments, a polynucleotide provided herein comprises a nucleotide sequence that encodes any one or more of the amino acid sequences set forth in **TABLE 10**.

[0279] In some embodiments, the polynucleotide is one that hybridizes to any one or more of the polynucleotide sequences provided herein. In some of the embodiments, the hybridization is under moderate conditions. In some embodiments, the hybridization is under highly stringent conditions, such as: at least about 6X SSC and 1% SDS at 65°C, with a first wash for 10 minutes at about 42°C with about 20% (v/v) formamide in 0.1X SSC, and with a subsequent wash with 0.2 X SSC and 0.1% SDS at 65°C.

[0280] DNA or RNA encoding the FAP binding agent (*e.g.*, anti-FAP antibody or antigen-binding fragment thereof, a bispecific comprising the same, or the fusion protein described above) or a portion thereof is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). The encoding DNA or RNA may also be obtained by synthetic methods.

[0281] The isolated polynucleotide can be inserted into a construct for further cloning (amplification of the DNA) or for expression, using recombinant techniques known in the art.

[0282] Many constructs are available. The construct components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter (*e.g.*, SV40, CMV, EF-1α), and a transcription termination sequence.

Constructs and Vectors

[0283] Provided herein is a construct includes the isolated polynucleotide provided above. A method of constructing the construct is known to those skilled in the art. For example, the construct can be obtained by *in vitro* recombinant DNA technology, DNA synthesis technology, or *in vivo* recombinant technology. More specifically, it can be constructed by inserting the isolated polynucleotide into a polyclonal site of an expression vector. The expression vector in the present disclosure generally refers to various commercially available expression vectors well known in the art, for example, bacterial plasm ids, bacteriophages, yeast plasmids, plant cell-infected viruses, mammalian cell-infected viruses such as adenovirus, retrovirus or other vectors.

[0284] In some embodiments, a vector can include any one or more of the polynucleotides described herein. For example, a vector can include a polynucleotide encoding a FAP binding agent (*e.g.*, anti-FAP antibody or antigen-binding fragment thereof, a bispecific comprising the same, or the fusion

protein described above), or more than one vector can include any combination of components of a FAP binding agent (*e.g.*, anti-FAP antibody or antigen-binding fragment thereof, a bispecific comprising the same, or the fusion protein described above, a heavy chain, optionally a second heavy chain, a light chain, a first targeting moiety, or a second targeting moiety, or any combination thereof).

[0285] The vector may also include one or more regulatory sequences operably linked to the polynucleotide sequence, where the regulatory sequence may include a suitable promoter sequence. The promoter sequence is usually operably linked to a sequence coding the amino acid sequence to be expressed. The promoter can be any nucleotide sequence that exhibits transcriptional activity in the selected host cell, including mutated, truncated and hybrid promoters, and can be obtained from a gene encoding an extracellular or intracellular polypeptide homologous or heterologous to the host cell.

[0286] The regulatory sequence may further include a suitable transcription terminator sequence, a sequence recognized by the host cell to terminate the transcription. The terminator sequence is linked to the 3' end or terminus of the nucleotide sequence encoding the polypeptide, and any terminator that is functional in the host cell of choice may be used in the present disclosure.

[0287] Generally, a suitable vector may contain an origin of replication capable in at least one organism, a promoter sequence, a convenient restriction enzyme site and one or more selectable markers. For example, these promoters may include, but not limited to, the lac or trp promoter of Escherichia coli (E. coli); the lambda phage PL promoter; and eukaryotic promoters (including CMV immediate-early promoter, HSV thymidine kinase promoter, early and late SV40 promoters, methanol oxidase promoter of Pichia pastoris), and some other known promoters that are capable of controlling gene expression in prokaryotic cells or eukaryotic cells or viruses.

[0288] Marker genes or selectable markers can be used to provide phenotypic characters for selection of transformed host cells. For example, marker genes may include, but not limited to, dihydrofolate

reductase, neomycin resistance and green fluorescent protein (GFP) for eukaryotic cell culture, or tetracycline resistance or ampicillin resistance for *E. coli*.

[0289] When the polynucleotide is expressed, the expression vector may further include an enhancer sequence. If an enhancer sequence is inserted into the vector, the transcription will be enhanced. Enhancer is a cis-acting factor of DNA, typically containing about 10 to 300 base pairs. Enhancer acts on a promoter to enhance gene transcription.

[0290] If desired, one or more polynucleotides also optionally comprise nucleotide sequences encoding secretory signal peptides fused in frame with the polypeptide sequences. The secretory signal peptides direct secretion of the antibody polypeptides by the cells that express the one or more nucleic acids, and are cleaved by the cell from the secreted polypeptides. The one or more nucleic acids may further optionally comprise sequences whose only intended function is to facilitate large scale production of the vector. One can manufacture and administer nucleic acids for gene therapy using procedures that have been described in the literature for a variety of transgenes. See, *e.g.*, Isner *et al.*, Circulation, 91: 2687-2692 (1995); and Isner *et al.*, Human Gene Therapy, 7: 989-1011 (1996).

[0291] In some embodiments, one or more polynucleotides may further comprise additional sequences to facilitate uptake by host cells and expression of the antibody or fragment thereof (and/or any other peptide). In some embodiments, a “naked” transgene encoding a FAP binding agent or portion thereof described herein (*e.g.*, a transgene without a viral, liposomal, or other vector to facilitate transfection) is employed.

[0292] Any suitable vectors may be used to introduce one or more polynucleotides that encode a FAP binding agent into the host. Exemplary vectors that have been described include replication deficient retroviral vectors, including but not limited to lentivirus vectors (see, *e.g.*, Kim *et al.*, J. Virol., 72(1): 811-816 (1998); Kingsman & Johnson, Scrip Magazine, October, 1998, pp. 43-46); parvoviral vectors, such as adeno-associated viral (AAV) vectors (U.S. Patent Nos. 5,474,935l; 5,139,941;

5,622,856; 5,658,776; 5,773,289; 5,789,390; 5,834,441; 5,863,541; 5,851,521; 5,252,479; Gnatenko *et al.*, J. Invest. Med., 45: 87-98, (1997)); adenoviral (AV) vectors (see, *e.g.*, U.S. Patent Nos. 5,792,453; 5,824,544; 5,707,618; 5,693,509; 5,670,488; 5,585,362; Quantin *et al.*, Proc. Natl. Acad. Sci. USA, 89: 2581-2584 (1992); Stratford Perricaudet *et al.*, J. Clin. Invest., 90: 626-630 (1992); and Rosenfeld *et al.*, Cell, 68: 143-155, (1992)); an adenoviral adeno-associated viral chimeric (U.S. Patent No. 5,856,152) or a vaccinia viral or a herpesviral vector (U.S. Patent Nos. 5,879,934; 5,849,571; 5,830,727; 5,661,033; 5,328,688); Lipofectin mediated gene transfer (BRL); liposomal vectors (U.S. Patent No. 5,631,237); and combinations thereof. Optionally, viral vectors are rendered replication-deficient by, for example, deleting or disrupting select genes required for viral replication.

[0293] Any of these expression vectors can be prepared using standard recombinant DNA techniques described in, for example, Sambrook *et al.*, Molecular Cloning, a Laboratory Manual, 2d edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), and Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994).

[0294] Other non-viral delivery mechanisms contemplated include calcium phosphate precipitation (Graham and Van Der Eb, Virology, 52: 456-467 (1973); Chen and Okayama, Mol. Cell Biol., 7: 2745-2752, 1987; Rippe *et al.*, Mol. Cell Biol., 10: 689-695 (1990)) DEAE-dextran (Gopal, Mol. Cell Biol., 5: 1188-1190, (1985)), electroporation (Tur-Kaspa *et al.*, Mol. Cell Biol., 6: 716-718 (1986); Potter *et al.*, Proc. Nat. Acad. Sci. USA, 81: 7161-7165 (1984)), direct microinjection (Harland and Weintraub, J. Cell Biol., 101: 1094-1099 (1985), DNA-loaded liposomes (Nicolau and Sene, Biochim. Biophys. Acta, 721: 185-190 (1982); Fraley *et al.*, Proc. Natl. Acad. Sci. USA, 76: 3348-3352 (1979); Felgner, Sci Am., 276(6): 102-6, 1997; Felgner, Hum Gene Ther., 7(15): 1791-3 (1996)), cell sonication (Fechheimer *et al.*, Proc. Natl. Acad. Sci. USA, 84: 8463-8467 (1987)), gene bombardment using high velocity microprojectiles (Yang *et al.*, Proc. Natl. Acad. Sci USA, 87: 9568-9572 (1990)), and

receptor-mediated transfection (Wu and Wu, J. Biol. Chem., 262: 4429-4432 (1987); Wu and Wu, Biochemistry, 27: 887-892 (1988); Wu and Wu, Adv. Drug Delivery Rev., 12: 159-167 (1993)).

[0295] An expression vector (or an antibody or fragment thereof described herein) may be entrapped in a liposome. See, *e.g.*, Ghosh and Bachhawat, In: Liver diseases, targeted diagnosis and therapy using specific receptors and ligands, Wu G, Wu C ed., New York: Marcel Dekker, pp. 87-104 (1991); Radler *et al.*, Science, 275(5301): 810-814 (1997). Also contemplated are various commercial approaches involving "lipofection" technology. In some embodiments, the liposome may be complexed with a hemagglutinating virus (HVJ). This has been shown to facilitate fusion with the cell membrane and promote cell entry of liposome-encapsulated DNA (see, *e.g.*, Kaneda *et al.*, Science, 243: 375-378 (1989). In some embodiments, the liposome is complexed or employed in conjunction with nuclear nonhistone chromosomal proteins (HMG-1) (see, *e.g.*, Kato *et al.*, J. Biol. Chem., 266: 3361-3364 (1991). In some embodiments, the liposomes are complexed or employed in conjunction with both HVJ and HMG-1. Such expression constructs have been successfully employed in transfer and expression of nucleic acid *in vitro* and *in vivo*. In some embodiments, a FAP binding agent (*e.g.*, an antibody), including a human FAP binding agent, is included in the liposome to target the liposome to cells (such as tumor cells) expressing FAP on their surface.

Antibody Expressing System

[0296] The present disclosure provides an antibody expression system is provided, which includes a construct provided above or incorporates an exogenous polynucleotide provided above, or more than one exogenous polynucleotide provided above, in the genome of a cell. Accordingly, provided herein is a cell, such as a host cell, or a use thereof.

[0297] A cell described herein, and specifically a host cell described herein can refer to a cell that is used to receive, maintain, reproduce and amplify a vector as provided herein. A host cell also can be

used to comprise a FAP binding agent provided herein, or express a FAP binding agent (*e.g.*, anti-FAP antibody or antigen-binding fragment thereof, a bispecific comprising the same, or the fusion protein described above) provided herein, encoded by the vector. The polynucleotide contained in the vector is replicated when the host cell divides, thereby amplifying the nucleic acids contained herein.

[0298] For recombinant production of a FAP binding agent, nucleic acids encoding the FAP binding agent, *e.g.*, as described above, are isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acids may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the FAP binding agent) or produced by recombinant methods or obtained by chemical synthesis.

[0299] Introduction of one or more nucleic acids into a desired host cell may be accomplished by any method, including but not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, *etc*. Nonlimiting exemplary methods are described, for example, in Sambrook *et al*., Molecular Cloning, A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press (2001). Nucleic acids may be transiently or stably transfected in the desired host cells, according to any suitable method. Therefore, methods for constructing the expression system should be known to those skilled in the art, for example, including, but not limited to, microinjection, gene gun method, electroporation, virus-mediated transformation, electron bombardment, precipitation with calcium phosphate, or a combination thereof.

[0300] Any cell suitable for the expression of an expression vector can be used as a host cell. For example, the host cell can be a prokaryotic cell, such as a bacterial cell; or a lower eukaryotic cell, such as a yeast cell; or a higher eukaryotic cell, such as a mammalian cell, specifically including, but not limited to, *Escherichia coli, Streptomyces*; bacterial cells of *Salmonella typhimurium*; or fungal

cells such as yeast, and filamentous fungi; plant cells; insect cells derived from Drosophila S2 or Sf9; animal cells such as CHO, COS, HEK293 cells, or *Bowes melanoma* cells, or a combination thereof.

[0301] For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, *e.g.*, U.S. Pat. Nos. 5,648,237, 5,789,199, and 5,840,523. (*See* also Charlton, K. A., In: Methods in Molecular Biology, Vol. 248, Lo, B. K. C. (ed.), Humana Press, Totowa, N.J., pp. 245-254 (2003), describing expression of antibody fragments in *E. coli.)* After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0302] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for FAP binding agent-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized", resulting in the production of a FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) with a partially or fully human glycosylation pattern. *See* Gemgross, T. U., Nat. Biotech. 22:1409-1414 (2004); and Li, H. *et al.*, Nat. Biotech. 24:210-215 (2006).

[0303] Suitable host cells for the expression of (glycosylated) FAP binding agent are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of Spodoptera frugiperda cells.

[0304] Plant cell cultures can also be utilized as hosts. See, *e.g.*, U.S. Pat. Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

[0305] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293T

cells as described, *e.g.*, in Graham, F. L. *et al.*, J. Gen Virol. 36:59-74 (1977)) or Epi293 cells as used herein; baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, *e.g.*, in Mather, J. P., Biol. Reprod. 23: 243-252 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells (as described, *e.g.*, in Mather, J. P. *et al.*, Annals N.Y. Acad. Sci. 383: 44-68 (1982)); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR-CHO cells (Urlaub, G. *et al.*, Proc. Natl. Acad. Sci. USA 77: 4216-4220 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, *e.g.*, Yazaki, P. and Wu, A. M., Methods in Molecular Biology, Vol. 248, Lo, B. K. C. (ed.), Humana Press, Totowa, N.J., pp. 255-268 (2004). In some embodiments, the host cell is eukaryotic, *e.g.*, a Chinese Hamster Ovary (CHO) cell or lymphoid cell (*e.g.*, Y0, NS0, Sp20 cell).

[0306] FAP binding agents provided herein can be purified by any suitable method. Such methods include, but are not limited to, the use of affinity matrices or hydrophobic interaction chromatography. Suitable affinity ligands include the ROR1 ECD and ligands that bind antibody constant regions. For example, a Protein A, Protein G, Protein A/G, or an antibody affinity column may be used to bind the constant region or Fc region and to purify an antibody or fusion protein. Hydrophobic interactive chromatography, for example, a butyl or phenyl column, may also suitable for purifying some polypeptides. Ion exchange chromatography (for example anion exchange chromatography and/or cation exchange chromatography) may also suitable for purifying some polypeptides. Mixed-mode chromatography (for example reversed phase/anion exchange, reversed phase/cation exchange, hydrophilic interaction/anion exchange, hydrophilic interaction/cation exchange, *etc.*) may also

suitable for purifying some polypeptides. Many methods of purifying polypeptides are known in the art.

[0307] In some embodiments, a FAP binding agent is produced in a cell-free system. Nonlimiting exemplary cell-free systems are described, for example, in Sitaraman *et al.*, Methods Mol. Biol. 498: 229-44 (2009); Spirin, Trends Biotechnol. 22: 538-45 (2004); Endo *et al.*, Biotechnol. Adv. 21: 695-713 (2003).

Systems of Use

[0308] Provided herein are systems of using a FAP binding agent described herein. Systems provided herein comprise components, wherein the components comprise: any one or more of the FAP binding agent provided herein, including anti-FAP antibody or antigen-binding fragment thereof, a bispecific comprising the same, a fusion protein described herein, variants thereof, derivatized versions thereof, nucleic acids encoding the same, and/or vectors comprising such nucleic acids.

[0309] In some embodiments, systems provided herein further comprise a sample obtained from a subject. A sample is biological sample. In some embodiments, a sample is obtained from a subject which is in need thereof of treatment with a FAP binding agent provided herein. In some embodiments, a sample is a tumor sample.

[0310] In some embodiments, systems provided herein further comprise one or more system solutions comprising compositions, pharmaceutical compositions, buffers, reagents including detection and/or amplification reagents, or any combination thereof. Such compositions, pharmaceutical compositions, buffers, and reagents are further described herein. In some embodiments, systems provided herein further comprise one or more support mediums, containers and other articles of manufacture, or any combination thereof. Such support mediums, containers and other articles of manufacture are further described herein.

[0311] In some embodiments, system components described herein are each comprised in a composition, or any combination of components are comprised in single composition. In some embodiments, system components, *e.g.*, in a composition, described herein are each comprised in a container, or any combination of components are comprised in single container. In some embodiments, systems comprise kits. In some embodiments, the systems comprising kits are referred to as kits. In some embodiments, systems comprise devices. In some embodiments, the systems comprising the devices are referred to as devices.

Compositions

[0312] Provided herein are compositions that can be useful for detecting FAP, diagnosing a FAP related disease or disorder, mediating activity in a diseased microenvironment characterized by the expression of FAP and/or FAP expressing cells, treatments of a FAP related disease or disorder, or any combination thereof. Compositions provided herein comprise: any one or more of the FAP binding agents provided herein, including anti-FAP antibodies, antigen-binding fragments thereof, variants thereof, derivatized versions thereof, and binding polypeptides directed to the same, nucleic acids encoding the same, and/or vectors comprising such nucleic acids. Optionally, compositions comprise buffers, reagents, carriers, excipients, and stabilizers and described herein.

[0313] In some embodiments, a composition is a cell culture media comprising a FAP binding agent. In some embodiments, a host cell culture fluid comprising a FAP binding agent. In some embodiments, a composition is a detection reagent comprising a FAP binding agent. In some embodiments, a composition is a pharmaceutical composition comprising a FAP binding agent.

Pharmaceutical Compositions

[0314] The present disclosure relates to a pharmaceutical composition including the anti-FAP antibody or antigen-binding fragment thereof described above, and a pharmaceutically acceptable carrier.

[0315] The present disclosure relates to a pharmaceutical composition including the fusion protein described above, and a pharmaceutically acceptable carrier. The pharmaceutically acceptable carriers include, but are not limited to, buffers, excipients, stabilizers or preservatives. Such carrier includes (but is not limited to) saline, buffer, glucose, water, glycerol, ethanol and combinations thereof. Employed carriers, excipients, or stabilizers are usually nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the carrier is an aqueous pH buffered solution. Examples of carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (*e.g.*, less than about 10 amino acid residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0316] A carrier can also refer to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete or incomplete)), excipient, or vehicle with which the therapeutic is administered. Such carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a exemplary carrier when a composition (*e.g.*, a pharmaceutical composition) is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable excipients (*e.g.*, pharmaceutical excipients) include

starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

[0317] Generally, these substances can be formulated in a non-toxic, inert and pharmaceutically acceptable aqueous carrier medium, wherein the pH is usually about 5-8, preferably about 6-8, although the pH can be changed according to properties of the formulated substances and disease conditions to be treated.

[0318] Compositions can take the form of solutions, suspensions, emulsion, sustained-release formulations and the like. Suitable routes of administering a composition comprising an antibody are well known in the art. Although more than one route can be used to administer a FAP binding agent (*e.g.*, an anti-FAP antibody, a FAP antigen-binding moiety, bispecific antibody comprising the FAP antigen-binding moiety, or a fusion protein thereof), a particular route can provide a more immediate and more effective reaction than another route. Depending on the circumstances, a composition comprising a FAP binding agent (*e.g.*, an anti-FAP antibody, a FAP antigen-binding moiety, bispecific antibody comprising the FAP antigen-binding moiety, or a fusion protein thereof), such as a human FAP binding agent, is applied or instilled into body cavities and/or introduced into circulation.

[0319] For example, it may be desirable to deliver a composition comprising a FAP binding agent, through injection by intravenous, subcutaneous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intraarterial, intraportal, intralesional, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, by sustained release systems, or by implantation devices. If desired, a FAP binding agent, is administered regionally via intraarterial or intravenous administration feeding the region of interest, for example, via the hepatic artery for

delivery to the liver. In other aspects, a FAP binding agent is administered directly to exposed tissue during tumor resection or other surgical procedures.

[0320] As described herein, the formulated pharmaceutical composition can be administered by conventional routes, including (but not limited to) intratumoral administration, intraperitoneal administration, intravenous administration, or topical administration. A pharmaceutical preparation should be matched with the administration mode. The pharmaceutical composition of the present application can be prepared into an injection form, for example, the pharmaceutical composition is prepared by conventional methods with physiological saline or an aqueous solution containing glucose and other adjuvants.

[0321] The pharmaceutical composition such as an injection and a solution should be manufactured under sterile conditions.

[0322] The pharmaceutical composition of the present disclosure contains a safe and effective amount (such as 0.001-99 wt %, 0.01-95 wt %, or 0.1-90 wt %) of the single domain antibody or the fusion protein provided and a pharmaceutically acceptable carrier or excipient.

[0323] In some embodiments, the dosage of active ingredients is a therapeutically effective amount, such as about 10 μg/kg body weight to about 100 mg/kg body weight per day. In addition, the FAP binding agent (*e.g.*, anti-FAP antibody or antigen-binding fragment thereof, a bispecific comprising the same, or the fusion protein described above) can also be used with other therapeutic agents. Accordingly, the dosage of active ingredients can refer to the amount of the FAP binding agent (*e.g.*, anti-FAP antibody or antigen-binding fragment thereof, a bispecific comprising the same, or the fusion protein described above) alone or in combination with the other therapeutic agent. Additional therapeutic agents compatible with systems, compositions, and/or pharmaceutical compositions provided herein are further described herein in the Combination Treatments section.

Detection and Diagnostics

[0324] The present disclosure provides methods for detection and diagnosis of a disease or disorder, *e.g.,* a tumor disease, in a subject using the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), the isolated polynucleotide, the construct, and/or the pharmaceutical composition provided herein. In some embodiments, the methods for detection and diagnosis may be useful to determine whether the antibodies or polypeptides described herein are an appropriate treatment for the subject.

[0325] Samples (*e.g.,* test biological samples) from a subject (*e.g.*, an individual suspected of having or known to have a tumor disease with FAP expression, or suspected of having or known to have another disease or condition), can be analyzed for FAP presence, absence, expression, and/or levels. For example, such samples can be collected and analyzed by detecting the presence or absence of binding of the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) provided herein, to substance (*e.g.*, protein) in the sample. In some examples, the methods further include comparing the amount of binding detected to an amount of binding to a control sample, or comparing the detected level of FAP to a control level of FAP. In some cases, the methods indicate the presence, absence, or severity of a FAP-associated disease or condition, such as one described herein.

[0326] This analysis can be performed prior to the initiation of treatment using the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), the isolated polynucleotide, the construct, and/or the pharmaceutical composition provided herein, or can be done as part of monitoring of progress of cancer treatment. In some embodiments, provided are methods of treatment, carried out by performing the detection assays and initiating, altering, or discontinuing treatment of the subject, for example, based on the results of the diagnostic assay. Such diagnostic analysis can be performed using any sample, including but not

limited to tissue, cells isolated from such tissues, and the like. In some cases, the methods are performed on liquid samples, such as blood, plasma, serum, whole blood, saliva, urine, or semen. Tissue samples include, for example, formalin-fixed or frozen tissue sections.

[0327] Any suitable method for detection and analysis of FAP can be employed. Various diagnostic assay techniques known in the art can be adapted for such purpose, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogeneous phases. General techniques to be used in performing the various immunoassays noted above are known to those of ordinary skill in the art.

[0328] The FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) provided for use in detection and diagnosis methods can be labeled with a detectable moiety. The detectable moiety directly or indirectly produces a detectable signal. For example, the detectable moiety can be any of those described herein such as, for example, a radioisotope, such as 3H, 14C, 32P, 35S, or 125I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate (FITC), Texas red, cyanin, photocyan, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, β-galactosidase or horseradish peroxidase.

[0329] Detection can be accomplished by contacting a sample under conditions suitable for the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) provided to FAP, and assessing the presence (*e.g.*, level) of FAP in the sample. A level of FAP in the sample in comparison with a level of a reference sample can indicate the presence of a tumor or tumor-associated tissues having FAP activity. The reference sample can be a sample taken from the subject at an earlier time point or a sample from another individual.

Kit

[0330] The present disclosure provides a kit containing the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), the isolated polynucleotide, the construct, and/or the pharmaceutical composition provided herein. Such kits can further include, if desired, one or more of various conventional pharmaceutical kit components, such as, for example, containers with one or more pharmaceutically acceptable carriers, additional containers etc., as will be readily apparent to a person skilled in the art. Instructions, either as inserts or a labels, indicating quantities of the components to be administered, guidelines for administration, and/or guidelines for mixing the components, can also be included in the kit. In some embodiments, the kits provided herein further include instructions for using the, the isolated polynucleotide, the construct, and/or the pharmaceutical composition provided herein, for use in a method provided herein. The instructions for use are generally written instructions, although electronic storage media (*e.g.,* magnetic diskette or optical disk) containing instructions are also acceptable.

[0331] In some embodiments, the kit comprises the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), the isolated polynucleotide, the construct, and/or the pharmaceutical composition provided herein, in suitable packaging or container for use in the methods described herein. Suitable packaging and containers are known in the art and includes, for example, vials, vessels, ampules, bottles, syringes (*e.g.,* pre-filled syringes), jars, flexible packaging and the like. An article of manufacture may further be sterilized and/or sealed. Each component (if there is more than one component) can be packaged in separate containers or some components can be combined in one container where cross-reactivity and shelf-life permit. The kits may be in unit dosage forms, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. Kits may also include multiple unit doses of the compounds and instructions for use and be packaged in quantities sufficient for storage and use in pharmacies (*e.g.*, hospital pharmacies and compounding pharmacies).

[0332] In some embodiment, the kit comprises one or more pharmaceutical packs comprising one or more containers (*e.g.*, vials, ampules, pre-filled syringes) containing the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), the isolated polynucleotide, the construct, and/or the pharmaceutical composition provided herein. In some instances, the kits contain a pharmaceutical composition described herein. In some embodiments, the kit comprises the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), the isolated polynucleotide, the construct, and/or the pharmaceutical composition provided herein, in lyophilized form. Associated with such container(s) can also be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Method of Use

[0333] The present disclosure provides the use of the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), or the pharmaceutical composition described above in the manufacture of a therapeutic agent for preventing, diagnosing, or treating a disease, disorder, or condition. Thus, provided herein is a method of treating a disease, the method comprising administering an effective amount or dose of the FAP binding agent (*e.g.*, an anti-FAP antibody, a FAP antigen-binding moiety, bispecific antibody comprising the FAP antigen-binding moiety, or a fusion protein thereof), including modified agents thereof (*e.g.*, conjugated anti-FAP antibodies), compositions or pharmaceutical compositions comprising the same, to a subject in need thereof.

[0334] Methods of treating can also comprise detecting FAP in a sample obtained from the subject being treated, and/or methods of diagnosing a disease or diseased microenvironment is characterized by or associated with cells expressing FAP. Methods of detecting and/or diagnosing can be executed

by using such methods provided herein, however, a person of ordinary skill in the art would understand that any suitable method could be utilized to detect FAP in a sample or diagnose a disease characterized by or associated with cells expressing FAP.

[0335] Multiple modes of action can be envisioned for the FAP binding agents provided herein. For example, mode of action is the conjugation of a FAP binding agents thereof to a drug in the form of an antibody drug conjugate (ADC). Another mode of action is the ability of a FAP binding agents to induce the formation of SLO. A third mode of action resides in the ability of a FAP binding agents to induce the formation of TLS. An additional mode of action is the ability of a FAP binding agent to activate CAFs. Another mode of action resides in the ability of a FAP binding agents to induce tumor cell death. Modes of action are further described herein.

[0336] In some embodiments, an effective amount or dose of FAP binding agents, refers to the amount of FAP binding agent described herein, or the amount of a composition or pharmaceutical composition comprising said FAP binding agent that will elicit the biological or medical response of or desired therapeutic effect on a tissue, system, animal, mammal, or human that is being sought by the researcher, medical doctor, or other clinician. An effective amount of the FAP binding agent may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the FAP+ molecule to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effect of the FAP binding agent is outweighed by the therapeutically beneficial effects. Such benefit includes improving signs or symptoms of cancer. An effective amount can be readily determined by one skilled in the art, by the use of known techniques, and by observing results obtained under analogous circumstances. An effective amount of a FAP binding agent described herein may be administered in a single dose or in multiple doses. In determining the effective amount for a patient, a number of factors are considered by the attending medical practitioner, including, but not limited to: the patient's size (*e.g.*, weight or mass), body surface area, age, and

general health; the specific disease or disorder involved; the degree of, or involvement, or the seventy' of the disease or disorder; the response of the individual patient; the particular compound administered; the mode of administration; the bioavailability characteristics of the preparation administered; the dose regimen selected; the use of concomitant medication; and other relevant circumstances known to medical practitioners. In some embodiments, the subject is mammal animals including human and non-human animals, such as human, mouse, and cynomolgus monkey.

[0337] In some embodiments, provided herein is a method for treating a subject having cancer, including administrating to the subject a therapeutically effective amount of the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), or the pharmaceutical composition provided herein.

[0338] The "therapeutically effective amount" of the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), or a pharmaceutical composition provided in the present disclosure, in some embodiments, causes a reduction in the severity of disease symptoms and increased frequency and duration of asymptomatic period of a disease, disorder or condition, or prevents injury or disability due to illness or suffering.

[0339] For example, for the treatment of FAP related tumors (including, for example, melanoma, lymphoma, bladder cancer, non-small cell lung cancer, head and neck cancer, and colon cancer), relative to untreated subjects, the "therapeutically effective amount" in some embodiments inhibits the cell growth or tumor growth by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, or at least about 80%.

[0340] The ability to inhibit tumor growth can be evaluated in an animal model system that predicts the efficacy against human tumors, or evaluated by detecting the ability to inhibit cell growth. Such inhibition can be determined in vitro by assays well known to those skilled in the art. The therapeutically effective amount of the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-

binding fragment thereof, a bispecific antibody, or a fusion protein thereof), and the pharmaceutical compositions is often able to reduce the tumor size, or otherwise relieve the symptoms of a subject. Those skilled in the art can select an appropriate therapeutically effective dose according to the actual situation, for example, the tumor size of the subject, the severity of the subject's symptoms, and the particular composition or route of administration chosen. A prescription for treatment (*e.g.*, decision on dosage, etc.) may be determined by a physician commonly considering factors including, but not limited to, the disease being treated, status of the patient, delivery site, route of administration and other factors. A prophylactically effective amount refers to an amount effective for achieving the desired prophylactic effect at a dose and for a period of time required. Usually, but not necessarily, since a prophylactic dose is administered to a subject before the onset of a disease or at an early stage of the disease, the "prophylactically effective amount" is usually lower than the "therapeutically effective amount".

[0341] For therapeutic applications, the FAP binding agent can be administered to a patient or subject, *e.g.* to a human or non-human subject, in a pharmaceutically acceptable dosage form. For example, administration may occur intravenously as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intra-cerebrospinal, subcutaneous, intra-articular, intrasynovial, or intrathecal. The FAP binding agents and pharmaceutical compositions thereof according to the current disclosure are particularly suitable to be administered by intra-tumoral, peri-tumoral, intra-lesional, or peri-lesional routes, to exert local as well as systemic therapeutic effects.

[0342] Exemplary administration routes include parenteral (*e.g.*, intramuscular, intravenous, intra-arterial, intraperitoneal, or subcutaneous). In addition, the antibodies, fragments, conjugates and pharmaceutical compositions might be administered by pulse infusion, with, *e.g.*, declining doses of the antibody, fragment or conjugate. In some embodiments, the dosing is given by injections, intravenous or subcutaneous injections, depending in part on whether the administration is brief or

chronic. The amount to be administered may depend on a variety of factors such as the clinical symptoms, weight of the patient or subject, and whether other drugs are administered. The skilled artisan will recognize that the route of administration will vary depending on the disorder or condition to be treated.

[0343] An FAP binding agent can be administered as needed to subjects. Determination of the frequency of administration can be made by persons skilled in the art, such as an attending physician based on considerations of the condition being treated, age of the subject being treated, severity of the condition being treated, general state of health of the subject being treated and the like. In some embodiments, an effective dose of a FAP binding agent is administered to a subject one or more times. In some embodiments, an effective dose of a FAP binding agent is administered to the subject once a month, less than once a month, such as, for example, every two months or every three months. In some embodiments, an effective dose of a FAP binding agent is administered less than once a month, such as, for example, once every three weeks, once every two weeks, or once every week. An effective dose of a FAP binding agent is administered to the subject at least once. In some embodiments, the effective dose of a FAP binding agent may be administered multiple times, including for periods of at least a month, at least six months, or at least a year.

[0344] In some embodiments, pharmaceutical compositions are administered in an amount effective for treatment of (including prophylaxis of) cancer. The therapeutically effective amount is typically dependent on the weight of the subject being treated, his or her physical or health condition, the extensiveness of the condition to be treated, or the age of the subject being treated. Indications

[0345] In some embodiments, the disease, disorder, or condition includes tumor diseases. In some embodiments, least a tumor cell expresses FAP.

[0346] In certain embodiments, tumor diseases are solid tumors. In some embodiment, the tumor disease comprises gastric cancer, liver cancer, lung cancer, colon cancer, colorectal cancer, spleen

cancer, rectal cancer, kidney cancer breast cancer, prostate cancer, skin cancer, bone cancer, blood cancer, multiple myeloma, glioma, ovarian cancer, uterine cancer, endometrial cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, bladder cancer, brain cancer, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, esophageal cancer, salivary gland cancer and lymphoma. Other examples of cancer include carcinoma, squamous cell carcinoma, lymphoma (*e.g.*, Hodgkin's and non Hodgkin's lymphoma), blastoma, sarcoma, and leukemia

[0347] Other cell proliferation disorders that can be treated using the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, spleen, pancreas, lung, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic region, and urogenital system. Also included are pre-cancerous conditions or lesions and cancer metastases. In certain embodiments the cancer is chosen from the group consisting of renal cell cancer, skin cancer, lung cancer, pancreatic cancer, colorectal cancer, breast cancer, brain cancer, head and neck cancer.

[0348] The present disclosure provides a method of decreasing the rate of tumor growth or the number of tumor cells, including contacting a tumor cell with an effective amount of the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), or the pharmaceutical composition described above.

[0349] The present disclosure provides a method of killing a tumor cell, including contacting a tumor cell with an effective amount of the FAP binding agent (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof), or the pharmaceutical composition described above.

Combination Treatments

[0350] FAP binding agents (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) provided herein can be administered in combination with one or more therapeutic agents. Accordingly, provided herein are combination treatments or co-treatments for use in systems, compositions, and/or methods of treatment as described herein. It is understood that any of the combination therapies provided herein can be used in combination with any one or more of the compounds provided herein, for example, with any of the FAP binding agents (*e.g.*, an anti-FAP antibody or antigen-binding fragment thereof, a bispecific antibody, or a fusion protein thereof) provided herein, ADCs thereof, or combination therapies comprising the same.

[0351] In some embodiments, co-treatments encompassed by the present disclosure can be administered simultaneously, separately, or in sequential combination with one or more further therapeutically active compounds, including FAP binding agents provided herein. Any suitable co-treatments can be used in the systems, compositions, and/or methods of the present disclosure, and which include co-treatments for the treatment of cancer. For example, a therapeutic agent may comprise any active ingredients suitable for the particular indication being treated, preferably those with complementary activities that do not adversely affect the FAP binding agent and/or other therapeutic agents in said co-treatment.

[0352] In some embodiments, an additional therapeutic agent is another anti-cancer agent, for example a microtubule disruptor, an antimetabolite, a topoisomerase inhibitor, a DNA intercalator, an alkylating agent, a hormonal therapy, a kinase inhibitor, a receptor antagonist, an activator of tumor cell apoptosis, or an anti angiogenic agent. In some embodiments, an additional therapeutic agent is an immunomodulator, a cytostatic agent, an inhibitor of cell adhesion, a cytotoxic or cytostatic agent, an activator of cell apoptosis, or an agent that increases the sensitivity of cells to apoptotic inducers.

Immunomodulators

[0353] Examples of inhibitors of PD-L1 (CD274) or PD-1 (PDCD1) that can be co-administered include pembrolizumab, nivolumab, cemiplimab, pidilizumab, AMP-224, MEDI0680 (AMP-514), spartalizumab, atezolizumab, avelumab, durvalumab, BMS-936559, cosibelimab (CK-301), sasanlimab (PF-06801591), tislelizumab (BGB-A317), GLS-010 (WBP-3055), AK-103 (HX-008), AK-105, CS-1003, HLX-10, retifanlimab (MGA-012), BI-754091, balstilimab (AGEN-2034), AMG-404, toripalimab (JS-001), cetrelimab (JNJ-63723283), genolimzumab (CBT-501), LZM-009, prolgolimab (BCD-100), lodapolimab (LY-3300054), SHR-1201, camrelizumab (SHR-1210), Sym-021, budigalimab (ABBV-181), PD1-PIK, BAT-1306, avelumab (MSB0010718C), CX-072, CBT-502, dostarlimab (TSR-042), MSB-2311, JTX-4014, BGB-A333, SHR-1316, CS-1001 (WBP-3155, envafolimab (KN-035), sintilimab (IBI-308), HLX-20, KL-A167, STI-A1014, STI-A1015 (IMC-001), BCD-135, FAZ-053, TQB-2450, MDX1105-01, GS-4224, GS-4416, INCB086550, MAX10181, zimberelimab (AB122), spartalizumab (PDR-001), and compounds disclosed in WO2018195321, WO2020014643, WO2019160882, or WO2018195321, as well as multi-specific inhibitors FPT-155 (CTLA4/PD-L1/CD28), PF-06936308 (PD-1/ CTLA4), MGD-013 (PD-1/LAG-3), FS-118 (LAG-3/PD-L1), RO-7247669 (PD-1/LAG-3), MGD-019 (PD-1/CTLA4), KN-046 (PD-1/CTLA4), MEDI-5752 (CTLA4/PD-1), RO-7121661 (PD-1/TIM-3), RG7769 (PD-1/TIM-3), TAK-252 (PD-1/OX40L), XmAb-20717 (PD-1/CTLA4), AK-104 (CTLA4/PD-1), FS-118 (LAG-3/PD-L1), FPT-155 (CTLA4/PD-L1/CD28), GEN-1046 (PD-L1/4-1BB), bintrafusp alpha (M7824; PD-L1/TGFβ-EC domain), CA-170 (PD-L1/VISTA), CDX-527 (CD27/PD-L1), LY-3415244 (TIM3/PDL1), and INBRX-105 (4-1BB/PDL1). In some embodiments the PD-L1 inhibitor is a small molecule inhibitor, such as CA-170, GS-4224, GS-4416 and lazertinib (GNS-1480; PD-L1/EGFR).

[0354] Examples of inhibitors of TIGIT that can be co-administered include tiragolumab (RG-6058), vibostolimab, domvanalimab (AB154), AB308, BMS-986207, AGEN-1307, COM-902, or etigilimab.

[0355] Examples of inhibitors of CTLA4 that can be co-administered include ipilimumab, tremelimumab, BMS-986218, AGEN1181, zalifrelimab (AGEN1884), BMS-986249, MK-1308, REGN-4659, ADU-1604, CS-1002 (ipilimumab biosimilar), BCD-145, APL-509, JS-007, BA-3071, ONC-392, AGEN-2041, HBM-4003, JHL-1155, KN-044, CG-0161, ATOR-1144, PBI-5D3H5, BPI-002, as well as multi-specific inhibitors FPT-155 (CTLA4/PD-L1/CD28), PF-06936308 (PD-1/CTLA4), MGD-019 (PD-1/CTLA4), KN-046 (PD-1/CTLA4), MEDI-5752 (CTLA4/PD-1), XmAb-20717 (PD-1/CTLA4), and AK-104 (CTLA4/PD-1).

[0356] Inhibition of regulatory T-cell (Treg) activity or Treg depletion can alleviate their suppression of antitumor immune responses and have anticancer effects. See, *e.g.*, Plitas and Rudensky, Annu. Rev. Cancer Biol. (2020) 4:459-77; Tanaka and Sakaguchi, Eur. J. Immunol. (2019) 49:1140-1146. In some embodiments, a compound of Formula (I), (Ia), (Ib), or (Ic) provided herein, or pharmaceutically acceptable salt thereof, provided herein is administered with one or more inhibitors of Treg activity or a Treg depleting agent. Treg inhibition or depletion can augment the effect of immune checkpoint inhibitors in cancer therapeutics.

[0357] In some embodiments compound or pharmaceutically acceptable salt thereof provided herein is administered with one or more Treg inhibitors. In some embodiments the Treg inhibitor can suppress the migration of Tregs into the tumor microenvironment. In some embodiments Treg inhibitor can reduce the immunosuppressive function of Tregs. In some embodiments, the Treg inhibitor can modulate the cellular phenotype and induce production of proinflammatory cytokines. Exemplary Treg inhibitors include, without limitation, CCR4 (NCBI Gene ID: 1233) antagonists and degraders of Ikaros zinc-finger proteins (*e.g.*, Ikaros (IKZF1; NCBI Gene ID: 10320), Helios (IKZF2; NCBI Gene ID: 22807), Aiolos (IKZF3; NCBI Gene ID: 22806), and Eos (IKZF4; NCBI Gene ID: 64375).

[0358] Examples of Helios degraders that can be co-administered include without limitation I-57 (Novartis) and compounds disclosed in WO2019038717, WO2020012334, WO20200117759, WO2021101919, and WO2023178181.

[0359] In some embodiments a compound or pharmaceutically acceptable salt thereof provided herein is administered with one or more Treg depleting agents. In some embodiments the Treg depleting agent is an antibody. In some embodiments the Treg depleting antibody has antibody-dependent cytotoxic (ADCC) activity. In some embodiments, the Treg depleting antibody is Fc-engineered to possess an enhanced ADCC activity. In some embodiments the Treg depleting antibody is an antibody-drug conjugate (ADC). Illustrative targets for Treg depleting agents include without limitation CD25 (IL2RA; NCBI Gene ID: 3559), CTLA4 (CD152; NCBI Gene ID: 1493); GITR (TNFRSF18; NCBI Gene ID: 8784); 4-1BB (CD137; NCBI Gene ID: 3604), OX-40 (CD134; NCBI Gene ID: 7293), LAG3 (CD223; NCBI Gene ID: 3902), TIGIT (NCBI Gene ID: 201633), CCR4 (NCBI Gene ID: 1233), and CCR8 (NCBI Gene ID: 1237).

[0360] In some embodiments the Treg inhibitor or Treg depleting agent that can be co-administered comprises an antibody or antigen-binding fragment thereof that selectively binds to a cell surface receptor selected from the group consisting of C-C motif chemokine receptor 4 (CCR4), C-C motif chemokine receptor 7 (CCR7), C-C motif chemokine receptor 8 (CCR8), C-X-C motif chemokine receptor 4 (CXCR4; CD184), TNFRSF4 (OX40), TNFRSF18 (GITR, CD357), TNFRSF9 (4-1BB, CD137), cytotoxic T-lymphocyte associated protein 4 (CTLA4, CD152), programmed cell death 1 (PDCD1, PD-1), Sialyl Lewis x (CD15s), CD27, ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1; CD39), protein tyrosine phosphatase receptor type C (PTPRC; CD45), neural cell adhesion molecule 1 (NCAM1; CD56), selectin L (SELL; CD62L), integrin subunit alpha E (ITGAE; CD103), interleukin 7 receptor (IL7R; CD127), CD40 ligand (CD40LG; CD154), folate receptor alpha (FOLR1), folate receptor beta (FOLR2), leucine rich repeat containing 32 (LRRC32; GARP),

IKAROS family zinc finger 2 (IKZF2; HELIOS), inducible T cell costimulatory (ICOS; CD278), lymphocyte activating 3 (LAG3; CD223), transforming growth factor beta 1 (TGFB1), hepatitis A virus cellular receptor 2 (HAVCR2; CD366; TIM3), T cell immunoreceptor with Ig and ITIM domains (TIGIT), TNF receptor superfamily member 1B (CD120b; TNFR2), IL2RA (CD25) or a combination thereof.

[0361] Examples of Treg depleting anti-CCR8 antibodies that can be administered include without limitation anti-CCR8 antibodies described in references disclosed herein, the entire contents of each of which are incorporated by reference herein.

[0362] In some embodiments, a CCR8 antibody is a monoclonal antibody having ADCC activity. Such antibodies are known in the art, for example from WO2020138489 Al, which is included herein by reference. In some embodiments, a CCR8 antibody is selected from an antibody disclosed in WO2020138489 Al, in particular an antibody as presented in the claims of WO2020138489 Al. In some embodiments, a CCR8 antibody is selected from a humanized antibody disclosed in WO2020138489 Al, in particular a humanized antibody as presented in the claims of WO2020138489 Al. In some embodiments, a CCR8 antibody is antibody 10A11, 2C7 or 19D7 from WO2020138489 Al or its humanized variant; in particular 10A11 or its humanized variant; more in particular the humanized 10A11 antibody. In some embodiments, a CCR8 antibody is 19D7 or the humanized 19D7 antibody.

[0363] In some embodiments, a CCR8 antibody includes BMS-986340 (Bristol Myers Squibb), LM-108 (LaNova Medicines), S-531011 (Shionogi), FPA157 (Five Prime, Amgen), IPG-7236 (Immunophage Biomedical), ICP-B05 (InnoCare Pharma Tech), SRF-114 (Surface Oncology), HBM1022 (Harbour BioMed), HFB1011 (HiFiBio), BAY-3375968 (Bayer), IO-1 (Oncurious), ZL-1218 (Zai Lab), GB2101 (Genor), PSB-114 (Sound Biologics), IPG-A05 (Immunophage Biomedical Co Ltd), PM-1024 (Biotheus Inc, Adimab LLC), DT-7012 (Domain Therapeutics SA), BCG-005

(Biocytogen LLC, Liberothera Co Ltd), GS-1811 (Jounce Therapeutics Inc, Gilead Sciences Inc), ABBC-514 (AbbieVie Inc), GNUV-202 (Genuv Inc), CHS-3318 (Coherus BioSciences Inc), CTM-033 (Lepu Biopharma), the anti-CCR8 monoclonal antibody by Integral Molecular Inc, the anti-CCR8 antibody by iBio Inc., the anti-CCR8 antibody by Bristol-Myers Squibb, the anti-CCR8 monoclonal antibody by iTeos therapeutics, and/or the anti-CCR8 therapy by Flanders Institute for Biotechnology VZW and Oncurious NV.

[0364] In some embodiments, a CCR8 antibody is an antibody described in WO2022078277, WO2022081718, WO2022000443, WO2022042690, WO2022003156, WO07044756, CN110835371, CN110835374, WO20138489, WO21142002, WO21152186, WO21163064, WO21178749, WO21194942, WO22136649, WO22136650, WO22136647, WO22241034, WO22256563, WO22256559, WO22268192, WO23288241, WO23010054, WO23020621, WO22211046, WO23098888, WO23116880, WO23137466, TW202330599, WO23174396, WO23193732, WO23208182, WO23206350, WO23219147, US11427640B1, US20210277129A1, US20230119066A1, US20230049152A1, US20220403037A1, US10087259B1, WO2013131010A2, WO2021183685A2, US20230176060A1, US20230101029A1, EP4118105A2, US20220389394A1, US20220365091A1, US20210324028A1, EP3589657A1, WO2018112032A1, EP4114862A2, WO2022216965A1, US20230270857A1, EP4232463A2, EP4225373A1, WO2023147488A1, WO2022200303A1, US20190092875A1, EP2656069A2, US6416954B1, WO2023125729A1, US20230160009A1, WO2023076574A1, US20230107291A1, WO2023046156A1, WO2023044402A1, WO2023036246A1, WO2022165260A1, WO2022256628A1, EP3458473B8, EP4090686A2, EP4081548A1, WO2022216702A1, WO2022192457A1, WO2022192895A1, US20220289838A1, EP4041758A1, EP4025255A1, US20220202950A1, EP3994270A1, EP3990476A1, WO2022063194A1, EP3917966A1, WO2021231327A2, EP3908601A1, US11173325B2, EP3793613A1, EP3762421A2, WO2020214718A1, EP2717941B1, US10401357B2,

US20200215111A1, EP2652508B1, US9233120B2, US8512701B2, US20200157518A1, EP3554550A1, US20190255107A1, US20190153115A1, EP3471752A1, WO2019036043A2, US20190041389A1, US20180318417A1, US20170218091A1, WO2017011342A1, WO2015183837A1, US20150259418A1, EP2872170A2, WO2015037000A1, EP2654792A2, EP2655415A2, US20130004416A1, EP2337795A2, US20090220486A1, US20090118175A1, EP1948694A2, WO2007005605A2, WO2005010153A2, or WO2002067771A2.

[0365] In some embodiments, a CCR8 antibody is an antibody that can be obtained from the hybridoma having ATCC Accession No. PTA-6940, PTA-6938, or PTA-6939.

[0366] In some embodiments, a CCR8 antibody is the HBM1022 antibody as disclosed in Lu *et al.* HBM1022, a novel anti-CCR8 antibody depletes tumor-infiltrating regulatory T cells via enhanced ADCC activity, mediates potent anti-tumor activity with Keytruda. Journal for ImmunoTherapy of Cancer 2020;8:doi: 10.1136/jitc-2020-SITC2020.0711.

[0367] In some embodiments, a CCR8 antibody is the FPA157 antibody as disclosed in Rankin A, Naik E861 Development of FPA157, an anti-CCR8 depleting antibody engineered to preferentially eliminate tumor-infiltrating T regulatory cells. Journal for ImmunoTherapy of Cancer 2020;8:doi: 10.1136/jitc-2020-SITC2020.0861.

[0368] In some embodiments, a CCR8 antibody is the SRFl 14 antibody as disclosed in Lake A, Warren M, Das S, *et al.* Journal for ImmunoTherapy of Cancer 2020;8:doi: 10.1136/jitc-2020-SITC2020.0726.

[0369] In some embodiments, a CCR8 antibody is the anti-CCR8 hIgGl-nonfucosylated BMS-986340 antibody as disclosed in Lan, Ruth, *et al.* "Highly selective anti-CCR8 antibody-mediated depletion of regulatory T cells leads to potent antitumor activity alone and in combination with anti-PD-1 in preclinical models." (2020): 6694-6694 and in Bayati F, Mohammadi M, Valadi M, Jamshidi S, Foma AM, Sharif-Paghaleh E. The Therapeutic Potential of Regulatory T Cells: Challenges and

Opportunities. Front Immunol. 2021;11:585819. Published 2021 Jan 15. doi: 10.3389/fimmu.2020.585819.

[0370] In some embodiments, a CCR8 antibody is the nanobody as disclosed in Van Damme H, Dombrecht B, Kiss M, Roose H, Allen E, Van Overmeire E, Kancheva D, Martens L, Murgaski A, Bardet PMR, Blancke G, Jans M, Bolli E, Martins MS, Elkrim Y, Dooley J, Boon L, Schwarze JK, Tacke F, Movahedi K, Vandamme N, Neyns B, Ocak S, Scheyltjens I, Vereecke L, Nana FA, Merchiers P, Laoui D, Van Ginderachter JA. Therapeutic depletion of CCR8+ tumor-infiltrating regulatory T cells elicits antitumor immunity and synergizes with anti-PD-1 therapy. J Immunother Cancer. 2021 Feb;9(2):e001749. doi: 10.1136/j itc-2020-001749. PMID: 33589525; PMCID: PMC7887378.

[0371] In some embodiments, a CCR8 antibody is azirkitug, cafelkibart, denikitug, lanerkitug,

[0372] Examples of Treg depleting anti-CCR4 antibodies that can be administered include mogamulizumab.

Chemotherapeutic Agents

[0373] In some embodiments a compound provided herein is administered with a chemotherapeutic agent or anti-neoplastic agent.

[0374] As used herein, the term “chemotherapeutic agent” or “chemotherapeutic” (or “chemotherapy” in the case of treatment with a chemotherapeutic agent) is meant to encompass any non-proteinaceous (*e.g.*, non-peptidic) chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include but not limited to: alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan, and piposulfan; aziridines such as benzodepa, carboquone, meturedepa, and uredepa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide, and trimemylolomelamine; acetogenins, *e.g.*, bullatacin and

bullatacinone; a camptothecin, including synthetic analog topotecan; bryostatin, callystatin; CC-1065, including its adozelesin, carzelesin, and bizelesin synthetic analogs; cryptophycins, particularly cryptophycin 1 and cryptophycin 8;dolastatin; duocarmycin, including the synthetic analogs KW-2189 and CBI-TMI; eleutherobin; 5-azacytidine; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cyclophosphamide, glufosfamide, evofosfamide, bendamustine, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, and uracil mustard; nitrosoureas such as carmustine, chlorozotocin, foremustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin phiI1), dynemicin including dynemicin A, bisphosphonates such as clodronate, an esperamicin, neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomophores, aclacinomycins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carrninomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as demopterin, methotrexate, pteropterin, and trimetrexate; purine analogs such as cladribine, pentostatin, fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, and testolactone; anti-adrenals such as aminoglutethimide, mitotane, and trilostane; folic acid replinishers such as frolinic acid;

radiotherapeutic agents such as Radium-223; trichothecenes, especially T-2 toxin, verracurin A, roridin A, and anguidine; taxoids such as paclitaxel (TAXOL®), abraxane, docetaxel (TAXOTERE®), cabazitaxel, BIND-014, tesetaxel; sabizabulin (Veru-111); platinum analogs such as cisplatin and carboplatin, NC-6004 nanoplatin; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; hestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformthine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; leucovorin; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; fluoropyrimidine; folinic acid; podophyllinic acid; 2-ethylhydrazide; procarbazine; polysaccharide-K (PSK); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; trabectedin, triaziquone; 2,2',2"-trichlorotriemylamine; urethane; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiopeta; chlorambucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; methotrexate; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitroxantrone; vancristine; vinorelbine (NAVELBINE®); novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeoloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DFMO); retinoids such as retinoic acid; capecitabine; NUC-1031; FOLFOX (folinic acid, 5-fluorouracil, oxaliplatin); FOLFIRI (folinic acid, 5-fluorouracil, irinotecan); FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin, irinotecan), FOLFIRINOX (folinic acid, 5-fluorouracil, irinotecan, oxaliplatin), and pharmaceutically acceptable salts, acids, or derivatives of any of the above. Such agents can be conjugated onto an antibody or any targeting agent described herein to create an antibody-drug conjugate (ADC) or targeted drug conjugate.

[0375] In some embodiments, ADCs that can be co-administered include belantamab mafodotin, brentuximab vedotin, camidanlumab tesirine, trastuzumab deruxtecan, trasuzumab emtansine,

mirvetuximab soravtansine, ladiratuzumab vedotin, loncastuximab tesirine, sacituzumab govitecan, datopotamab deruxtecan (DS-1062; Dato-DXd), inotuzumab ozogamicin, gemtuzumab ozogamicin, loncastuzumab tesirine, belantamab mafodotin, mirvetuximab soravtansine, moxetumomab pasudotox, patritumab deruxtecan, polatuzumab vedotin, upifitamab rilsodotin, rovalpituzumab tesirine, enfortumab vedotin, tisotumab vedotin, tusamitamab ravtansine, disitamab vedotin, telisotuzumab vedotin (ABBV-399).

Cytotoxic or Cytostatic agent

[0376] In some embodiments, the co-treatment is a cytotoxic or cytostatic agent. Examples of cytotoxic or cytostatic agents include chemotherapeutic agents and cytolytic agents described herein. In some embodiments, the co-treatment is an antibody drug conjugate (ADC). In some embodiments, the antibody moiety of the ADC issacituzumab, wherein the antibody moiety targets TROP2 and the drug moiety is govitecan. In some embodiments, the antibody moiety of the ADC istisotumab and the drug moiety is vedotinTissue Factor. In some embodiments, the antibody moiety of the ADC isenfortumab and the drug moiety is vedotinNectin4. In some embodiments, the antibody moiety of the ADC isbrentuximab and the drug moiety is vedotinCD30. In some embodiments, the antibody moiety of the ADC istrastuzumab and the drug moiety is deruxtecanHER2. In some embodiments, the antibody moiety of the ADC istrastuzumab and the drug moiety is emtansineHER2. In some embodiments, the antibody moiety of the ADC ispolatuzumab and the drug moiety is vedotinCD79. In some embodiments, the antibody moiety of the ADC isinotuzumab and the drug moiety is ozogamicinCD22. In some embodiments, the antibody moiety of the ADC isgemtuzumab and the drug moiety is ozogamicinCD33. In some embodiments, the antibody moiety of the ADC isloncastuximab and the drug moiety is tesirineCD19. In some embodiments, the antibody moiety of the ADC isbelantamab and the drug moiety is mafodotinBCMA. In some embodiments, the antibody moiety of

the ADC ismirvetuximab and the drug moiety is soravtansineFRα. In some embodiments, the antibody moiety of the ADC ismoxetumomab and the drug moiety is pasudotoxCD22.

Exemplified Combination Therapies

Breast Cancer Combination Therapy

[0377] Therapeutic agents used to treat breast cancer include albumin-bound paclitaxel, anastrozole, atezolizumab, capecitabine, carboplatin, cisplatin, cyclophosphamide, docetaxel, doxorubicin, epirubicin, everolimus, exemestane, fluorouracil, fulvestrant, gemcitabine, Ixabepilone, lapatinib, letrozole, methotrexate, mitoxantrone, paclitaxel, pegylated liposomal doxorubicin, pertuzumab, tamoxifen, toremifene, trastuzumab, vinorelbine, and any combinations thereof. In some embodiments therapeutic agents used to treat breast cancer (*e.g.*, HR+/-/HER2 +/-) include trastuzumab (Herceptin®), pertuzumab (Perjeta®), docetaxel, carboplatin, palbociclib (Ibrance®), letrozole, trastuzumab emtansine (Kadcyla®), fulvestrant (Faslodex®), olaparib (Lynparza®), eribulin, tucatinib, capecitabine, lapatinib, everolimus (Afinitor®), exemestane, eribulin mesylate (Halaven®), and combinations thereof. In some embodiments therapeutic agents used to treat breast cancer include trastuzumab + pertuzumab + docetaxel, trastuzumab + pertuzumab + docetaxel + carboplatin, palbociclib + letrozole, tucatinib + capecitabine, lapatinib + capecitabine, palbociclib + fulvestrant, or everolimus + exemestane. In some embodiments therapeutic agents used to treat breast cancer include trastuzumab deruxtecan (Enhertu®), datopotamab deruxtecan (DS-1062), enfortumab vedotin (Padcev®), balixafortide, elacestrant, or a combination thereof. In some embodiments therapeutic agents used to treat breast cancer include balixafortide + eribulin.

Triple Negative Breast Cancer (TNBC) Combination Therapy

[0378] Therapeutic agents used to treat TNBC include atezolizumab, cyclophosphamide, docetaxel, doxorubicin, epirubicin, fluorouracil, paclitaxel, and combinations thereof. In some embodiments therapeutic agents used to treat TNBC include olaparib (Lynparza®), atezolizumab (Tecentriq®), paclitaxel (Abraxane®), eribulin, bevacizumab (Avastin®), carboplatin, gemcitabine, eribulin

mesylate (Halaven®), sacituzumab govitecan (Trodelvy®), pembrolizumab (Keytruda®), cisplatin, doxorubicin, epirubicin, or a combination thereof. In some embodiments therapeutic agents to treat TNBC include atezolizumab + paclitaxel, bevacizumab + paclitaxel, carboplatin + paclitaxel, carboplatin + gemcitabine, or paclitaxel + gemcitabine. In some embodiments therapeutic agents used to treat TNBC include eryaspase, capivasertib, alpelisib, rucaparib + nivolumab, atezolumab + paclitaxel + gemcitabine+ capecitabine + carboplatin, ipatasertib + paclitaxel, ladiratuzumab vedotin + pembrolimab, durvalumab + DS-8201a, trilaciclib + gemcitabine +carboplatin. In some embodiments therapeutic agents used to treat TNBC include trastuzumab deruxtecan (Enhertu®), datopotamab deruxtecan (DS-1062), enfortumab vedotin (Padcev®), balixafortide, adagloxad simolenin, nelipepimut-s (NeuVax®), nivolumab (Opdivo®), rucaparib, toripalimab (Tuoyi®), camrelizumab, capivasertib, durvalumab (Imfinzi®), and combinations thereof. In some embodiments therapeutic agents use to treat TNBC include nivolumab + rucaparib, bevacizumab (Avastin®) + chemotherapy, toripalimab + paclitaxel, toripalimab + albumin-bound paclitaxel, camrelizumab + chemotherapy, pembrolizumab + chemotherapy, balixafortide + eribulin, durvalumab + trastuzumab deruxtecan, durvalumab + paclitaxel, or capivasertib + paclitaxel.

Bladder Cancer Combination Therapy

[0379] Therapeutic agents used to treat bladder cancer include datopotamab deruxtecan (DS-1062), trastuzumab deruxtecan (Enhertu®), erdafitinib, eganelisib, lenvatinib, bempegaldesleukin (NKTR-214), or a combination thereof. In some embodiments therapeutic agents used to treat bladder cancer include eganelisib + nivolumab, pembrolizumab (Keytruda®) + enfortumab vedotin (Padcev®), nivolumab + ipilimumab, duravalumab + tremelimumab, lenvatinib + pembrolizumab, enfortumab vedotin (Padcev®) + pembrolizumab, and bempegaldesleukin + nivolumab.

Colorectal Cancer (CRC) Combination Therapy

[0380] Therapeutic agents used to treat CRC include bevacizumab, capecitabine, cetuximab, fluorouracil, irinotecan, leucovorin, oxaliplatin, panitumumab, ziv-aflibercept, and any combinations thereof. In some embodiments therapeutic agents used to treat CRC include bevacizumab (Avastin®), leucovorin, 5-FU, oxaliplatin (FOLFOX), pembrolizumab (Keytruda®), FOLFIRI, regorafenib (Stivarga®), aflibercept (Zaltrap®), cetuximab (Erbitux®), Lonsurf (Orcantas®), XELOX, FOLFOXIRI, or a combination thereof. In some embodiments therapeutic agents used to treat CRC include bevacizumab + leucovorin + 5-FU + oxaliplatin (FOLFOX), bevacizumab + FOLFIRI, bevacizumab + FOLFOX, aflibercept + FOLFIRI, cetuximab + FOLFIRI, bevacizumab + XELOX, and bevacizumab + FOLFOXIRI. In some embodiments therapeutic agents used to treat CRC include binimetinib + encorafenib + cetuximab, trametinib + dabrafenib + panitumumab, trastuzumab + pertuzumab, napabucasin + FOLFIRI + bevacizumab, nivolumab + ipilimumab.

Esophageal and Esophagogastric Junction Cancer Combination Therapy

[0381] Therapeutic agents used to treat esophageal and esophagogastric junction cancer include capecitabine, carboplatin, cisplatin, docetaxel, epirubicin, fluoropyrimidine, fluorouracil, irinotecan, leucovorin, oxaliplatin, paclitaxel, ramucirumab, trastuzumab, and any combinations thereof. In some embodiments therapeutic agents used to treat gastroesophageal junction cancer (GEJ) include herceptin, cisplatin, 5-FU, ramicurimab, or paclitaxel. In some embodiments therapeutic agents used to treat GEJ cancer include ALX-148, AO-176, or IBI-188.

Gastric Cancer Combination Therapy

[0382] Therapeutic agents used to treat gastric cancer include capecitabine, carboplatin, cisplatin, docetaxel, epirubicin, fluoropyrimidine, fluorouracil, Irinotecan, leucovorin, mitomycin, oxaliplatin, paclitaxel, ramucirumab, trastuzumab, and any combinations thereof.

Head and Neck Cancer Combination Therapy

[0383] Therapeutic agents used to treat head & neck cancer include afatinib, bleomycin, capecitabine, carboplatin, cetuximab, cisplatin, docetaxel, fluorouracil, gemcitabine, hydroxyurea, methotrexate, nivolumab, paclitaxel, pembrolizumab, vinorelbine, and any combinations thereof.

[0384] Therapeutic agents used to treat head and neck squamous cell carcinoma (HNSCC) include pembrolizumab, carboplatin, 5-FU, docetaxel, cetuximab (Erbitux®), cisplatin, nivolumab (Opdivo®), and combinations thereof. In some embodiments therapeutic agents used to treat HNSCC include pembrolizumab + carboplatin + 5-FU, cetuximab + cisplatin + 5-FU, cetuximab + carboplatin + 5-FU, cisplatin + 5-FU, and carboplatin + 5-FU. In some embodiments therapeutic agents used to treat HNSCC include durvalumab, durvalumab + tremelimumab, nivolumab + ipilimumab, rovaluecel, pembrolizumab, pembrolizumab + epacadostat, GSK3359609 + pembrolizumab, lenvatinib + pembrolizumab, retifanlimab, retifanlimab + enobituzumab, ADU-S100 + pembrolizumab, epacadostat + nivolumab+ ipilimumab/lirilumab.

Non-Small Cell Lung Cancer Combination Therapy

[0385] Therapeutic agents used to treat non-small cell lung cancer (NSCLC) include afatinib, albumin-bound paclitaxel, alectinib, atezolizumab, bevacizumab, bevacizumab, cabozantinib, carboplatin, cisplatin, crizotinib, dabrafenib, docetaxel, erlotinib, etoposide, gemcitabine, nivolumab, paclitaxel, pembrolizumab, pemetrexed, ramucirumab, trametinib, trastuzumab, vandetanib, vemurafenib, vinblastine, vinorelbine, and any combinations thereof. In some embodiments therapeutic agents used to treat NSCLC include alectinib (Alecensa®), dabrafenib (Tafinlar®), trametinib (Mekinist®), osimertinib (Tagrisso®), entrectinib (Tarceva®), crizotinib (Xalkori®), pembrolizumab (Keytruda®), carboplatin, pemetrexed (Alimta®), nab-paclitaxel (Abraxane®), ramucirumab (Cyramza®), docetaxel, bevacizumab (Avastin®), brigatinib, gemcitabine, cisplatin, afatinib (Gilotrif®), nivolumab (Opdivo®), gefitinib (Iressa®), and combinations thereof. In some

embodiments therapeutic agents used to treat NSCLC include dabrafenib + trametinib, pembrolizumab + carboplatin + pemetrexed, pembrolizumab + carboplatin + nab-paclitaxel, ramucirumab + docetaxel, bevacizumab + carboplatin + pemetrexed, pembrolizumab + pemetrexed + carboplatin, cisplatin + pemetrexed, bevacizumab + carboplatin + nab-paclitaxel, cisplatin + gemcitabine, nivolumab + docetaxel, carboplatin + pemetrexed, carboplatin + nab-paclitaxel, or pemetrexed + cisplatin + carboplatin. In some embodiments therapeutic agents used to NSCLC include datopotamab deruxtecan (DS-1062), trastuzumab deruxtecan (Enhertu®), enfortumab vedotin (Padcev®), durvalumab, canakinumab, cemiplimab, nogapendekin alfa, avelumab, tiragolumab, domvanalimab, vibostolimab, ociperlimab, or a combination thereof. In some embodiments therapeutic agents used to treat NSCLC include datopotamab deruxtecan + pembrolizumab, datopotamab deruxtecan + durvalumab, durvalumab + tremelimumab, pembrolizumab + lenvatinib + pemetrexed, pembrolizumab + olaparib, nogapendekin alfa (N-803) + pembrolizumab, tiragolumab + atezolizumab, vibostolimab + pembrolizumab, or ociperlimab + tislelizumab.

Small Cell Lung Cancer Combination Therapy

[0386] Therapeutic agents used to treat small cell lung cancer (SCLC) include atezolizumab, bendamustime, carboplatin, cisplatin, cyclophosphamide, docetaxel, doxorubicin, etoposide, gemcitabine, ipillimumab, irinotecan, nivolumab, paclitaxel, temozolomide, topotecan, vincristine, vinorelbine, and any combinations thereof. In some embodiments therapeutic agents used to treat SCLC include atezolizumab, carboplatin, cisplatin, etoposide, paclitaxel, topotecan, nivolumab, durvalumab, trilaciclib, or combinations thereof. In some embodiments therapeutic agents used to treat SCLC include atezolizumab + carboplatin + etoposide, atezolizumab + carboplatin, atezolizumab + etoposide, or carboplatin + paclitaxel.

Ovarian Cancer Combination Therapy

[0387] Therapeutic agents used to treat ovarian cancer include 5-flourouracil, albumin bound paclitaxel, altretamine, anastrozole, bevacizumab, capecitabine, carboplatin, cisplatin, cyclophosphamide, docetaxel, doxorubicin, etoposide, exemestane, gemcitabine, ifosfamide, irinotecan, letrozole, leuprolide acetate, liposomal doxorubicin, megestrol acetate, melphalan, olaparib, oxaliplatin, paclitaxel, pazopanib, pemetrexed, tamoxifen, topotecan, vinorelbine, and any combinations thereof.

Pancreatic Cancer Combination Therapies

[0388] Therapeutic agents used to treat pancreatic cancer include 5-FU, leucovorin, oxaliplatin, irinotecan, gemcitabine, nab-paclitaxel (Abraxane®), FOLFIRINOX, and combinations thereof. In some embodiments therapeutic agents used to treat pancreatic cancer include 5-FU + leucovorin + oxaliplatin + irinotecan, 5-FU + nanoliposomal irinotecan, leucovorin + nanoliposomal irinotecan, and gemcitabine + nab-paclitaxel.

Prostate Cancer Combination Therapies

[0389] Therapeutic agents used to treat prostate cancer include enzalutamide (Xtandi®), leuprolide, trifluridine, tipiracil (Lonsurf), cabazitaxel, prednisone, abiraterone (Zytiga®), docetaxel, mitoxantrone, bicalutamide, LHRH, flutamide, ADT, sabizabulin (Veru-111), and combinations thereof. In some embodiments therapeutic agents used to treat prostate cancer include enzalutamide + leuprolide, trifluridine + tipiracil (Lonsurf), cabazitaxel + prednisone, abiraterone + prednisone, docetaxel + prednisone, mitoxantrone + prednisone, bicalutamide + LHRH, flutamide + LHRH, leuprolide + flutamide , and abiraterone + prednisone + ADT.

Exemplary Embodiments

[0390] Embodiment 1. An anti-FAP antibody or antigen-binding fragment thereof, comprising heavy chain complementarity determining regions HCDR1, HCDR2 and HCDR3, and light chain complementarity determining regions LCDR1, LCDR2 and LCDR3, wherein

LCDR1 comprises an amino acid sequence as shown in KTNQNVDYX$_1$GNTFMH (SEQ ID NO: 23), wherein X$_1$ is N or S, LCDR2 comprises an amino acid sequence as shown in LASNLAS (SEQ ID NO: 24), LCDR3 comprises an amino acid sequence as shown in QQSRNLPYT (SEQ ID NO: 25); HCDR1 comprises an amino acid sequence as shown in IYGVN (SEQ ID NO: 26), HCDR2 comprises an amino acid sequence as shown in AIWSGGRKDYX$_2$LSLKS (SEQ ID NO: 27), wherein X$_2$ is N or S, and HCDR3 comprises an amino acid sequence as shown in SQDMPGYFDY (SEQ ID NO: 28); or

LCDR1 comprises an amino acid sequence as shown in SASSRVGYMH (SEQ ID NO: 29), LCDR2 comprises an amino acid sequence as shown in DTSKLAS (SEQ ID NO: 30), LCDR3 comprises an amino acid sequence as shown in FQGSGYPFT (SEQ ID NO: 31); HCDR1 comprises an amino acid sequence as shown in TAGMSVG (SEQ ID NO: 32), HCDR2 comprises an amino acid sequence as shown in DIWWDDKKHYNPSLKD (SEQ ID NO: 33), HCDR3 comprises an amino acid sequence as shown in DMIFNFYFDV (SEQ ID NO: 34).

[0391] Embodiment 2. The anti-FAP antibody or antigen-binding fragment thereof of embodiment 1, comprising a heavy chain variable region (VH) and a light chain (VL), wherein the VH includes the HCDR1, HCDR2 and HCDR3 of embodiment 1, and/or the VL includes the LCDR1, LCDR2 and LCDR3 of embodiment 1.

[0392] Embodiment 3. The anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 1-2, comprising a fragment crystallizable (Fc) region derived from immunoglobulin.

[0393] Embodiment 4. The anti-FAP antibody or antigen-binding fragment thereof of embodiment 3, wherein the Fc fragment is derived from IgG1, IgG2, IgG3, or IgG4, optionally the Fc fragment is derived from IgG4.

[0394] Embodiment 5. The anti-FAP antibody or antigen-binding fragment thereof of embodiment 3 or 4, wherein the Fc fragment comprises mutation S228P.

[0395] Embodiment 6. The anti-FAP antibody or antigen-binding fragment thereof of embodiment 5, comprising

(1) a heavy chain having an amino acid sequence set forth in SEQ ID NO: 1, and a light chain having an amino acid sequence set forth in SEQ ID NO: 2; or

(2) a heavy chain having an amino acid sequence set forth in SEQ ID NO: 4, and a light chain having an amino acid sequence set forth in SEQ ID NO: 3.

[0396] Embodiment 7. The anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 1-6, wherein the anti-FAP antibody or antigen-binding fragment thereof cross-reacts with human, cynomolgus and mouse FAP.

[0397] Embodiment 8. A bispecific antibody or antigen-binding fragment thereof, comprising a FAP antigen-binding moiety, and a cytokine moiety capable of stimulating immune cells, wherein the FAP antigen-binding moiety comprises:

LCDR1 comprises an amino acid sequence as shown in KTNQNVDYX$_1$GNTFMH (SEQ ID NO: 23), wherein X$_1$ is N or S, LCDR2 comprises an amino acid sequence as shown in LASNLAS (SEQ ID NO: 24), LCDR3 comprise an amino acid sequence as shown in QQSRNLPYT (SEQ ID NO: 25); HCDR1 comprises an amino acid sequence as shown in IYGVN (SEQ ID NO: 26), HCDR2 comprises an amino acid sequence as shown in AIWSGGRKDYX$_2$LSLKS (SEQ ID NO: 27), wherein X$_2$ is N or S , HCDR3 comprise an amino acid sequence as shown in SQDMPGYFDY (SEQ ID NO: 28); or

LCDR1 comprises an amino acid sequence as shown in SASSRVGYMH (SEQ ID NO: 29), LCDR2 comprises an amino acid sequence as shown in DTSKLAS (SEQ ID NO: 30), LCDR3 comprise an amino acid sequence as shown in FQGSGYPFT (SEQ ID NO: 31); HCDR1 comprises an amino acid sequence as shown in TAGMSVG (SEQ ID NO: 32), HCDR2 comprises an amino acid sequence as shown in DIWWDDKKHYNPSLKD (SEQ ID NO: 33), HCDR3 comprise an amino acid sequence as shown in DMIFNFYFDV (SEQ ID NO: 34).

[0398] Embodiment 9. The bispecific antibody or antigen-binding fragment thereof of embodiment 8, wherein the FAP antigen-binding moiety comprises at least one VH paired with a VL, wherein the VH includes the HCDR1, HCDR2 and HCDR3 of embodiment 8, and/or the VL includes the LCDR1, LCDR2 and LCDR3 of embodiment 8.

[0399] Embodiment 10. The bispecific antibody or antigen-binding fragment thereof of embodiment 8 or 9, comprising a Fc fragment derived from immunoglobulin at N-terminus of the FAP antigen-binding moiety.

[0400] Embodiment 11. The bispecific antibody or antigen-binding fragment thereof of embodiment 10, wherein the Fc fragment is derived from IgG1, IgG2, IgG3, or IgG4, optionally the Fc fragment is derived from IgG4.

[0401] Embodiment 12. The bispecific antibody or antigen-binding fragment thereof of embodiment 10 or 11, wherein the Fc fragment comprises mutation S228P.

[0402] Embodiment 13. The bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-12, wherein the Fc fragment comprises one or more modifications selected from the group consisting of knobs-into-holes, DDKK, electrostatic steering of CH3, DuoBody, SEEDbodies, cFAE, XmAb, Azymetric, and BEAT®, optionally, the Fc fragment comprises modifications knobs-into-holes and/or DDKK.

[0403] Embodiment 14. The bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-13, wherein the cytokine moiety is at C-terminus of the bispecific antibody or antigen-binding fragment thereof.

[0404] Embodiment 15. The bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-14, wherein the cytokine moiety is operably linked to the C-terminus of the Fc fragment.

[0405] Embodiment 16. The bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-15, wherein the cytokine moiety links to the Fc fragment via a linker.

[0406] Embodiment 17. The bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-16, wherein the cytokine moiety comprises a first cytokine moiety and a second cytokine moiety, the first cytokine moiety comprises one LIGHT mutant and the second cytokine moiety comprises tandem-linked two LIGHT mutants, the LIGHT mutant includes an amino acid sequence set forth in SEQ ID NO: 17 or 18.

[0407] Embodiment 18. The bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-17, comprising a first heavy chain, a second heavy chain, and two light chains paired with the first heavy chain and the second heavy chain, respectively, wherein

(1) the first heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 5; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 6; the light chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 3;

(2) the first heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 8; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 9; the light chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 7;

(3) the first heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 10; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 11; the light chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to an amino acid sequence set forth in SEQ ID NO: 3.

[0408] Embodiment 19. The bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-18, comprising the following:

(1) the first heavy chain having an amino acid sequence set forth in SEQ ID NO: 5, the second heavy chain having an amino acid sequence set forth in SEQ ID NO: 6, and the light chain having an amino acid sequence set forth in SEQ ID NO: 3;

(2) the first heavy chain having an amino acid sequence set forth in SEQ ID NO: 8, the second heavy chain having an amino acid sequence set forth in SEQ ID NO: 9, and the light chain having an amino acid sequence set forth in SEQ ID NO: 7;

(3) the first heavy chain having an amino acid sequence set forth in SEQ ID NO: 10, the second heavy chain having an amino acid sequence set forth in SEQ ID NO: 11, and the light chain having an amino acid sequence set forth in SEQ ID NO: 3.

[0409] Embodiment 20. The bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-19, wherein the bispecific antibody or antigen-binding fragment thereof binds to FAP with dissociation constant (KD) no more than 20 nM, 15 nM, 10 nM or 5 nM.

[0410] Embodiment 21. The bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-20, wherein the bispecific antibody or antigen-binding fragment thereof binds to LTßR with dissociation constant (KD) no more than 20 nM, 15 nM, 10 nM or 5 nM.

[0411] Embodiment 22. The bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-21, wherein the bispecific antibody or antigen-binding fragment thereof barely binds to human or cynomolgus HVEM.

[0412] Embodiment 23. The bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-22, wherein the LIGHT mutant in bispecific antibody or antigen-binding fragment thereof is capable to reduce binding affinity to DcR3.

[0413] Embodiment 24. The bispecific antibody or antigen-binding fragment thereof of any one of embodiment 8-23, wherein the bispecific antibody or antigen-binding fragment thereof specifically binds to human FAP, and/or dose not bind to DPPIV.

[0414] Embodiment 25. An isolated polynucleotide encoding any sequence of the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 1-7 or the bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-24.

[0415] Embodiment 26. A construct comprising the polynucleotide of embodiment 25.

[0416] Embodiment 27. An antibody expressing system, comprising the construct including an isolated polynucleotide of embodiment 25 or having a genome integrated with an exogenous polynucleotide of embodiment 25, wherein preferably, the expressing system is a cell expression system.

[0417] Embodiment 28. A method for producing the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 1-7 or the bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-24, comprising: under conditions suitable for expressing the antibody, expressing the antibody or fusion protein using the antibody expressing system of embodiment 27.

[0418] Embodiment 29. A pharmaceutical composition comprising the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 1-7 or the bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-24, and a pharmaceutically acceptable carrier.

[0419] Embodiment 30. A kit comprising the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 1-7, the bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-24, the isolated polynucleotide of embodiment 25, or the construct of embodiment 26.

[0420] Embodiment 31. Use of the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 1-7, the bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-24 or the pharmaceutical composition of embodiment 29 in the manufacture of a therapeutic agent for preventing, diagnosing, or treating a disease, disorder, or condition.

[0421] Embodiment 32. The use of embodiment 31, wherein the disease, disorder, or condition comprises tumor diseases.

[0422] Embodiment 33. The use of embodiment 31 or 32, wherein at least a tumor cell expresses FAP.

[0423] Embodiment 34. The use of any one of embodiments 31-33, wherein the tumor diseases are solid tumors.

[0424] Embodiment 35. The use of any one of embodiments 31-34, wherein the tumor comprises gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, skin cancer, bone cancer, multiple myeloma, glioma, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer and lymphoma.

[0425] Embodiment 36. A method for treating a subject in need, comprising administrating to the subject a therapeutically effective amount of the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 1-7, the bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-24 or the pharmaceutical composition of embodiment 29.

[0426] Embodiment 37. A method for decreasing the rate of tumor growth or the number of tumor cells, comprising contacting a tumor cell with an effective amount of the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 1-7, the bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-24 or the pharmaceutical composition of embodiment 29.

[0427] Embodiment 38. A method of killing a tumor cell, comprising contacting a tumor cell with an effective amount of the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 1-7, the bispecific antibody or antigen-binding fragment thereof of any one of embodiments 8-24 or the pharmaceutical composition of embodiment 29.

[0428] Embodiment 39. An anti-FAP antibody or antigen-binding fragment thereof, comprising a heavy chain variable region (VH) including heavy chain complementarity determining regions HCDR1, HCDR2 and HCDR3, and a light chain variable region (VL) including light chain complementarity determining regions LCDR1, LCDR2 and LCDR3, wherein

LCDR1 comprises an amino acid sequence as shown in KTNQNVDYX$_1$GNTFMH (SEQ ID NO: 23), wherein X$_1$ is N or S , LCDR2 comprises an amino acid sequence as shown in LASNLAS (SEQ ID NO: 24), LCDR3 comprises an amino acid sequence as shown in QQSRNLPYT (SEQ ID NO: 25), HCDR1 comprises an amino acid sequence as shown in IYGVN (SEQ ID NO: 26), HCDR2 comprises an amino acid sequence as shown in AIWSGGRKDYX$_2$LSLKS (SEQ ID NO: 27), wherein X$_2$ is N or S , HCDR3 comprises an amino acid sequence as shown in SQDMPGYFDY (SEQ ID NO: 28); or

LCDR1 comprises an amino acid sequence as shown in SASSRVGYMH (SEQ ID NO: 29), LCDR2 comprises an amino acid sequence as shown in DTSKLAS (SEQ ID NO: 30), LCDR3 comprises an amino acid sequence as shown in FQGSGYPFT (SEQ ID NO: 31), HCDR1 comprises an amino acid sequence as shown in TAGMSVG (SEQ ID NO: 32), HCDR2 comprises an amino acid sequence as shown in DIWWDDKKHYNPSLKD (SEQ ID NO: 33), HCDR3 comprises an amino acid sequence as shown in DMIFNFYFDV (SEQ ID NO: 34).

[0429] Embodiment 40. The anti-FAP antibody or antigen-binding fragment thereof of embodiment 39, comprising

(1) a VH having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence (QVQLKESGPGMVQPSRTLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDYNLSLKSRLIISGDTSKSQVLLTMNSLQSEDTAMYFCARSQDMPGYFDYWGQGVMVTVSS), and a VL having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence (DIVLTQSPALAVSLGQRATISCKTNQNVDYNGNTFMHWYQQKPGQQPKLLLYLASNLASGIPDRFSGRGSGTDFTLTIDPVEADDTATYYCQQSRNLPYTFGAGTKLEIK); or

(2) a VH having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence (EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDYSLSLKSRLTISGDTSKNQVSLKLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS), and a VL having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence (SIVLTQPPSVSVAPGQTARITCKTNQNVDYSGNTFMHWYQQKPGQQPVLLLYLASNLASGIPERFSGSGSGTTFTLTISRVEAGDEADYYCQQSRNLPYTFGTGTKVTVL).

[0430] Embodiment 41. The anti-FAP antibody or antigen-binding fragment thereof of embodiment 39 or 40, comprising a fragment crystallizable (Fc) region derived from immunoglobulin.

[0431] Embodiment 42. The anti-FAP antibody or antigen-binding fragment thereof of embodiment 41, wherein the Fc fragment is derived from IgG1, IgG2, IgG3, or IgG4, optionally the Fc fragment is derived from IgG4.

[0432] Embodiment 43. The anti-FAP antibody or antigen-binding fragment thereof of embodiment 41 or 42, wherein the Fc fragment comprises mutation S228P or LALAPG mutation.

[0433] Embodiment 44. The anti-FAP antibody or antigen-binding fragment thereof of embodiment 43, comprising

(1) a heavy chain having an amino acid sequence set forth in SEQ ID NO: 1, and a light chain having an amino acid sequence set forth in SEQ ID NO: 2; or

(2) a heavy chain having an amino acid sequence set forth in SEQ ID NO: 3, and a light chain having an amino acid sequence set forth in SEQ ID NO: 4.

[0434] Embodiment 45. The anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 39-44, wherein the anti-FAP antibody or antigen-binding fragment thereof cross-reacts with human, cynomolgus and mouse FAP

[0435] Embodiment 46. Fusion protein, comprising a FAP antigen-binding moiety, and a cytokine moiety capable of stimulating immune cells, wherein the FAP antigen-binding moiety comprises a heavy chain variable region (VH) including heavy chain complementarity determining regions HCDR1, HCDR2 and HCDR3, and a light chain variable region (VL) including light chain complementarity determining regions LCDR1, LCDR2 and LCDR3, wherein

LCDR1 comprises an amino acid sequence as shown in KTNQNVDYX$_1$GNTFMH (SEQ ID NO: 23), wherein X$_1$ is N or S, LCDR2 comprises an amino acid sequence as shown in LASNLAS (SEQ ID

NO: 24), LCDR3 comprise an amino acid sequence as shown in QQSRNLPYT (SEQ ID NO: 25); HCDR1 comprises an amino acid sequence as shown in IYGVN (SEQ ID NO: 26), HCDR2 comprises an amino acid sequence as shown in AIWSGGRKDYX_2LSLKS (SEQ ID NO: 27), wherein X_2 is N or S, HCDR3 comprise an amino acid sequence as shown in SQDMPGYFDY (SEQ ID NO: 28); or LCDR1 comprises an amino acid sequence as shown in SASSRVGYMH (SEQ ID NO: 29), LCDR2 comprises an amino acid sequence as shown in DTSKLAS (SEQ ID NO: 30), LCDR3 comprise an amino acid sequence as shown in FQGSGYPFT (SEQ ID NO: 31); HCDR1 comprises an amino acid sequence as shown in TAGMSVG (SEQ ID NO: 32), HCDR2 comprises an amino acid sequence as shown in DIWWDDKKHYNPSLKD (SEQ ID NO: 33), HCDR3 comprise an amino acid sequence as shown in DMIFNFYFDV (SEQ ID NO: 34).

[0436] Embodiment 47. The fusion protein of embodiment 46, wherein the FAP antigen-binding moiety comprises

(1) a VH having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence (QVQLKESGPGMVQPSRTLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDYNLSLKSRLIISGDTSKSQVLLTMNSLQSEDTAMYFCARSQDMPGYFDYWGQGVMVTVSS), and a VL having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence (DIVLTQSPALAVSLGQRATISCKTNQNVDYNGNTFMHWYQQKPGQQPKLLLYLASNLASGIPDRFSGRGSGTDFTLTIDPVEADDTATYYCQQSRNLPYTFGAGTKLEIK); or

(2) a VH having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence (EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDYSLSLKSRLTISGDTSKNQVSLKLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS), and a

VL having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence (SIVLTQPPSVSVAPGQTARITCKTNQNVDYSGNTFMHWYQQKPGQQPVLLLYLASNLASGIPERFSGSGSGTTFTLTISRVEAGDEADYYCQQSRNLPYTFGTGTKVTVL).

[0437] Embodiment 48. The fusion protein of embodiment 46 or 47, comprising a Fc fragment derived from immunoglobulin at N-terminus of the FAP antigen-binding moiety.

[0438] Embodiment 49. The fusion protein of embodiment 48, wherein the Fc fragment is derived from IgG1, IgG2, IgG3, or IgG4, optionally the Fc fragment is derived from IgG4.

[0439] Embodiment 50. The fusion protein of embodiment 48 or 49, wherein the Fc fragment comprises mutation S228P or LALAPG mutation.

[0440] Embodiment 51. The fusion protein of any one of embodiments 46-50, wherein the Fc fragment comprises one or more modifications selected from the group consisting of knobs-into-holes, DDKK, electrostatic steering of CH3, DuoBody, SEEDbodies, cFAE, XmAb, Azymetric, and BEAT®, optionally, the Fc fragment comprises modifications knobs-into-holes and/or DDKK.

[0441] Embodiment 52. The fusion protein of any one of embodiments 46-51, wherein the cytokine moiety is at C-terminus of the fusion protein; optionally the cytokine moiety is operably linked to the C-terminus of the Fc fragment; optionally, the cytokine moiety links to the Fc fragment via a linker

[0442] Embodiment 53. The fusion protein of any one of embodiments 46-52, wherein the cytokine moiety comprises a first cytokine moiety and a second cytokine moiety, the first cytokine moiety comprises one cytokine mutant and the second cytokine moiety comprises tandem-linked two cytokine mutants, the first cytokine moiety and the second cytokine moiety link to different C-terminus of the protein fragments within the Fc fragment.

[0443] Embodiment 54. The fusion protein of embodiment 53, wherein the first cytokine moiety links to the C-terminus of the Fc fragment with hole mutations, and the second cytokine moiety links to the C-terminus of the Fc fragment with knob mutations

[0444] Embodiment 55. The fusion protein of embodiment 53 or 54, wherein the cytokine mutant is a LIGHT mutant, the LIGHT mutant includes an amino acid sequence set forth in SEQ ID NO: 17 or 18; or

the first cytokine moiety comprises one Lymphotoxin-β mutant, and the second cytokine moiety comprises tandem-linked Lymphotoxin-αβ mutant, the Lymphotoxin-β mutant includes an amino acid sequence (LSPGLPAAHLIGAPLKGQGLGWETTKEQAFLTSGTQFSDAEGLALPQDGLYYLYCLVGYR GRAPPGGGDPQGRSVTLRSSLYRAGGAYGPGTPELLLEGAETVTPVLDPARRQGYGPLWYT SVGFGGLVQLRRGERVYVNISHPDMVDFARGKTFFGAVMVG), the Lymphotoxin-α mutant includes an amino acid sequence (AHSTLKPAAHLIGDPSKQNSLLWRANTDRAFLQDGFSLSNNSLLVPTSGIYFVYSQVVFSGK AYSPKATSSPLYLAHEVQLFSSQYPFHVPLLSSQKMVYPGLQEPWLHSMYHGAAFQLTQGD QLSTHTDGIPHLVLSPSTVFFGAFAL).

[0445] Embodiment 56. The fusion protein of any one of embodiments 46-55, comprising a first heavy chain, a second heavy chain, and two light chains paired with the first heavy chain and the second heavy chain, respectively, wherein

(1) the first heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence set forth in SEQ ID NO: 3; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence set forth in SEQ ID NO: 5; the light chain comprising an amino acid sequence having at least 80%,

85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence set forth in SEQ ID NO: 6;

(2) the first heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence set forth in SEQ ID NO: 7; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% , 100% identity to an amino acid sequence set forth in SEQ ID NO: 8; the light chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence set forth in SEQ ID NO: 9;

(3) the first heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence set forth in SEQ ID NO: 3; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence set forth in SEQ ID NO: 10; the light chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence set forth in SEQ ID NO: 11;

(4) the first heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence set forth in SEQ ID NO: 3; the second heavy chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence set forth in SEQ ID NO: 15; the light chain comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity to an amino acid sequence set forth in SEQ ID NO: 16.

[0446] Embodiment 57. The fusion protein of any one of embodiments 46-56, comprising the following:

(1) the first heavy chain having an amino acid sequence set forth in SEQ ID NO: 3, the second heavy chain having an amino acid sequence set forth in SEQ ID NO: 5, and the light chain having an amino acid sequence set forth in SEQ ID NO: 6;

(2) the first heavy chain having an amino acid sequence set forth in SEQ ID NO: 7, the second heavy chain having an amino acid sequence set forth in SEQ ID NO: 8, and the light chain having an amino acid sequence set forth in SEQ ID NO: 9;

(3) the first heavy chain having an amino acid sequence set forth in SEQ ID NO: 3, the second heavy chain having an amino acid sequence set forth in SEQ ID NO: 10, and the light chain having an amino acid sequence set forth in SEQ ID NO: 11;

(4) the first heavy chain having an amino acid sequence set forth in SEQ ID NO: 3, the second heavy chain having an amino acid sequence set forth in SEQ ID NO: 15, and the light chain having an amino acid sequence set forth in SEQ ID NO: 16.

[0447] Embodiment 58. The fusion protein of any one of embodiments 46-57, wherein the fusion protein binds to FAP with dissociation constant (KD) no more than 20 nM, 15 nM, 10 nM or 5 nM.

[0448] Embodiment 59. The fusion protein of any one of embodiments 46-58, wherein the fusion protein binds to LTßR with dissociation constant (KD) no more than 20 nM, 15 nM, 10 nM or 5 nM.

[0449] Embodiment 60. The fusion protein of any one of embodiments 46-59, wherein the fusion protein barely binds to human or cynomolgus HVEM.

[0450] Embodiment 61. The fusion protein of any one of embodiments 46-60, wherein the LIGHT mutant in fusion protein is capable to reduce binding affinity to DcR3.

[0451] Embodiment 62. The fusion protein of any one of embodiment 46-61, wherein the fusion protein specifically binds to human FAP, and/or dose not bind to DPPIV.

[0452] Embodiment 63. An isolated polynucleotide encoding any sequence of the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 39-45 or the fusion protein of any one of embodiments 46-62.

[0453] Embodiment 64. A construct comprising the polynucleotide of embodiment 63.

[0454] Embodiment 65. An antibody expressing system, comprising the construct including an isolated polynucleotide of embodiment 63 or having a genome integrated with an exogenous polynucleotide of embodiment 63, wherein preferably, the expressing system is a cell expression system.

[0455] Embodiment 66. A method for producing the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 39-45 or the fusion protein of any one of embodiments 46-62, comprising: under conditions suitable for expressing the antibody, expressing the antibody or fusion protein using the antibody expressing system of embodiment 65.

[0456] Embodiment 67. A pharmaceutical composition comprising the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 39-45 or the fusion protein of any one of embodiments 46-62, and a pharmaceutically acceptable carrier.

[0457] Embodiment 68. A kit comprising the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 39-45, the fusion protein of any one of embodiments 46-62, the isolated polynucleotide of embodiment 63, or the construct of embodiment 64.

[0458] Embodiment 69. Use of the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 39-45, the fusion protein of any one of embodiments 46-62 or the pharmaceutical

composition of embodiment 67 in the manufacture of a therapeutic agent for preventing, diagnosing, or treating a disease, disorder, or condition.

[0459] Embodiment 70. The use of embodiment 69, wherein the disease, disorder, or condition comprises tumor diseases.

[0460] Embodiment 71. The use of embodiment 69 or 70, wherein at least a tumor cell expresses FAP.

[0461] Embodiment 72. The use of any one of embodiments 69-72, wherein the tumor diseases are solid tumors.

[0462] Embodiment 73. The use of any one of embodiments 69-72, wherein the tumor comprises gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, skin cancer, bone cancer, multiple myeloma, glioma, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer and lymphoma.

[0463] Embodiment 74. A method for treating a subject in need, comprising administrating to the subject a therapeutically effective amount of the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 39-45, the fusion protein of any one of embodiments 46-62 or the pharmaceutical composition of embodiment 67.

[0464] Embodiment 75. A method for decreasing the rate of tumor growth or the number of tumor cells, comprising contacting a tumor cell with an effective amount of the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 39-45, the fusion protein of any one of embodiments 46-62 or the pharmaceutical composition of embodiment 67.

[0465] Embodiment 76. A method of killing a tumor cell, comprising contacting a tumor cell with an effective amount of the anti-FAP antibody or antigen-binding fragment thereof of any one of embodiments 39-45, the fusion protein of any one of embodiments 46-62 or the pharmaceutical composition of embodiment 67.

SEQUENCES

[0466] The sequences in following **TABLE 5** are exemplary sequences of FAP and epitopes thereof as described herein.

TABLE 5: EXEMPLARY AMINO ACID SEQUENCES OF FAPS AND EPITOPES THEREOF

SEQ ID NO:	Description	Amino Acid Sequence
43	Human FAP (Uniprot Q12884)	MKTWVKIVFGVATSAVLALLVMCIVLRPSRVHNSEENTMRALTLKDILNGTFSYKTFFPNWISGQEYLHQSADNNIVLYNIETGQ SYTILSNRTMKSVNASNYGLSPDRQFVYLESDYSKLWRYSYTATYYIYDLSNGEFVRGNELPRPIQYLCWSPVGSKLAYVYQNNI YLKQRPGDPPFQITFNGRENKIFNGIPDWVYEEEMLATKYALWWSPNGKFLAYAEFNDTDIPVIAYSYYGDEQYPRTINIPYPKA GAKNPVVRIFIIDTTYPAYVGPQEVPVPAMIASSDYYFSWLTWVTDERVCLQWLKRVQNVSVLSICDFREDWQTWDCPKTQEHI EESRTGWAGGFFVSTPVFSYDAISYYKIFSDKDGYKHIHYIKDTVENAIQITSGKWEAINIFRVTQDSLFYSSNEFEEYPGRRNIYRI SIGSYPPSKKCVTCHLRKERCQYYTASFSDYAKYYALVCYGPGIPISTLHDGRTDQEIKILEENKELENALKNIQLPKEEIKKLEVD EITLWYKMILPPQFDRSKKYPLLIQVYGGPCSQSVRSVFAVNWISYLASKEGMVIALVDGRGTAFQGDKLLYAVYRKLGVYEVE DQITAVRKFIEMGFIDEKRIAIWGWSYGGYVSSLALASGTGLFKCGIAVAPVSSWEYYASVYTERFMGLPTKDDNLEHYKNSTV MARAEYFRNVDYLLIHGTADDNVHFQNSAQIAKALVNAQVDFQAMWYSDQNHGLSGLSTNHLYTHMTHFLKQCFSLSD
44	Cynomolgus FAP (NCBI XP_005573377.1)	MKTWVKIVFGVATSAVLALLVMCIVLRPPRVHNSEENTMRALTLKDILNGTFSYKTFFPNWISGQEYLHQSADNNIVLYNIETGQ SYTILSNRTMKSVNASNYGLSPDRQFVYLESDYSKLWRYSYTATYYIYDLSNGEFVRGNELPRPIQYLCWSPVGSKLAYVYQNNI YLKQRPGDPPFQITFNGRENKIFNGIPDWVYEEEMLATKYALWWSPNGKFLAYAEFNDTDIPVIAYSYYGDEQYPRTINIPYPKA GAKNPFVRIFIIDTTYPAYVGPQEVPVPAMIASSDYYFSWLTWVTDERVCLQWLKRVQNVSVLSICDFREDWQTWDCPKTQEHI EESRTGWAGGFFVSTPVFSYDAISYYKIFSDKDGYKHIHYIKDTVENAIQITSGKWEAINIFRVTQDSLFYSSNEFEDYPGRRNIYRI SIGSYPPSKKCVTCHLRKERCQYYTASFSDYAKYYALVCYGPGIPISTLHDGRTDQEIKILEENKELENALKNIQLPKEEIKKLEVD EITLWYKMILPPQFDRSKKYPLLIQVYGGPCSQSVRSVFAVNWISYLASKEGMVIALVDGRGTAFQGDKLLYAVYRKLGVYEVE DQITAVRKFIEMGFIDEKRIAIWGWSYGGYVSSLALASGTGLFKCGIAVAPVSSWEYYASVYTERFMGLPTKDDNLEHYKNSTV MARAEYFRNVDYLLIHGTADDNVHFQNSAQIAKALVNAQVDFQAMWYSDQNHGLSGLSTNHLYTHMTHFLKQCFSLSD
45	Mouse FAP (Uniprot P97321)	MKTWLKTVFGVTTLAALALVVICIVLRPSRVYKPEGNTKRALTLKDILNGTFSYKTYFPNWISEQEYLHQSEDDNIVFYNIETRES YIILSNSTMKSVNATDYGLSPDRQFVYLESDYSKLWRYSYTATYYIYDLQNGEFVRGYELPRPIQYLCWSPVGSKLAYVYQNNIY LKQRPGDPPFQITYTGRENRIFNGIPDWVYEEEMLATKYALWWSPDGKFLAYVEFNDSDIPIIAYSYYGDGQYPRTINIPYPKAGA KNPVVRVFIVDTTYPHHVGPMEVPVPEMIASSDYYFSWLTWVSSERVCLQWLKRVQNVSVLSICDFREDWHAWECPKNQEHVE ESRTGWAGGFFVSTPAFSQDATSYYKIFSDKDGYKHIHYIKDTVENAIQITSGKWEAIYIFRVTQDSLFYSSNEFEGYPGRRNIYRI SIGNSPPSKKCVTCHLRKERCQYYTASFSYKAKYYALVCYGPGLPISTLHDGRTDQEIQVLEENKELENSLRNIQLPKVEIKKLKD GGLTFWYKMILPPQFDRSKKYPLLIQVYGGPCSQSVKSVFAVNWITYLASKEGIVIALVDGRGTAFQGDKFLHAVYRKLGVYEV EDQLTAVRKFIEMGFIDEERIAIWGWSYGGYVSSLALASGTGLFKCGIAVAPVSSWEYYASIYSERFMGLPTKDDNLEHYKNSTV MARAEYFRNVDYLLIHGTADDNVHFQNSAQIAKALVNAQVDFQAMWYSDQNHGISSGRSQNHLYTHMTHFLKQCFSLSD

[0467] The sequences in following **TABLE 5.1** are exemplary sequences of tumor associated cell receptors (TACRs) and epitopes thereof as described herein.

TABLE 5.1: EXEMPLARY AMINO ACID SEQUENCES OF TACRS AND EPITOPES THEREOF

SEQ ID NO:	Description	Amino Acid Sequence
49	Human LTβR (Uniprot P36941)	MLLPWATSAPGLAWGPLVLGLFGLLAASQPQAVPPYASENQTCRDQEKEYYEPQHRICCSRCPPGTYVSAKCSRIRDTVCATCAENSYNEHWNYLTICQLCRPCDPVMGLEEIAPCTSKRKTQCRCQPGMFCAAWALECTHCELLSDCPPGTEAELKDEVGKGNNHCVPCKAGHFQNTSSPSARCQPHTRCENQGLVEAAPGTAQSDTTCKNPLEPLPPEMSGTMLMLAVLLPLAFFLLLATVFSCIWKSHPSLCRKLGSLLKRRPQGEGPNPVAGSWEPPKAHPYFPDLVQPLLPISGDVSPVSTGLPAAPVLEAGVPQQQSPLDLTREPQLEPGEQSQVAHGTNGIHVTGGSMTITGNIYIYNGPVLGGPPGPGDLPATPEPPYPIPEEGDPGPPGLSTPHQEDGKAWHLAETEHCGATPSNRGPRNQFITHD
50	Cynomolgus LTβR (Uniprot A0A2K5VGQ6)	MRLPWATSAPGLAWGPLVLGLFGLLAASQPQVVRKGPVPPYGSENQTCRDQEKEYYEPRHRICCSRCPPGTYVSAKCSRSRDTVCATCAENSYNEHWNYLTICQLCRPCDPVMGLEEIAPCTSKRKTQCRCQPGMFCAAWALECTHCELLSDCPPGTEAELKDEVGKGNNHCVPCKAGHFQNTSSPSARCQPHTRCEDQGLVEAAPGTAQSDTTCRNPSESLPPEMSGTMLMLAILLPLAFFLLLATIFACIWKSHPSLCRKLGSLLKRHPQGEGPNPVAAGRDPPKANPQYPDLVEPLLPISGDVSPVSTGLPTALVSEEGVPQQQSPLDLTTEPQLEPGEQNQVAHGTNGIHVTGGSMTITGNIYIYNGPVLGGPPGPGDLPATPDPPYPIPEEGDPGPPGLSTPHQEDGKAWHLAETEHCGATPSNRGPRSQFITYD
51	Mouse LTβR (Uniprot P50284)	MRLPRASSPCGLAWGPLLLGLSGLLVASQPQLVPPYRIENQTCWDQDKEYYEPMHDVCCSRCPPGEFVFAVCSRSQDTVCKTCPHNSYNEHWNHLSTCQLCRPCDIVLGFEEVAPCTSDRKAECRCQPGMSCVYLDNECVHCEEERLVLCQPGTEAEVTDEIMDTDVNCVPCKPGHFQNTSSPRARCQPHTRCEIQGLVEAAPGTSYSDTICKNPPEPGAMLLLAILLSLVLFLLFTTVLACAWMRHPSLCRKLGTLLKRHPEGEESPPCPAPRADPHFPDLAEPLLPMSGDLSPSPAGPPTAPSLEEVVLQQQSPLVQARELEAEPGEHGQVAHGANGIHVTGGSVTVTGNIYIYNGPVLGGTRGPGDPPAPPEPPYPTPEEGAPGPSELSTPYQEDGKAWHLAETETLGCQDL

[0468] The sequences in the following **TABLE 6** illustrate consensus amino acid sequences that can be used to generate the polypeptide sequences (*e.g.*, anti-FAP antibodies, bispecific antibodies, or fusion proteins thereof) and/or systems and compositions and perform the methods described herein. In **TABLE 6**, the brackets indicate amino acids in the alternative, for example [Y/T] means Y or T. In another example, [Y/N/K] means Y, N or K. Also in **TABLE 6**, the subscript numbers indicate the instances of the amino acid indicated in the bracket, for example $[R]_{0\text{-}1}$ means no R or a single R. As needed, a nucleotide sequence can be readily deduced from the amino acid sequence.

TABLE 6: EXEMPLARY CONSENSUS AMINO ACID SEQUENCES

SEQ ID NO:	Description	Sequence	AB Consensus
26	HCDR1	IYGVN	9E3 CHIMERA (ABC1139), ABC1931, ABC1930, ABC1947, ABC2066, ABC2067, and ABC2097
27	HCDR2	AIWSGGRKDYX_2LSLKS	9E3 CHIMERA (ABC1139), ABC1931, ABC1930, ABC1947, ABC2066, ABC2067, and ABC2097
28	HCDR3	SQDMPGYFDY	9E3 CHIMERA (ABC1139), ABC1931, ABC1930, ABC1947, ABC2066, ABC2067, and ABC2097
23	LCDR1	KTNQNVDYX_1GNTFMH	9E3 CHIMERA (ABC1139), ABC1931, ABC1930, ABC1947, ABC2066, ABC2067, and ABC2097
24	LCDR2	LASNLAS	9E3 CHIMERA (ABC1139), ABC1931, ABC1930, ABC1947, ABC2066, ABC2067, and ABC2097
25	LCDR3	QQSRNLPYT	9E3 CHIMERA (ABC1139), ABC1931, ABC1930,

			ABC1947, ABC2066, ABC2067, and ABC2097
X_1 is N or S; X_2 is N or S			

[0469] The sequences in the following **TABLE 7** illustrate exemplary CDR amino acid sequences that can be used to generate the FAP binding agents of the present disclosure (*e.g.*, anti-FAP antibodies, bispecific antibodies, or fusion proteins thereof) and/or systems and compositions and perform the methods described herein. As needed, a nucleotide sequence can be readily deduced from the amino acid sequence.

TABLE 7: EXEMPLARY CDR AMINO ACID SEQUENCES

AB	Description	HC			LC		
		CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
9E3 CHIMERA (ABC 1139)	Chothia	GFSLSIY (SEQ ID NO: 52)	SGG (SEQ ID NO: 58)	QDMPGYFD (SEQ ID NO: 67)	NQNVDYNGNTF (SEQ ID NO: 73)	LAS (SEQ ID NO: 83)	SRNLPY (SEQ ID NO: 88)
	Honegger	VSGFSLSIYG (SEQ ID NO: 53)	IWSGGRKDYNLSLKSR (SEQ ID NO: 59)	SQDMPGYFD (SEQ ID NO: 68)	TNQNVDYNGNTF (SEQ ID NO: 74)	LASNLASGIPDR (SEQ ID NO: 84)	SRNLPY (SEQ ID NO: 88)
	IMGT	GFSLSIYG (SEQ ID NO: 54)	IWSGGRK (SEQ ID NO: 60)	ARSQDMPGYFDY (SEQ ID NO: 69)	QNVDYNGNTF (SEQ ID NO: 75)	LAS (SEQ ID NO: 83)	QQSRNLPYT (SEQ ID NO: 25)
	Kabat	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYNLSLKS (SEQ ID NO: 61)	SQDMPGYFDY (SEQ ID NO: 28)	KTNQNVDYNGNTFMH (SEQ ID NO: 76)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)
ABC1931	Chothia	GFSLSIY (SEQ ID NO: 52)	SGG (SEQ ID NO: 58)	QDMPGYFD (SEQ ID NO: 67)	TNQNVDYSGNTF (SEQ ID NO: 77)	LAS (SEQ ID NO: 83)	SRNLPY (SEQ ID NO: 88)
	Honegger	VSGFSLSIYG (SEQ ID NO: 53)	IWSGGRKDYSLSLKSR (SEQ ID NO: 62)	SQDMPGYFD (SEQ ID NO: 68)	TNQNVDYSGNTF (SEQ ID NO: 77)	LASNLASGIPER (SEQ ID NO: 85)	SRNLPY (SEQ ID NO: 88)
	IMGT	GFSLSIYG (SEQ ID NO: 54)	IWSGGRK (SEQ ID NO: 60)	ARSQDMPGYFDY (SEQ ID NO: 69)	NQNVDYSGNTF (SEQ ID NO: 78)	LAS (SEQ ID NO: 83)	QQSRNLPYT (SEQ ID NO: 25)
	Kabat	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)	KTNQNVDYSGNTFMH (SEQ ID NO: 79)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)
ABC1930	Chothia	GFSLSIY (SEQ ID NO: 52)	SGG (SEQ ID NO: 58)	QDMPGYFD (SEQ ID NO: 67)	TNQNVDYSGNTF (SEQ ID NO: 77)	LAS (SEQ ID NO: 83)	SRNLPY (SEQ ID NO: 88)
	Honegger	VSGFSLSIYG (SEQ ID NO: 53)	IWSGGRKDYSLSLKSR (SEQ ID NO: 62)	SQDMPGYFD (SEQ ID NO: 68)	TNQNVDYSGNTF (SEQ ID NO: 77)	LASNLASGIPER (SEQ ID NO: 85)	SRNLPY (SEQ ID NO: 88)

	IMGT	GFSLSIYG (SEQ ID NO: 54)	IWSGGRK (SEQ ID NO: 60)	ARSQDMPGYFDY (SEQ ID NO: 69)	NQNVDYSGNTF (SEQ ID NO: 78)	LAS (SEQ ID NO: 83)	QQSRNLPYT (SEQ ID NO: 25)
	Kabat	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)	KTNQNVDYSGNTFMH (SEQ ID NO: 79)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)
ABC1773	**Chothia**	GFSLSTAGM (SEQ ID NO: 55)	WDD (SEQ ID NO: 64)	MIFNFYFD (SEQ ID NO: 70)	SSRVGY (SEQ ID NO: 80)	DTS (SEQ ID NO: 86)	GSGYPF (SEQ ID NO: 89)
	Honegger	FSGFSLSTAGMS (SEQ ID NO: 56)	IWWDDKKHYNPSLKDR (SEQ ID NO: 65)	DMIFNFYFD (SEQ ID NO: 71)	ASSRVGY (SEQ ID NO: 81)	DTSKLASGVPSR (SEQ ID NO: 87)	GSGYPF (SEQ ID NO: 89)
	IMGT	GFSLSTAGMS (SEQ ID NO: 57)	IWWDDKK (SEQ ID NO: 66)	ARDMIFNFYFDV (SEQ ID NO: 72)	SRVGY (SEQ ID NO: 82)	DTS (SEQ ID NO: 86)	FQGSGYPFT (SEQ ID NO: 31)
	Kabat	TAGMSVG (SEQ ID NO: 32)	DIWWDDKKHYNPSLKD (SEQ ID NO: 33)	DMIFNFYFDV (SEQ ID NO: 34)	SASSRVGYMH (SEQ ID NO: 29)	DTSKLAS (SEQ ID NO: 30)	FQGSGYPFT (SEQ ID NO: 31)
ABC1947	**Chothia**	GFSLSIY (SEQ ID NO: 52)	SGG (SEQ ID NO: 58)	QDMPGYFD (SEQ ID NO: 67)	TNQNVDYSGNTF (SEQ ID NO: 77)	LAS (SEQ ID NO: 83)	SRNLPY (SEQ ID NO: 88)
	Honegger	VSGFSLSIYG (SEQ ID NO: 53)	IWSGGRKDYSLSLKSR (SEQ ID NO: 62)	SQDMPGYFD (SEQ ID NO: 68)	TNQNVDYSGNTF (SEQ ID NO: 77)	LASNLASGIPER (SEQ ID NO: 85)	SRNLPY (SEQ ID NO: 88)
	IMGT	GFSLSIYG (SEQ ID NO: 54)	IWSGGRK (SEQ ID NO: 60)	ARSQDMPGYFDY (SEQ ID NO: 69)	NQNVDYSGNTF (SEQ ID NO: 78)	LAS (SEQ ID NO: 83)	QQSRNLPYT (SEQ ID NO: 25)
	Kabat	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)	KTNQNVDYSGNTFMH (SEQ ID NO: 79)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)
ABC2066	**Chothia**	GFSLSIY (SEQ ID NO: 52)	SGG (SEQ ID NO: 58)	QDMPGYFD (SEQ ID NO: 67)	TNQNVDYSGNTF (SEQ ID NO: 77)	LAS (SEQ ID NO: 83)	SRNLPY (SEQ ID NO: 88)
	Honegger	VSGFSLSIYG (SEQ ID NO: 53)	IWSGGRKDYSLSLKSR (SEQ ID NO: 62)	SQDMPGYFD (SEQ ID NO: 68)	TNQNVDYSGNTF (SEQ ID NO: 77)	LASNLASGIPER (SEQ ID NO: 85)	SRNLPY (SEQ ID NO: 88)
	IMGT	GFSLSIYG (SEQ ID NO: 54)	IWSGGRK (SEQ ID NO: 60)	ARSQDMPGYFDY (SEQ ID NO: 69)	NQNVDYSGNTF (SEQ ID NO: 78)	LAS (SEQ ID NO: 83)	QQSRNLPYT (SEQ ID NO: 25)
	Kabat	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)	KTNQNVDYSGNTFMH (SEQ ID NO: 79)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)
ABC2067	**Chothia**	GFSLSIY (SEQ ID NO: 52)	SGG (SEQ ID NO: 58)	QDMPGYFD (SEQ ID NO: 67)	TNQNVDYSGNTF (SEQ ID NO: 77)	LAS (SEQ ID NO: 83)	SRNLPY (SEQ ID NO: 88)
	Honegger	VSGFSLSIYG (SEQ ID NO: 53)	IWSGGRKDYSLSLKSR (SEQ ID NO: 62)	SQDMPGYFD (SEQ ID NO: 68)	TNQNVDYSGNTF (SEQ ID NO: 77)	LASNLASGIPER (SEQ ID NO: 85)	SRNLPY (SEQ ID NO: 88)
	IMGT	GFSLSIYG (SEQ ID NO: 54)	IWSGGRK (SEQ ID NO: 60)	ARSQDMPGYFDY (SEQ ID NO: 69)	NQNVDYSGNTF (SEQ ID NO: 78)	LAS (SEQ ID NO: 83)	QQSRNLPYT (SEQ ID NO: 25)
	Kabat	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)	KTNQNVDYSGNTFMH (SEQ ID NO: 79)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)
ABC2097	**Chothia**	GFSLSIY (SEQ ID NO: 52)	SGG (SEQ ID NO: 58)	QDMPGYFD (SEQ ID NO: 67)	TNQNVDYSGNTF (SEQ ID NO: 77)	LAS (SEQ ID NO: 83)	SRNLPY (SEQ ID NO: 88)
	Honegger	VSGFSLSIYG (SEQ ID NO: 53)	IWSGGRKDYSLSLKSR (SEQ ID NO: 62)	SQDMPGYFD (SEQ ID NO: 68)	TNQNVDYSGNTF (SEQ ID NO: 77)	LASNLASGIPER (SEQ ID NO: 85)	SRNLPY (SEQ ID NO: 88)

	IMGT	GFSLSIYG (SEQ ID NO: 54)	IWSGGRK (SEQ ID NO: 60)	ARSQDMPGYFDY (SEQ ID NO: 69)	NQNVDYSGNTF (SEQ ID NO: 78)	LAS (SEQ ID NO: 83)	QQSRNLPYT (SEQ ID NO: 25)
	Kabat	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)	KTNQNVDYSGNTFMH (SEQ ID NO: 79)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)

[0470] The sequences in the following **TABLE 8** illustrate exemplary VH and VL amino acid sequences that can be used to generate the FAP binding agents of the present disclosure (*e.g.*, anti-FAP antibodies, bispecific antibodies, or fusion proteins thereof) and/or systems and compositions and perform the methods described herein. As needed, a nucleotide sequence can be readily deduced from the amino acid sequence.

TABLE 8: EXEMPLARY VH AND VL AMINO ACID SEQUENCES

AB	VH	AB	VL
9E3 CHIMERA (ABC1139)	QVQLKESGPGMVQPSRTLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDYNLSLKSRLIISGDTSKSQVLLTMNSLQSEDTAMYFCARSQDMPGYFDYWGQGVMVTVSS (SEQ ID NO: 35)	**9E3 CHIMERA (ABC1139)**	DIVLTQSPALAVSLGQRATISCKTNQNVDYNGNTFMHWYQQKPGQQPKLLLYLASNLASGIPDRFSGRGSGTDFTLTIDPVEADDTATYYCQQSRNLPYTFGAGTKLEIK (SEQ ID NO: 36)
ABC1930, ABC1931, ABC1947, ABC2066, ABC2067, ABC2097	EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDYSLSLKSRLTISGDTSKNQVSLKLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS (SEQ ID NO: 37)	**ABC1930, ABC1931, ABC1947, ABC2066, ABC2067, ABC2097**	SIVLTQPPSVSVAPGQTARITCKTNQNVDYSGNTFMHWYQQKPGQQPVLLLYLASNLASGIPERFSGSGSGTTFTLTISRVEAGDEADYYCQQSRNLPYTFGTGTKVTVL (SEQ ID NO: 38)
ABC1773	QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLADIWWDDKKHYNPSLKDRLTISKDTSKNQVVLKVTNMDPADTATYYCARDMIFNFYFDVWGQGTTVTVSS (SEQ ID NO: 41)	**ABC1773**	DIQMTQSPSTLSASVGDRVTITCSASSRVGYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCFQGSGYPFTFGGGTKVEIK (SEQ ID NO: 42)

[0471] The sequences in the following **TABLE 9** illustrate exemplary HC and LC amino acid sequences that can be used to generate the FAP binding agents of the present disclosure (*e.g.*, anti-FAP antibodies, bispecific antibodies, or fusion proteins thereof) and/or systems and compositions and perform the methods described herein. As needed, a nucleotide sequence can be readily deduced from the amino acid sequence.

TABLE 9: EXEMPLARY HC AND LC AMINO ACID SEQUENCES

AB	HC	LC
9E3 CHIMERA (ABC 1139)	QVQLKESGPGMVQPSRTLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDYNLSLKSRLIISGDTSKSQVLLTMNSLQSEDTAMYFCARSQDMPGYFDYWGQGVMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1)	DIVLTQSPALAVSLGQRATISCKTNQNVDYNGNTFMHWYQQKPGQQPKLLLYLASNLASGIPDRFSGRGSGTDFTLTIDPVEADDTATYYCQQSRNLPYTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 2)
ABC 1931	EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDYSLSLKSRLTISGDTSKNQVSLKLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 4)	SIVLTQPPSVSVAPGQTARITCKTNQNVDYSGNTFMHWYQQKPGQQPVLLLYLASNLASGIPERFSGSGSGTTFTLTISRVEAGDEADYYCQQSRNLPYTFGTGTKVTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO: 3)

AB	HC1	HC2	LC
ABC 1930	EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQP PGKGLEWIAAIWSGGRKDYSLSLKSRLTISGDTSKNQVSL KLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQE EMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLVSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSLGKGGGGSGGGGSEVNPAAHLTGANSS LTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKAGYYY IYSKVQLGGMGCPLGLASTITHGLYKRTPRYPEELELLVS QQSPCGRATSSSRVWQDSSFLGGVVHLEAGEKVVVRVL DERLVRLLDGTRSYFGAFMV (SEQ ID NO: 5)	EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQP PGKGLEWIAAIWSGGRKDYSLSLKSRLTISGDTSKNQVSL KLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQE EMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSLGKGGGGSGGGGSEVNPAAHLTGANSS LTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKAGYYY IYSKVQLGGMGCPLGLASTITHGLYKRTPRYPEELELLVS QQSPCGRATSSSRVWQDSSFLGGVVHLEAGEKVVVRVL DERLVRLLDGTRSYFGAFMVGGGGSGGGGSEVNPAAHL TGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTK AGYYYIYSKVQLGGMGCPLGLASTITHGLYKRTPRYPEE LELLVSQQSPCGRATSSSRVWQDSSFLGGVVHLEAGEKV VVRVLDERLVRLLDGTRSYFGAFMV (SEQ ID NO: 6)	SIVLTQPPSVSVAPGQT ARITCKTNQNVDYSGN TFMHWYQQKPGQQPV LLLYLASNLASGIPERFS GSGSGTTFTLTISRVEA GDEADYYCQQSRNLPY TFGTGTKVTVLGQPKA APSVTLFPPSSEELQAN KATLVCLISDFYPGAVT VAWKADSSPVKAGVET TTPSKQSNNKYAASSY LSLTPEQWKSHRSYSC QVTHEGSTVEKTVAPT ECS (SEQ ID NO: 3)
ABC 1773	QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIR QPPGKALEWLADIWWDDKKHYNPSLKDRLTISKDTSKN QVVLKVTNMDPADTATYYCARDMIFNFYFDVWGQGTT VTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTL PPSQEEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLVSRLTVDKSRWQEGNVFSCSVMH EALHNHYTQKSLSLSLGKGGGGSGGGGSEVNPAAHLTG ANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKA GYYYIYSKVQLGGMGCPLGLASTITHGLYKRTPRYPEEL ELLVSQQSPCGRATSSSRVWQDSSFLGGVVHLEAGEKVV VRVLDERLVRLLDGTRSYFGAFMV* (SEQ ID NO: 8)	QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIR QPPGKALEWLADIWWDDKKHYNPSLKDRLTISKDTSKN QVVLKVTNMDPADTATYYCARDMIFNFYFDVWGQGTT VTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL PPCQEEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM HEALHNHYTQKSLSLSLGKGGGGSGGGGSEVNPAAHLT GANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTK AGYYYIYSKVQLGGMGCPLGLASTITHGLYKRTPRYPEE LELLVSQQSPCGRATSSSRVWQDSSFLGGVVHLEAGEKV VVRVLDERLVRLLDGTRSYFGAFMVGGGGSGGGGSEVN PAAHLTGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGA LVVTKAGYYYIYSKVQLGGMGCPLGLASTITHGLYKRTP RYPEELELLVSQQSPCGRATSSSRVWQDSSFLGGVVHLE	DIQMTQSPSTLSASVGD RVTITCSASSRVGYMH WYQQKPGKAPKLLIYD TSKLASGVPSRFSGSGS GTEFTLTISSLQPDDFAT YYCFQGSGYPFTFGGG TKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLL NNFYPREAKVQWKVD NALQSGNSQESVTEQD SKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQ GLSSPVTKSFNRGEC* (SEQ ID NO: 7)

		AGEKVVVRVLDERLVRLLDGTRSYFGAFMV* (SEQ ID NO: 9)	
ABC 1947	EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQP PGKGLEWIAAIWSGGRKDYSLSLKSRLTISGDTSKNQVSL KLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQE EMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLVSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSLGKGGGGSGGGGSEVNPAAHLTGANSS LTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKAGYYY IYSKVQLGGVGCPLGLASTITHGLYKRTPRYPEELELLVS QQSPCGRATSSSRVWWDSSFLGGVVHLEAGEKVVVRVL DERLVRLRDGTRSYFGAFMV (SEQ ID NO: 10)	EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQP PGKGLEWIAAIWSGGRKDYSLSLKSRLTISGDTSKNQVSL KLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQE EMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSLGKGGGGSGGGGSEVNPAAHLTGANSS LTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKAGYYY IYSKVQLGGVGCPLGLASTITHGLYKRTPRYPEELELLVS QQSPCGRATSSSRVWWDSSFLGGVVHLEAGEKVVVRVL DERLVRLRDGTRSYFGAFMVGGGGSGGGGSEVNPAAHL TGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTK AGYYYIYSKVQLGGVGCPLGLASTITHGLYKRTPRYPEE LELLVSQQSPCGRATSSSRVWWDSSFLGGVVHLEAGEK VVVRVLDERLVRLRDGTRSYFGAFMV (SEQ ID NO: 11)	SIVLTQPPSVSVAPGQT ARITCKTNQNVDYSGN TFMHWYQQKPGQQPV LLLYLASNLASGIPERFS GSGSGTTFTLTISRVEA GDEADYYCQQSRNLPY TFGTGTKVTVLGQPKA APSVTLFPPSSEELQAN KATLVCLISDFYPGAVT VAWKADSSPVKAGVET TTPSKQSNNKYAASSY LSLTPEQWKSHRSYSC QVTHEGSTVEKTVAPT ECS (SEQ ID NO: 3)
ABC 2066	EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQP PGKGLEWIAAIWSGGRKDYSLSLKSRLTISGDTSKNQVSL KLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQE EMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSLGK* (SEQ ID NO: 13)	RRSHEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRG LSYHDGALVVTKTGYYYIYSKVQLGGVGCPLGLAGTITH GLYKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSSFL GGVVHLEAGEKVVVRVLGKRLVRLRDGTRSYFGAFMV GRRSHEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLR GLSYHDGALVVTKTGYYYIYSKVQLGGVGCPLGLAGTIT HGLYKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSSF LGGVVHLEAGEKVVVRVLGKRLVRLRDGTRSYFGAFM VGRRSHEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFL RGLSYHDGALVVTKTGYYYIYSKVQLGGVGCPLGLAGTI THGLYKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSS FLGGVVHLEAGEKVVVRVLGKRLVRLRDGTRSYFGAFM VGGGGSGGGGSGGGGSGGGGTGPPCPPCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEE MTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLVSRLTVDKSRWQEGNVFSCSVMHEALHN HYTQKSLSLSLGK* (SEQ ID NO: 12)	SIVLTQPPSVSVAPGQT ARITCKTNQNVDYSGN TFMHWYQQKPGQQPV LLLYLASNLASGIPERFS GSGSGTTFTLTISRVEA GDEADYYCQQSRNLPY TFGTGTKVTVLGQPKA APSVTLFPPSSEELQAN KATLVCLISDFYPGAVT VAWKADSSPVKAGVET TTPSKQSNNKYAASSY LSLTPEQWKSHRSYSC QVTHEGSTVEKTVAPT ECS (SEQ ID NO: 3)

ABC 2067	RRSHEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRG LSYHDGALVVTKTGYYYIYSKVQLGGVGCPLGLAGTITH GLYKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSSFL GGVVHLEAGEKVVVRVLGKRLVRLRDGTRSYFGAFMV GRRSHEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLR GLSYHDGALVVTKTGYYYIYSKVQLGGVGCPLGLAGTIT HGLYKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSSF LGGVVHLEAGEKVVVRVLGKRLVRLRDGTRSYFGAFM VGRRSHEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFL RGLSYHDGALVVTKTGYYYIYSKVQLGGVGCPLGLAGTI THGLYKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSS FLGGVVHLEAGEKVVVRVLGKRLVRLRDGTRSYFGAFM VGGGGSGGGGSGGGGSGGGGTCPPCPAPEFLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMT KNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLVSRLTVDKSRWQEGNVFSCSVMHEALHNHY TQKSLSLSLGK* (SEQ ID NO: 14)	EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQP PGKGLEWIAAIWSGGRKDYSLSLKSRLTISGDTSKNQVSL KLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQE EMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSLGK* (SEQ ID NO: 13)	SIVLTQPPSVSVAPGQT ARITCKTNQNVDYSGN TFMHWYQQKPGQQPV LLLYLASNLASGIPERFS GSGSGTTFTLTISRVEA GDEADYYCQQSRNLPY TFGTGTKVTVLGQPKA APSVTLFPPSSEELQAN KATLVCLISDFYPGAVT VAWKADSSPVKAGVET TTPSKQSNNKYAASSY LSLTPEQWKSHRSYSC QVTHEGSTVEKTVAPT ECS (SEQ ID NO: 3)
ABC 2097	EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQP PGKGLEWIAAIWSGGRKDYSLSLKSRLTISGDTSKNQVSL KLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQE EMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSLGKGGGGSGGGGSLSPGLPAAHLIGAPL KGQGLGWETTKEQAFLTSGTQFSDAEGLALPQDGLYYL YCLVGYRGRAPPGGGDPQGRSVTLRSSLYRAGGAYGPG TPELLLEGAETVTPVLDPARRQGYGPLWYTSVGFGGLVQ LRRGERVYVNISHPDMVDFARGKTFFGAVMVG* (SEQ ID NO: 15)	EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQP PGKGLEWIAAIWSGGRKDYSLSLKSRLTISGDTSKNQVSL KLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQE EMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLVSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSLGKGGGGSGGGGSAHSTLKPAAHLIGDP SKQNSLLWRANTDRAFLQDGFSLSNNSLLVPTSGIYFVYS QVVFSGKAYSPKATSSPLYLAHEVQLFSSQYPFHVPLLSS QKMVYPGLQEPWLHSMYHGAAFQLTQGDQLSTHTDGIP HLVLSPSTVFFGAFALLSPGLPAAHLIGAPLKGQGLGWET TKEQAFLTSGTQFSDAEGLALPQDGLYYLYCLVGYRGR APPGGGDPQGRSVTLRSSLYRAGGAYGPGTPELLLEGAE TVTPVLDPARRQGYGPLWYTSVGFGGLVQLRRGERVYV NISHPDMVDFARGKTFFGAVMVG* (SEQ ID NO: 16)	SIVLTQPPSVSVAPGQT ARITCKTNQNVDYSGN TFMHWYQQKPGQQPV LLLYLASNLASGIPERFS GSGSGTTFTLTISRVEA GDEADYYCQQSRNLPY TFGTGTKVTVLGQPKA APSVTLFPPSSEELQAN KATLVCLISDFYPGAVT VAWKADSSPVKAGVET TTPSKQSNNKYAASSY LSLTPEQWKSHRSYSC QVTHEGSTVEKTVAPT ECS (SEQ ID NO: 3)

[0472] The sequences in the following **TABLE 10** illustrate exemplary tumor associated cell receptor ligand amino acid sequences that can be used to generate the FAP binding agents of the present disclosure (*e.g.*, anti-FAP antibodies, bispecific antibodies, or fusion proteins thereof) and/or systems and compositions and perform the methods described herein. As needed, a nucleotide sequence can be readily deduced from the amino acid sequence.

TABLE 10: EXEMPLARY AMINO ACID SEQUENCES

SEQ ID NO:	Description	Sequence
17	LIGHT1 mutant	EVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKAGYYYIYSKVQLGGMGCPLGLASTITHG LYKRTPRYPEELELLVSQQSPCGRATSSSRVWQDSSFLGGVVHLEAGEKVVVRVLDERLVRLLDGTRSYFGAFMV
18	wild type LIGHT	EVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKAGYYYIYSKVQLGGVGCPLGLASTITHGL YKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSSFLGGVVHLEAGEKVVVRVLDERLVRLRDGTRSYFGAFMV
39	Lymphotoxin B mutant	LSPGLPAAHLIGAPLKGQGLGWETTKEQAFLTSGTQFSDAEGLALPQDGLYYLYCLVGYRGRAPPGGGDPQGRSVT LRSSLYRAGGAYGPGTPELLLEGAETVTPVLDPARRQGYGPLWYTSVGFGGLVQLRRGERVYVNISHPDMVDFAR GKTFFGAVMVG
40	Lymphotoxin A mutant	AHSTLKPAAHLIGDPSKQNSLLWRANTDRAFLQDGFSLSNNSLLVPTSGIYFVYSQVVFSGKAYSPKATSSPLYLAH EVQLFSSQYPFHVPLLSSQKMVYPGLQEPWLHSMYHGAAFQLTQGDQLSTHTDGIPHLVLSPSTVFFGAFAL

EXAMPLES

[0473] The invention is further described in the following examples, which do not limit the scope of the invention described in the claims. The reagents used in examples are commercially available.

[0474] Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, pathology, oncology, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, *e.g.*, Green and Sambrook et al., Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012); Therapeutic Monoclonal Antibodies: From Bench to Clinic, Zhiqiang An (Editor), Wiley, (2009); and Antibody Engineering, 2nd Ed., Vols 1 and 2, Ontermann and Dubel, eds., Springer-Verlag, Heidelberg (2010).

Example 1: Generation of anti-FAP antibodies

1.1. Hybridoma generation and screening

[0475] To generate antibodies against FAP, Sprague-Dawley rats were immunized with FAP-overexpressing cells. To monitor immune responses, titrated serum was screened for binding to multiple FAP-overexpressing cell lines by FACS, typically after 4-6 weeks of immunization. Animals with sufficient titers of anti-FAP IgG were selected for the final immunization.

[0476] For hybridoma generation, cells from lymphoid organs, such as spleens and lymph nodes were collected, isolated, and fused with SP2/0 myeloma cells. The resulting cells were plated in flat-bottom 96 well plates in Medium E (StemCell Technologies) supplemented with HAT (Sigma H0262-10VL) to select for hybridomas. After one week of culture, hybridoma supernatants were collected and

screened against FAP-overexpressing cells by flow cytometry. The selected hybridomas were subsequently subcloned and further screened to identify anti-FAP monoclonal hybridomas.

1.2. Generation of expression vectors

[0477] To obtain the sequences of the heavy chain and light chain variable domains, mRNA from the hybridomas was purified using Dynabeads mRNA Direct Micro Purification kit (ThermoFisher #61021) and the cDNAs were synthesized and amplified using template-switching oligos (Pinto et al., Anal. Biochem. 397, 2010). The variable domain fragments of the heavy and light chains were subsequently amplified, purified, and sequenced.

[0478] To generate the expression vectors, the variable regions of heavy and light chain DNA sequences were synthesized and subcloned in-frame with either the human IgG1 constant heavy chain or the human IgG1 kappa constant light chain pre-inserted into the pCI- vector (Promega #E1841). The CDRs and the variable regions of 9E3 chimera (ABC1139) are shown below.

[0479] Heavy Chain (AP1333):

QVQLKESGPGMVQPSRTLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKD
YNLSLKSRLIISGDTSKSQVLLTMNSLQSEDTAMYFCARSQDMPGYFDYWGQGVMVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1)

Light Chain (AP1334):

DIVLTQSPALAVSLGQRATISCKTNQNVDYNGNTFMHWYQQKPGQQPKLLLYLASNL
ASGIPDRFSGRGSGTDFTLTIDPVEADDTATYYCQQSRNLPYTFGAGTKLEIKRTVAAPS

VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 2)

CDRs are underlined and defined using the Kabat system. The variable regions are in bold.

1.3. Humanization of antibodies

[0480] To humanize antibodies, the CDR residues of the light and heavy chains were grafted onto the human frameworks with high homology. To restore affinity, some mutations were designed by changing human framework residues to their murine counterparts at specific positions. Post-translational modifications (PTM) were removed.

[0481] The CDRs, and variable regions of humanized antibody ABC1931 are shown below.

Light Chain (AP2463):

SIVLTQPPSVSVAPGQTARITCKTNQNVDYSGNTFMHWYQQKPGQQPVLLLYLASNLA SGIPERFSGSGSGTTFTLTISRVEAGDEADYYCQQSRNLPYTFGTGTKVTVLGQPKAAPS VTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASS YLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO: 3)

Heavy Chain (AP1992):

EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKD YSLSLKSRLTISGDTSKNQVSLKLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS VMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 4)

CDRs are underlined and defined using the Kabat system. The variable regions are in bold.

1.4 Production of antibodies

[0482] Antibodies were produced by co-transfecting plasmids into Expi293F cells (ThermoFisher A14527). Specifically, 30 μg of total DNA was diluted into Opti-MEM medium (Life Technologies), and then incubated with Expifectamine 293 transfection reagent (Gibco #A14525) in the same medium

for 20 min. The mixture was then added to 30 ml of Expi293F cells growing in suspension at a density of 2.5 million cells/ml at 37°C and 8% CO_2.

[0483] The cells were grown in suspension for about six days after transfection and then harvested by centrifugation at 7,000 rpm, 4℃ for 20 minutes. The supernatant was filtered through a 0.22 μm filter, and affinity purification was performed using protein A resins. The protein was eluted with elution buffer (1 M Glycine pH 3.0, 10% glycerol) and neutralized to pH 6.0 with 1M Tris pH 7.5. Size exclusion chromatography (SEC) was performed on AKTA to polish the product, and the antibody concentration was measured with NanoDrop at 280 nm. The antibodies were analyzed by electrophoresis on 4-20% Tris-glycine SDS-PAGE gels (Bio-Rad) under reducing (R) and non-reducing (NR) conditions.

1.5. Generating stable cell lines overexpressing receptors or targets

[0484] The 293T-FAP cell line was generated by transfecting full-length human, cynomolgus or mouse FAP (Table 1) into HEK-293T cells using lipofectamine 3000 following standard protocols. After three days, the cells were treated with hygromycin (Millipore Sigma) for 14 days to generate stable cell lines. 293T-HVEM and 293T-LTβR cell lines were generated with a similar procedure.

TABLE 1: FAP, HVEM, LTBR PROTEINS AND SEQUENCE SOURCES

Protein name	Sequence source
Human FAP	Uniprot Q12884
Cynomolgus FAP	NCBI XP_005573377.1
Mouse FAP	Uniprot P97321
Human HVEM	Uniprot Q92956
Cynomolgus HVEM	NCBI XP_005545061.1
Mouse HVEM	Uniprot Q80WM9
Human LTβR	Uniprot P36941
Cynomolgus LTβR	Uniprot A0A2K5VGQ6
Mouse LTβR	Uniprot P50284

[0485] Cell binding analysis by FACS:

[0486] The stably transfected HEK-293T cells expressing FAP were used to evaluate the binding activities of anti-FAP monoclonal antibodies (mAb). The cells were incubated with serially diluted anti-FAP monoclonal antibodies for 30 minutes on ice, with a maximum concentration of 50 nM in flow cytometry buffer (PBS, 0.5% BSA, 2 mM EDTA). The cells were then washed three times with PBS, and the bound mAbs were stained with FITC-conjugated mouse anti-human IgG antibody for 15 minutes on ice. The cells were further washed with PBS three times, and the fluorescence was measured using a Cytek Aurora cytometer (Cytek) and analyzed using FlowJo and GraphPad Prism 9.0 software to determine the EC_{50}. Results are summarized in Tables 2.

TABLE 2: EC50S OF ANTI-FAP MABS BINDING TO 293T CELLS EXPRESSING FAP

EC_{50} (nM)	9E3 chimera mAb	ABC1931 (humanized 9E3)
hFAP	1.001	3.7
cynoFAP	0.616	1.4
mFAP	0.470	0.84

Human FAP, cynomolgus FAP and mouse FAP are abbreviated to hFAP, cynoFAP and mFAP, respectively.

[0487] As shown in Figures 1A-1C and Table 2, the chimera antibody 9E3 cross-reacts with human, cynomolgus and mouse FAP, while the humanized antibody ABC1931 maintains cross-reactivity and affinities.

Example 2: Immunocytokine generation and selection

2.1. Generation of Immunocytokine

[0488] The variable domain of the humanized 9E3 was used to generate immunocytokine in both format A and format D. Immunocytokines were produced by co-transfection of plasmids with different heavy chain (HC) plasmid ratios into Expi293F cells (ThermoFisher#A14527) following the manufacturer's recommendations. Expi293F cells were grown in suspension culture. The supernatant was harvested by centrifugation six days post-transfection and further filtered with 0.22 μm filter, and

purified by protein A beads and analyzed under non-reducing and reducing conditions by SDS-PAGE gel.

[0489] The tested immunocytokines are shown below in **TABLE 4, TABLE 4.1,** and **TABLE 4.2**.

TABLE 4: Assayed Antibody and Heavy Chains/Light chains

Protein Concept ID	chain	chain code	Protein Concept ID	chain	chain code
9E3 chimera (ABC1139)	HC	AP1333	ABC1947	LC	AP2463
				HC1	AP1981
	LC	AP1334		HC2	AP2464
ABC1931	LC	AP2463	ABC2066	LC	AP2463
				HC1	AP2586
	HC	AP1992		HC2	AP2584
ABC1930	LC	AP2463	ABC2067	LC	AP2463
	HC1	AP1979		HC1	AP2585
	HC2	AP2271		HC2	AP2586
ABC1773	LC	AP672	ABC2097	LC	AP2463
	HC1	AP2267		HC1	AP2555
	HC2	AP2269		HC2	AP2556

TABLE 4.1: Assayed Antibody and Kabat CDRs

Protein Concept ID	chain	CDR1	CDR2	CDR3
9E3 chimera (ABC1139)	HC	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYNLSLKS (SEQ ID NO: 61)	SQDMPGYFDY (SEQ ID NO: 28)
	LC	KTNQNVDYNGNTFMH (SEQ ID NO: 76)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)
ABC1931	LC	KTNQNVDYSGNTFMH (SEQ ID NO: 79)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)
	HC	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)
ABC1930	LC	KTNQNVDYSGNTFMH (SEQ ID NO: 79)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)
	HC1	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)
	HC2	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)
ABC1773	LC	SASSRVGYMH (SEQ ID NO: 29)	DTSKLAS (SEQ ID NO: 30)	FQGSGYPFT (SEQ ID NO: 31)
	HC1	TAGMSVG (SEQ ID NO: 32)	DIWWDDKKHYNPSLKD (SEQ ID NO: 33)	DMIFNFYFDV (SEQ ID NO: 34)
	HC2	TAGMSVG (SEQ ID NO: 32)	DIWWDDKKHYNPSLKD (SEQ ID NO: 33)	DMIFNFYFDV (SEQ ID NO: 34)
ABC1947	LC	KTNQNVDYSGNTFMH (SEQ ID NO: 79)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)

	HC1	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)
	HC2	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)
ABC2066	LC	KTNQNVDYSGNTFMH (SEQ ID NO: 79)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)
	HC1	/	/	/
	HC2	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)
ABC2067	LC	KTNQNVDYSGNTFMH (SEQ ID NO: 79)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)
	HC1	/	/	/
	HC2	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)
ABC2097	LC	KTNQNVDYSGNTFMH (SEQ ID NO: 79)	LASNLAS (SEQ ID NO: 24)	QQSRNLPYT (SEQ ID NO: 25)
	HC1	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)
	HC2	IYGVN (SEQ ID NO: 26)	AIWSGGRKDYSLSLKS (SEQ ID NO: 63)	SQDMPGYFDY (SEQ ID NO: 28)

TABLE 4.2: Assayed Antibody and chain amino acid sequences

chain code	Single code amino acid sequence (Variable regions are in bold)
AP1979 (SEQ ID NO: 5)	**EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDY SLSLKSRLTISGDTSKNQVSLKLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS** ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGKGGGGSGGGGSEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRGL SYHDGALVVTKAGYYYIYSKVQLGGMGCPLGLASTITHGLYKRTPRYPEELELLVSQQSPCG RATSSSRVWQDSSFLGGVVHLEAGEKVVVRVLDERLVRLLDGTRSYFGAFMV
AP2271 (SEQ ID NO: 6)	**EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDY SLSLKSRLTISGDTSKNQVSLKLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS** ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLSLGKGGGGSGGGGSEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRG LSYHDGALVVTKAGYYYIYSKVQLGGMGCPLGLASTITHGLYKRTPRYPEELELLVSQQSPC GRATSSSRVWQDSSFLGGVVHLEAGEKVVVRVLDERLVRLLDGTRSYFGAFMVGGGGSGG GGSEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKAGYYYIYSKVQ LGGMGCPLGLASTITHGLYKRTPRYPEELELLVSQQSPCGRATSSSRVWQDSSFLGGVVHLEA GEKVVVRVLDERLVRLLDGTRSYFGAFMV
AP672 (SEQ ID NO: 7)	**DIQMTQSPSTLSASVGDRVTITCSASSRVGYMHWYQQKPGKAPKLLIYDTSKLASGVPS RFSGSGSGTEFTLTISSLQPDDFATYYCFQGSGYPFTFGGGTKVEIK**RTVAAPSVFIFPPSD

	EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC*
AP2267 (SEQ ID NO: 8)	**QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLADIWWDDK KHYNPSLKDRLTISKDTSKNQVVLKVTNMDPADTATYYCARDMIFNFYFDVWGQGTTV TVSS**ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLS CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSRWQEGNVFSCSVM HEALHNHYTQKSLSLSLGKGGGGSGGGGSEVNPAAHLTGANSSLTGSGGPLLWETQLGLAF LRGLSYHDGALVVTKAGYYYIYSKVQLGGMGCPLGLASTITHGLYKRTPRYPEELELLVSQQ SPCGRATSSSRVWQDSSFLGGVVHLEAGEKVVVRVLDERLVRLLDGTRSYFGAFMV*
AP2269 (SEQ ID NO: 9)	**QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLADIWWDDK KHYNPSLKDRLTISKDTSKNQVVLKVTNMDPADTATYYCARDMIFNFYFDVWGQGTTV TVSS**ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSL WCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGKGGGGSGGGGSEVNPAAHLTGANSSLTGSGGPLLWETQLGLA FLRGLSYHDGALVVTKAGYYYIYSKVQLGGMGCPLGLASTITHGLYKRTPRYPEELELLVSQ QSPCGRATSSSRVWQDSSFLGGVVHLEAGEKVVVRVLDERLVRLLDGTRSYFGAFMVGGGG SGGGGSEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKAGYYYIYS KVQLGGMGCPLGLASTITHGLYKRTPRYPEELELLVSQQSPCGRATSSSRVWQDSSFLGGVV HLEAGEKVVVRVLDERLVRLLDGTRSYFGAFMV*
AP1981 (SEQ ID NO: 10)	**EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDY SLSLKSRLTISGDTSKNQVSLKLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS** ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGKGGGGSGGGGSEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRGL SYHDGALVVTKAGYYYIYSKVQLGGVGCPLGLASTITHGLYKRTPRYPEELELLVSQQSPCG RATSSSRVWWDSSFLGGVVHLEAGEKVVVRVLDERLVRLRDGTRSYFGAFMV
AP2464 (SEQ ID NO: 11)	E**VQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDY SLSLKSRLTISGDTSKNQVSLKLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS** ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLSLGKGGGGSGGGGSEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRG LSYHDGALVVTKAGYYYIYSKVQLGGVGCPLGLASTITHGLYKRTPRYPEELELLVSQQSPC GRATSSSRVWWDSSFLGGVVHLEAGEKVVVRVLDERLVRLRDGTRSYFGAFMVGGGGSGG GGSEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKAGYYYIYSKVQ LGGVGCPLGLASTITHGLYKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSSFLGGVVHLE AGEKVVVRVLDERLVRLRDGTRSYFGAFMV
AP2584 (SEQ ID NO: 12)	RRSHEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKTGYYYIYSKV QLGGVGCPLGLAGTITHGLYKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSSFLGGVVHL EAGEKVVVRVLGKRLVRLRDGTRSYFGAFMV**G**RRSHEVNPAAHLTGANSSLTGSGGPLLWE TQLGLAFLRGLSYHDGALVVTKTGYYYIYSKVQLGGVGCPLGLAGTITHGLYKRTPRYPEEL ELLVSQQSPCGRATSSSRVWWDSSFLGGVVHLEAGEKVVVRVLGKRLVRLRDGTRSYFGAF MVGRRSHEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKTGYYYIY SKVQLGGVGCPLGLAGTITHGLYKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSSFLGGV VHLEAGEKVVVRVLGKRLVRLRDGTRSYFGAFMVGGGGSGGGGSGGGGSGGGGTGPPCPP CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTK

	PREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLP PSQEEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDK SRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK*
AP2586 (SEQ ID NO: 13)	**EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDY SLSLKSRLTISGDTSKNQVSLKLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS** ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLSLGK*
AP2585 (SEQ ID NO: 14)	RRSHEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKTGYYYIYSKV QLGGVGCPLGLAGTITHGLYKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSSFLGGVVHL EAGEKVVVRVLGKRLVRLRDGTRSYFGAFMVGRRSHEVNPAAHLTGANSSLTGSGGPLLWE TQLGLAFLRGLSYHDGALVVTKTGYYYIYSKVQLGGVGCPLGLAGTITHGLYKRTPRYPEEL ELLVSQQSPCGRATSSSRVWWDSSFLGGVVHLEAGEKVVVRVLGKRLVRLRDGTRSYFGAF MVGRRSHEVNPAAHLTGANSSLTGSGGPLLWETQLGLAFLRGLSYHDGALVVTKTGYYYIY SKVQLGGVGCPLGLAGTITHGLYKRTPRYPEELELLVSQQSPCGRATSSSRVWWDSSFLGGV VHLEAGEKVVVRVLGKRLVRLRDGTRSYFGAFMVGGGGSGGGGSGGGGSGGGGTCPPCPA PEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQ EEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSRW QEGNVFSCSVMHEALHNHYTQKSLSLSLGK*
AP2555 (SEQ ID NO: 15)	**EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDY SLSLKSRLTISGDTSKNQVSLKLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS** ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLSLGKGGGGSGGGGSLSPGLPAAHLIGAPLKGQGLGWETTKEQAFLTSGT QFSDAEGLALPQDGLYYLYCLVGYRGRAPPGGGDPQGRSVTLRSSLYRAGGAYGPGTPELLL EGAETVTPVLDPARRQGYGPLWYTSVGFGGLVQLRRGERVYVNISHPDMVDFARGKTFFGA VMVG*
AP2556 (SEQ ID NO: 16)	**EVQLQESGPGLVKPSETLSLTCTVSGFSLSIYGVNWVRQPPGKGLEWIAAIWSGGRKDY SLSLKSRLTISGDTSKNQVSLKLSSVTAADTAVYYCARSQDMPGYFDYWGQGTLVTVSS** ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGKGGGGSGGGGSAHSTLKPAAHLIGDPSKQNSLLWRANTDRAFLQDGF SLSNNSLLVPTSGIYFVYSQVVFSGKAYSPKATSSPLYLAHEVQLFSSQYPFHVPLLSSQKMV YPGLQEPWLHSMYHGAAFQLTQGDQLSTHTDGIPHLVLSPSTVFFGAFALLSPGLPAAHLIGA PLKGQGLGWETTKEQAFLTSGTQFSDAEGLALPQDGLYYLYCLVGYRGRAPPGGGDPQGRS VTLRSSLYRAGGAYGPGTPELLLEGAETVTPVLDPARRQGYGPLWYTSVGFGGLVQLRRGE RVYVNISHPDMVDFARGKTFFGAVMVG*

[0490] Figures 2 and 6 show the formats of above immunocytokines. Format A: ABC2066, ABC2067; Format D: ABC1930, ABC1773, ABC1947 and ABC2097.

[0491] In this study, three tandem-linked human mutant LIGHT (hmLIGHT) units were linked to the N-terminal of the Fc region to assemble with the anti-FAP humanized 9E3 halfbody. The structure (Format A) and hmLIGHT mutation are based on a previously reported immunocytokine (Cancer Cell 29, 285-296, 2016). However, constructs (ABC2066 and ABC2067) in both Format A, with slight differences in the hIgG4 Fc starting residues, failed to assemble correctly, due to the chain encoding three tandem hmLIGHT units exhibiting low expression, even with different ratios.

[0492] In contrast, ABC1930 and ABC1947 in Format D, sharing the same anti-FAP variable domain with ABC2066 and ABC2067, displayed correct assembly. Successful expression and correct assembly of the LIGHT-based immunocytokines were demonstrated by the presence of a dominant band in the non-reducing gel that corresponded to the molecular weight of correctly assembled molecules. The correct assembly was further supported by three bands revealed by SDS-PAGE analysis of reduced proteins, which represented two heavy chains with different molecular weights and one light chain (Figure 6).

[0493] Moreover, ABC1930 with the LIGHT1 mutant (abbreviated as mu1 or LIGHTmu1 or LIGHT1) exhibited 3-fold higher expression than ABC1947 with wild type LIGHT (abbreviated as wt or LIGHTwt), in addition to the desired receptor selectivity (Figure 7A and Figure 7B).

[0494] Lymphotoxin-αββ, has similar receptor binding selectivity for LTßR over HVEM and DcR3, compared to LIGHTmu1, potentially offering enhanced safety and efficacy in providing an anti-tumor effect. ABC2097, an immunocytokine equipped with Lymphotoxin-αββ in Format D, can also be generated expressed, albeit with a lower yield (Figure 7A and Figure 7B).

[0495] The LIGHTmu1 mutant in ABC1930 has an amino acid sequence as shown in SEQ ID NO: 17, and wild type LIGHT in ABC1947 has an amino acid sequence as shown in SEQ ID NO: 18.

2.2. FAP binding by BLI

[0496] Kinetic assays were performed at 30°C on Octet RH16 system (Sartorius) and samples were diluted in PBST buffer (PBS with 0.02% Tween-20, pH 7.4) with an orbital shake speed of 1000 rpm. To assess the binding kinetics between ABC1930 and FAP recombinant proteins, the Ni-NTA biosensor (Sartorius, 18-5101) was loaded with 10 ug/mL His-tagged FAP antigen protein (human or cynomolgus, or mouse) to a density of 1.0 nm, followed by a baseline step of 60 seconds in PBST buffer. The FAP-captured biosensors were then immersed in wells containing different concentrations of ABC1930 for 2 minutes followed by 4-minute of dissociation period in PBST buffer. The FAP-captured sensors were also dipped in wells containing PBST buffer to allow single reference subtraction to compensate for the natural dissociation of captured FAP. The sensorgrams were blank-referenced against the buffer and globally fitted with a 1:1 model to extract kinetic parameters using Data Analysis software (Fortebio/Sartorius). See Figure 3A, and Table 3.

2.3 LTßR and HVEM binding by BLI

[0497] LTßR-Fc proteins were biotinylated using an EZ-Link Sulfo-NHS-biotin kit (#21925, Thermo scientific). Assays were carried out by soaking the Streptavidin Biosensors (ForteBio) in PBST buffer. First, sensors were rinsed in PBST buffer for 180 seconds which served as the baseline. Second, sensors were immobilized for 200 sec with biotinylated target protein (20 μg/ml). Then, sensors were washed in PBST buffer for another 60 seconds. Finally, sensors were exposed to a series of diluted immunocytokines, with a maximum concentration of 100 nM. The association of each concentration of the immunocytokine was monitored for 180 sec, followed by dissociation in PBST buffer for 180 seconds. To wash off the immunocytokine, the SA biosensors were dipped into 3M $MgCl_2$ 10 sec, repeated 3 times. The sensorgrams were blank-referenced against the buffer and globally fitted with a 1:1 model to extract kinetic parameters using Data Analysis software (Fortebio/Sartorius).

[0498] HVEM-His proteins were purchased from Sino Biological (catalog numbers 10334-H08H, 90109-C08B, 10567-M08H). The Ni-NTA biosensor (Sartorius, 18-5101) was loaded with 10 ug/mL

His-tagged HVEM protein (human or cynomolgus, or mouse) to a density of 1.0 nm, followed by a 60-second baseline step in PBST buffer. The biosensors were then immersed in wells containing different concentrations of ABC1930 or ABC1947 for 2 minutes, followed by 4-minute dissociation period in PBST buffer. To account for the dissociation of captured HVEM, the sensors were also dipped in wells containing PBST buffer for single-reference subtraction. The sensorgrams were blank-referenced against the buffer and globally fitted with a 1:1 model to extract kinetic parameters using Data Analysis software (Fortebio/Sartorius). See Figures 3B-3C, and Table 3.

2.4 DcR3 binding by ELISA

[0499] DcR3 is a soluble protein expressed in humans and cynomolgus macaques but is absent in mice. DcR3 negatively regulates the ability of LIGHT to activate LTβR and HVEM (Liu et al., 2021) and is upregulated in certain autoinflammatory diseases and cancer patients (Fuchsberger et al., 2021). Consistent with the lower binding affinities of DcR3 to LIGHTmu1than wild-type LIGHT, DcR3 had significantly weaker binding to ABC1930 than to ABC1947.

[0500] The recombinant DcR3 protein was generated by fusing human DcR3 residues 33-300 to the N-terminal of rabbit Fc. The recombinant proteins were expressed in Expi293F and affinity-purified by protein A column, and the monomer was collected based on Superdex 200.

[0501] DcR3 proteins were immobilized onto maxiSorp 96-well ELISA plates (Thermo Fisher) at a concentration of 2 μg/mL and then blocked with 0.5% BSA-PBST buffer for one hour. Biotinylated LIGHT-based immunocytokines (EZ-Link Sulfo-NHS-biotin kit, #21925, Thermo scientific, Rockford, IL, USA) were added to the plates at various dilutions, with a maximum concentration of 10 nM. The plates were incubated for an hour, washed four times with PBST, and further incubated with HRP-conjugated streptavidin (Acrobiosystems, #STN-NH913), and washed again with PBST. The TMB substrate (#34029, Thermo Fisher) was added to the plates, which were read at 450 nM on a SpectraMax M5 microplate reader (Molecular Devices). See Figure 4.

[0502] Results of FAP, LTßR, HVEM and DcR3 binding are summarized in Table 3.

TABLE 3: FAP, LTßR, HVEM AND DCR3 BINDING

	ABC1930				ABC1947		
Target	FAP	LTßR	HVEM	DcR3(ELISA)	LTßR	HVEM	DcR3(ELISA)
Species	K_D (nM)	K_D (nM)	K_D (nM)	EC50 (nM)	K_D (nM)	K_D (nM)	EC50 (nM)
Human	3.0	1.2	No binding	Reduced	0.6	1.3	0.056
Cynomolgus	4.7	1.8	No binding	Reduced	1.1	1.0	0.044
Mouse	2.6	4.3	142 nM	NA	8	4.8	NA

[0503] ABC1930 binds to LTßR without engaging HVEM and DcR3, potentially offering enhanced safety and efficacy in providing an anti-tumor effect.

2.5 DPPIV binding by ELISA

[0504] The protein with the highest similarity to FAP in human is DPPIV. To test the specificity of ABC1930, DPPIV ELISA was performed.

[0505] Human FAP and DPPIV proteins were immobilized onto MaxiSorp 96-well ELISA plates at a concentration of 0.5 μg/mL and then blocked with 0.5% BSA-PBST buffer for one hour. ABC1930 was added to the plates at a series of dilutions, with a maximum concentration of 50 nM. The plates were incubated for an hour, washed four times with PBST, and further incubated with an HRP-conjugated donkey anti-human IgG (H+L) antibody (Jackson Immunoresearch, #709-035-149), followed by four additional times with PBST. The TMB substrate (#34029, Thermo Fisher) was added to the plates, which were read at 450 nM on a SpectraMax M5 microplate reader (Molecular Devices).

[0506] As shown in Figure 5, ABC1930 binds to human FAP, but not DPPIV.

Example 3: Functional assays with engineered cell lines

3.1 Evaluation of the cis-activation of the endogenous murine LTβR pathway by ABC1931, ABC1773 and ABC1930 using CCL2 as a surrogate readout

[0507] Two murine cell lines, BALB/c-3T3-FAP (LTβR+ HVEM- FAP+) and BALB/c-3T3 (LTβR+ HVEM- FAP-) were used to investigate the effect of ABC1931, ABC1773 and ABC1930 to activate

endogenous mouse LTβR. BALB/c-3T3-FAP cell line was generated by transfecting BALB/c-3T3 cells (ATCC) with mouse Fap (abbreviated as mFAP), which was selected and confirmed for mFAP expression. The cells were plated at 4,000 cells per well in a 96-well tissue culture plate and incubated with different concentrations of anti-FAP-LIGHT immunocytokines for 24 hours at 37°C. After centrifugation, supernatants were collected to quantify the level of CCL2 protein by ELISA (Cat#432704, Biolegend) with a Tecan Spark reader.

[0508] Figures 8A-8C showed that ABC1931 did not induce CCL2 production regardless of FAP expression. However, ABC1930 activated the LTβR signaling much more prominently (>1000x) when cells expressed both FAP and LTβR, suggesting a cis-activation. The non-targeted control ABC1773 (anti-FAP-LIGHTmu1) failed to demonstrate this cis-activation. Since cancer-associate fibroblasts (CAFs) in the tumor microenvironment play an important role in tumor biology and has the potential to co-express both FAP and LTβR, ABC1930 would preferentially stimulate LTβR in FAP+ LTβR + CAFs in the tumor microenvironment.

3.2 Evaluation of the trans-activation of the endogenous murine LTβR pathway by ABC1931, ABC1773, and ABC1930 using CCL2 as a surrogate readout

[0509] To study the effect of trans-expressed FAP on the activation of endogenous LTβR pathway in BALB/c-3T3-WT (LTβR+ HVEM- FAP-) cells, a CT26-FAP cell line was generated by stably transfecting CT26 cells (ATCC) with a plasmid containing mouse FAP gene using lipofectamine 3000 and antibiotic selection. We confirmed that only 3T3 cells, but not CT26-WT or CT26-FAP, could express CCL2 after stimulation by LIGHT-based immunocytokines.

[0510] CT26-WT or CT26-FAP cells were plated at 10,000/well in a 96-well tissue culture plate and incubated with serially diluted ABC1931, ABC1773 and ABC1930 for one hour at 37°C, 5% CO_2. Then 4,000 BALB/c-3T3 cells were added to the culture as responder cells. ELISA assay (Cat#432704,

Biolegend) was performed to measure the CCL2 protein level in the media, as a surrogate readout of the LTβR pathway activation.

[0511] As shown in Figures 9A-9C, in the presence of CT26-FAP cells, ABC1930 activated the LTβR signaling much more prominently (>100x), suggesting a trans-activation. The non-targeted control ABC1773 (control antibody-LIGHTmu1) failed to show this trans-activation.

Example 4: *In vivo* evaluation in the KPC mouse model

[0512] The KPC0826 cell line originated from a commercially available KPC cell line, which is derived from a genetically modified KPC tumor model. KPC0826 was derived from aggressive KPC tumor masses established by this commercial cell line using a previously published protocol (Beatty et al., 2011). To establish a subcutaneous KPC0826 tumor model, 2.5 million KPC0826 cells in 50 µl HBSS were mixed with 50 µl of matrigel and implanted into the flank of C57BL/6 mice. The established subcutaneous KPC0826 tumor model showed a relatively high presence of FAP+ CAF cells, around 1-5% of total dissociated cells from tumor tissues (data not shown). On day 0, when tumor volumes reached an average of ~113 mm^3, the mice were divided into 6 groups based on body weight and tumor volume: G1_buffer, G2_GEM (gemcitabine), G3_ABC1931, G4_ABC1931 + GEM, G5_ABC1930 and G6_ABC1930 + GEM, with six mice per group. Mice were treated twice a week for 7 doses with 0.75 nanomoles of the respective large molecules, corresponding to a dose of 5.4 mg/kg for ABC1931 and 7.25 mg/kg for ABC1930. GEM was given at 75 mg/kg. Tumor growth was monitored by measuring tumor volumes using calipers at indicated time points until day 24. The tumor volume was calculated using the formula: length x $width^2/2$. Tumor growth inhibition (TGI) was compared to the control group (G1_buffer) and calculated using the DRAP R package (J Transl Med 17, 39, 2019).

[0513] As shown in Figures 10A-10C, the results demonstrated that ABC1930 exhibited a great single-agent anti-tumor activity in the KPC0826 model, with TGI reaching over 80%. The combination of ABC1930 with GEM further suppressed tumor growth.

Example 5: *In vivo* evaluation in the LL2 tumor model

[0514] To establish a subcutaneous LL2 tumor model, 0.12 million LL2 cells in 50 μl HBSS were mixed with 50 μl of matrigel and implanted into the flank of C57LB/6 mice. On day 0, when tumor volumes reached an average of ~40 mm^3, the mice were divided into 3 groups based on body weight and tumor volume: G1_Ctrl (Vehicle), G2_ABC1930 (anti-FAP-LIGHT mu1), G3_ABC1773 (control antibody-LIGHT mu1), with six mice per group. Mice were treated twice a week for 3 doses with 0.25 nanomoles of the respective large molecules, corresponding to a dose of 2.417 mg/kg for ABC1930 and ABC1773. Tumor growth was monitored by measuring tumor volumes using calipers at indicated time points until day 12. The tumor volume was calculated using the formula: length x $width^2/2$.

[0515] The results demonstrated that ABC1930 immunocytokine exhibited a significant single-agent anti-tumor activity in the LL2 model, more potent than the non-targeted control molecule ABC1773.

[0516] Figures 11A-11C demonstrate the *in vivo* anti-tumor activity of ABC1930 anti-FAP-LIGHT mu1 in the subcutaneous LL2 model. Figure 11A outlines the treatment timeline. Figure 11B shows individual tumor volumes of each group. Figure 11C displays the average tumor volumes for each group.

Example 6: *In vivo* evaluation in the LL2-OVA tumor model

[0517] To establish a subcutaneous LL2-OVA tumor model, 0.12 million LL2-OVA cells in 50 μl HBSS were mixed with 50 μl of matrigel and implanted into the flank of C57LB/6 mice. On day 0, when tumor volumes reached an average of ~80 mm^3, the mice were divided into 4 groups based on body weight and tumor volume: G1_Ctrl (Vehicle), G2_ABC092 (anti-PD1), G3_ABC1930 (anti-FAP-LIGHT mu1), G4_ABC1930 (anti-FAP-LIGHT mu1) + ABC092 (anti-PD1), with five mice per

group. Mice were treated for 2 doses on day 0 and day 5. Tumor growth was monitored by measuring tumor volumes using calipers at indicated time points until day 8. The tumor volume was calculated using the formula: length x width^2/2.

[0518] ABC092 is derived from a well-established and widely used anti-mouse anti-PD1 antibody, known as clone RPM1-14. The heavy chain of ABC092 includes an amino acid sequence as shown in SEQ ID NO: 19, the light chain of ABC 092 includes an amino acid sequence as shown in SEQ ID NO: 20.

[0519] ABC092 anti-PD1 alone did not show a significant effect within the short treatment time frame. Conversely, ABC1930 exhibited a significant single-agent activity and a slight synergy with anti-PD1.

[0520] Figures 12A-12C demonstrate the *in vivo* anti-tumor activity of ABC1930 anti-FAP-LIGHT mu1 in the subcutaneous LL2-OVA model. Figure 12A outlines the treatment timeline and doses. Figure 12B shows individual tumor volumes of each group. Figure 12C displays the average tumor volumes for each group.

Example 7: *In vivo* evaluation in the subcutaneous EMT6 tumor model

[0521] To establish a subcutaneous EMT6 tumor model, 0.5 million EMT6 cells in 50 µl HBSS were mixed with 50 µl of matrigel and implanted into the flank of BALB/c mice. On day 0, when tumor volumes reached an average of ~92 mm^3, the mice were divided into 4 groups based on body weight and tumor volume: G1_Vehicle (control), G2_ABC1930 (anti-FAP-LIGHT mu1), G3_ABC727 (anti-PDL1), G4_ABC1930 (anti-FAP-LIGHT mu1) + ABC727 (anti-PDL1), with six mice per group. Mice were treated twice a week for 4 doses. Tumor growth was monitored by measuring tumor volumes using calipers at indicated time points until day 14. The tumor volume was calculated using the formula: length x width^2/2.

[0522] ABC727 is derived from atezolizumab from Roche, having a heavy chain including the amino acid sequence as shown in SEQ ID NO: 21, and a light chain including the amino acid sequence as shown in SEQ ID NO: 22.

[0523] In the EMT6 subcutaneous model, both ABC1930 and anti-PDL1 exhibited single-agent activity. The combination further deepened the anti-tumor response. As a result, 1 out of 6 tumors was regressed in the anti-PDL1 group, and 2 out 6 were regressed in the combination group by day 14.

[0524] Figures 13A-13C demonstrate the *in vivo* anti-tumor activity of ABC1930 anti-FAP-LIGHT mu1 in the subcutaneous EMT6 model. Figure 13A outlines the treatment timeline and doses. Figure 13B shows individual tumor volumes of each group. Figure 13C displays the average tumor volumes foreach group.

CLAIMS

1. An isolated anti-FAP antibody or a FAP antigen-binding fragment thereof, comprising:

a)

i) a heavy chain variable region (VH) comprising

(1) a heavy chain complementarity determining region 1 (HCDR1) comprising the amino acid sequence of IYGVN (SEQ ID NO: 26),

(2) heavy chain complementarity determining region 2 (HCDR2) comprising the amino acid sequence of AIWSGGRKDYX_2LSLKS (SEQ ID NO: 27), wherein X_2 is N or S, and

(3) heavy chain complementarity determining region 3 (HCDR3) comprising the amino acid sequence of SQDMPGYFDY (SEQ ID NO: 28), and

ii) a light chain variable region (VL) comprising

(1) a light chain complementarity determining region 1 (LCDR1) comprising the amino acid sequence of KTNQNVDYX_1GNTFMH (SEQ ID NO: 23), wherein X_1 is N or S,

(2) a light chain complementarity determining region 2 (LCDR2) comprising the amino acid sequence of LASNLAS (SEQ ID NO: 24), and

(3) a light chain complementarity determining region 3 (LCDR3) comprising the amino acid sequence of QQSRNLPYT (SEQ ID NO: 25);

or

b)

i) a VH region comprising

(1) a HCDR1 comprising the amino acid sequence of TAGMSVG (SEQ ID NO: 32),

(2) a HCDR2 comprising the amino acid sequence of DIWWDDKKHYNPSLKD (SEQ ID NO: 33),

(3) a HCDR3 comprising the amino acid sequence of DMIFNFYFDV (SEQ ID NO: 34), and

ii) a VL region comprising

(1) LCDR1 comprising the amino acid sequence of SASSRVGYMH (SEQ ID NO: 29),

(2) LCDR2 comprising the amino acid sequence of DTSKLAS (SEQ ID NO: 30), and

(3) LCDR3 comprising the amino acid sequence of FQGSGYPFT (SEQ ID NO: 31).

2. The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof of claim 1, wherein the isolated anti-FAP antibody or a FAP antigen-binding fragment thereof comprises:
   a) a VH region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 35, and/or a VL region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 36;
   b) a VH region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 37, and/or a VL region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 38; or
   c) a VH region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 41, and/or a VL region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 42.

3. The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof of claim 1 or 2, wherein the isolated anti-FAP antibody or a FAP antigen-binding fragment thereof comprises:
   a) a heavy chain (HC) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 1, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, and 16, and/or
   b) a light chain (LC) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 2, 3, and 7.

4. The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof of any one of claims 1-3, wherein the isolated anti-FAP antibody or a FAP antigen-binding fragment thereof comprises:
   a) HC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 1, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 2; or
   b) a HC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 4, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;
   c) a first heavy chain (HC1) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 5, a second heavy chain (HC2) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 6, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;
   d) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 8, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 9, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 7;
   e) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 10, a HC2 region comprising an amino acid sequence

that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 11, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

f) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 13, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 12, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

g) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 14, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 13, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3; or

h) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 15, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 16, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3.

5. The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof of any one of claims 1-4, wherein the VH region and/or VL region further comprises human framework sequences.

6. The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof of any one of claims 1-5, wherein the VH region and/or VL region further comprises a framework 1 (FR1), a framework 2 (FR2), a framework 3 (FR3) and/or a framework 4 (FR4) sequence.
7. The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof of any one of claims 1-6, comprising a fragment crystallizable (Fc) region derived from immunoglobulin.
8. The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof of claim 7, wherein the Fc fragment is derived from IgG1, IgG2, IgG3, or IgG4, optionally the Fc fragment is derived from IgG4.
9. The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof of claim 7 or 8, wherein the Fc fragment comprises mutation S228P or LALAPG mutation.
10. The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof of any one of claims 1-9, wherein the antibody is a monoclonal antibody.
11. The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof of any one of claims 1-10, wherein the antibody is a humanized, human or chimeric antibody.
12. The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof of any one of claims 1-11, wherein the anti-FAP antibody or FAP antigen-binding fragment cross-reacts with human, cynomolgus and mouse FAP.
13. The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof of any one of claims 1-12, wherein the antibody or fragment thereof is a Fab, Fab', F(ab')2, Fv, scFv, (scFv)2, single chain antibody molecule, dual variable region antibody, single variable region antibody, linear antibody, V region, or a multispecific antibody formed from antibody fragments.
14. The isolated anti-FAP antibody or a FAP antigen-binding fragment thereof of any one of claims 1-13, wherein the isolated anti-FAP antibody or a FAP antigen-binding fragment thereof binds to FAP with dissociation constant (K_D) no more than 20 nM, 15 nM, 10 nM or 5 nM.
15. A bispecific antibody or antigen-binding fragment thereof comprising a FAP antigen-binding moiety and a second binding moiety, wherein the FAP antigen-binding moiety comprises:

    a)

    i) a heavy chain variable region (VH) comprising

    (1) a heavy chain complementarity determining region 1 (HCDR1) comprising the amino acid sequence of IYGVN (SEQ ID NO: 26),

(2) heavy chain complementarity determining region 2 (HCDR2) comprising the amino acid sequence of AIWSGGRKDYX_2LSLKS (SEQ ID NO: 27), wherein X_2 is N or S, and

(3) heavy chain complementarity determining region 3 (HCDR3) comprising the amino acid sequence of SQDMPGYFDY (SEQ ID NO: 28), and

ii) a light chain variable region (VL) comprising

(1) a light chain complementarity determining region 1 (LCDR1) comprising the amino acid sequence of KTNQNVDYX_1GNTFMH (SEQ ID NO: 23), wherein X_1 is N or S,

(2) a light chain complementarity determining region 2 (LCDR2) comprising the amino acid sequence of LASNLAS (SEQ ID NO: 24), and

(3) a light chain complementarity determining region 3 (LCDR3) comprising the amino acid sequence of QQSRNLPYT (SEQ ID NO: 25);

or

b)

i) a VH region comprising

(1) a HCDR1 comprising the amino acid sequence of TAGMSVG (SEQ ID NO: 32),

(2) a HCDR2 comprising the amino acid sequence of DIWWDDKKHYNPSLKD (SEQ ID NO: 33),

(3) a HCDR3 comprising the amino acid sequence of DMIFNFYFDV (SEQ ID NO: 34), and

ii) a VL region comprising

(1) LCDR1 comprising the amino acid sequence of SASSRVGYMH (SEQ ID NO: 29),

(2) LCDR2 comprising the amino acid sequence of DTSKLAS (SEQ ID NO: 30), and

(3) LCDR3 comprising the amino acid sequence of FQGSGYPFT (SEQ ID NO: 31).

16. The bispecific antibody or antigen-binding fragment thereof of claim 15, wherein the FAP antigen-binding moiety comprises:

a) a VH region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 35, and/or a VL region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,

or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 36;

b) a VH region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 37, and/or a VL region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 38; or

c) a VH region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 41, and/or a VL region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 42.

17. The bispecific antibody or antigen-binding fragment thereof of claim 15 or 16, wherein the FAP antigen-binding moiety comprises:

a) a heavy chain (HC) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 1, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, and 16, and/or

b) a light chain (LC) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in SEQ ID NO: 2, 3, and 7.

18. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 17, wherein the FAP antigen-binding moiety comprises:

a) HC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 1, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 2; or

b) a HC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 4, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

c) a first heavy chain (HC1) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 5, a second heavy chain (HC2) region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 6, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

d) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 8, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 9, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 7;

e) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 10, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 11, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

f) a first HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 13, a HC2 region comprising an amino acid

sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 12, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3;

g) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 14, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 13, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3; or

h) a HC1 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 15, a HC2 region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 16, and/or a LC region comprising an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to an amino acid sequence as shown in SEQ ID NO: 3.

19. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 18, comprising a Fc fragment derived from immunoglobulin at a N-terminus of the FAP antigen-binding moiety.

20. The bispecific antibody or antigen-binding fragment thereof of claim 19, wherein the Fc fragment is derived from IgG1, IgG2, IgG3, or IgG4, optionally the Fc fragment is derived from IgG4.

21. The bispecific antibody or antigen-binding fragment thereof of claim 19 or 20, wherein the Fc fragment comprises mutation S228P or LALAPG mutation.

22. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 21, wherein the bispecific antibody or antigen-binding fragment thereof comprises an Fc fragment and wherein the Fc fragment comprises one or more modifications selected from the group consisting of knobs-into-holes, DDKK, electrostatic steering of CH3, DuoBody, SEEDbodies, cFAE, XmAb,

Azymetric, and BEAT®, optionally, the Fc fragment comprises modifications knobs-into-holes and/or DDKK.

23. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 22, wherein the second binding moiety is at a C-terminus of the FAP antigen-binding moiety.
24. The bispecific antibody or antigen-binding fragment thereof of any one of claims 19 to 23, wherein the second binding moiety is operably linked to the C-terminus of the Fc fragment.
25. The bispecific antibody or antigen-binding fragment thereof of any one of claims 19 to 24, wherein the second binding moiety links to the Fc fragment by a linker.
26. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 25, wherein the second binding moiety binds and/or activates a second target.
27. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 26, wherein the second binding moiety binds and/or activates a tumor associated cell receptor.
28. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 27, wherein the second binding moiety comprises a first portion and a second portion, wherein each portion comprises one or more units.
29. The bispecific antibody or antigen-binding fragment thereof of claim 28, wherein the one or more units comprise a first unit, a second unit, and/or a third unit.
30. The bispecific antibody or antigen-binding fragment thereof of claim 28 or 29, wherein each of the one or more units are identical.
31. The bispecific antibody or antigen-binding fragment thereof of claim 28 or 29, wherein the one or more units are not identical.
32. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 31, wherein the second binding moiety is a tumor necrosis factor, interleukin, lymphokine, interferon, colony stimulating factor, chemokine or growth factor.
33. The bispecific antibody or antigen-binding fragment thereof of any one of claims 28 to 31, wherein each of the one or more units independently is a tumor necrosis factor, interleukin, lymphokine, interferon, colony stimulating factor, chemokine or growth factor.
34. The bispecific antibody or antigen-binding fragment thereof of any one or claims 29 to 31, wherein each of the one or more units independently comprise an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to any one of the amino acid sequences set forth in **TABLE 10**.

35. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 31, wherein the second binding moiety comprises a second antigen-binding moiety or a cytokine moiety.
36. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 35, wherein the second binding moiety comprises a cytokine moiety.
37. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 36, wherein the cytokine moiety comprises a first cytokine moiety and a second cytokine moiety.
38. The bispecific antibody or antigen-binding fragment thereof of claim 37, wherein the first cytokine moiety comprises a first cytokine unit.
39. The bispecific antibody or antigen-binding fragment thereof of claim 38, wherein the first cytokine unit comprises a LIGHT unit or a lymphotoxin-β unit.
40. The bispecific antibody or antigen-binding fragment thereof of claim 37, wherein the second cytokine moiety comprises a second cytokine unit and a third cytokine unit.
41. The bispecific antibody or antigen-binding fragment thereof of claim 40, wherein the second cytokine unit comprises a LIGHT unit, a lymphotoxin-α unit, or a lymphotoxin-β unit.
42. The bispecific antibody or antigen-binding fragment thereof of claim 40, wherein the third cytokine unit comprises a LIGHT unit, a lymphotoxin-α unit, or a lymphotoxin-β unit.
43. The bispecific antibody or antigen-binding fragment thereof of any one of claims 39 to 42, wherein the first cytokine moiety comprises a first LIGHT unit, the second cytokine unit comprises a second LIGHT unit, and the third cytokine unit comprises a third LIGHT unit.
44. The bispecific antibody or antigen-binding fragment thereof of claim 43, wherein the first, second, and/or third LIGHT unit each independently comprises an amino acid sequence set forth in **SEQ ID NO: 17** or **18**.
45. The bispecific antibody or antigen-binding fragment thereof of any one of claims 39 to 42, wherein the first cytokine moiety comprises a lymphotoxin-β unit, the second cytokine unit comprises a lymphotoxin-α unit, and the third cytokine unit comprises a lymphotoxin-β unit.
46. The bispecific antibody or antigen-binding fragment thereof of claim 45, wherein the lymphotoxin-β unit comprises an amino acid sequence set forth in **SEQ ID NO: 39**, the lymphotoxin-α unit comprises an amino acid sequence set forth in **SEQ ID NO: 40**.
47. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 46, wherein the bispecific antibody or antigen-binding fragment thereof binds to FAP with dissociation constant (K_D) no more than 20 nM, 15 nM, 10 nM or 5 nM.

48. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 47, wherein the bispecific antibody or antigen-binding fragment thereof binds to LTßR with dissociation constant (K_D) no more than 20 nM, 15 nM, 10 nM or 5 nM.

49. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 48, wherein the bispecific antibody or antigen-binding fragment thereof barely binds to human or cynomolgus HVEM.

50. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 49, wherein the second binding moiety in the bispecific antibody or antigen-binding fragment thereof is capable of reduced binding affinity to DcR3.

51. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 50, wherein the bispecific antibody or antigen-binding fragment thereof specifically binds to human FAP, and/or does not bind to DPPIV.

52. The bispecific antibody or antigen-binding fragment thereof of claim 15, wherein the second binding moiety binds and/or activates LTβR, HER2, PDL-1, PD-1, EGFR, VEGFR, VEGF, CCR8, OX-40, 418B, Angiopoietin-2, IL-4Ra, BCMA, Blys, BTNO2, C5, CD122, CD13, CD133, CD137, CD138, CD16a, CD19, CD20, CD22, CD27, CD28, CD3, CD30, CD33, CD38, CD40, CD47, CD-8, CEA, CGPR/CGRPR, CSPGs, CTLA4, CTLA-4, DLL-4, EpCAM, factor IXa, factor X, GITR, GP130, Her3, HSG, ICOS, IGFl, IGFl/2, IGF-lR, IGF2, IGFR, IL-1, IL- 12, IL-12p40, IL-13, IL-l 7A, IL-1~, IL-23, IL-5, IL-6, IL-6R, Lag-3, LAG3, MAG, Met, NgR, NogoA, OMGp, OX40, PDGFR, PSMA, RGMA, RGMB, SARS-CoV-2, Te38, TIM-3, TNF, TNFa, TROP-2, TWEAK, or TRAIL.

53. The bispecific antibody or antigen-binding fragment thereof of claim 52, wherein the second binding moiety is a second antigen-binding moiety.

54. The bispecific antibody or antigen-binding fragment thereof of claim 52, wherein the second antigen-binding moiety comprises anti-LTβR binding moiety, anti-HER2 binding moiety, anti-PDL-1 binding moiety, anti-PD-1 binding moiety, anti-EGFR binding moiety, anti-VEGFR binding moiety, anti-VEGF binding moiety, anti-CCR8 binding moiety, anti-OX-40 binding moiety, anti-418B binding moiety, anti-Angiopoietin-2 binding moiety, anti-IL-4Ra binding moiety, anti-BCMA binding moiety, anti-Blys binding moiety, anti-BTNO2 binding moiety, anti-C5 binding moiety, anti-CD122 binding moiety, anti-CD13 binding moiety, anti-CD133 binding moiety, anti-CD137 binding moiety, anti-CD138 binding moiety, anti-CD16a binding moiety, anti-CD19 binding moiety, anti-CD20 binding moiety, anti-CD22 binding moiety, anti-CD27 binding moiety, anti-CD28 binding moiety, anti-CD3 binding moiety, anti-CD30 binding moiety, anti-

CD33 binding moiety, anti-CD38 binding moiety, anti-CD40 binding moiety, anti-CD47 binding moiety, anti-CD-8 binding moiety, anti-CEA binding moiety, anti-CGPR/CGRPR binding moiety, anti-CSPGs binding moiety, anti-CTLA4 binding moiety, anti-CTLA-4 binding moiety, anti-DLL-4 binding moiety, anti-EpCAM binding moiety, anti-factor IXa binding moiety, anti-factor X binding moiety, anti-GITR binding moiety, anti-GP130 binding moiety, anti-Her3 binding moiety, anti-HSG binding moiety, anti-ICOS binding moiety, anti-IGFl binding moiety, anti-IGFl/2 binding moiety, anti-IGF-lR binding moiety, anti-IGF2 binding moiety, anti-IGFR binding moiety, anti-IL-1 binding moiety, anti-IL-12 binding moiety, anti-IL-12p40 binding moiety, anti-IL-13 binding moiety, anti-IL-l 7A binding moiety, anti-IL-1~ binding moiety, anti-IL-23 binding moiety, anti-IL-5 binding moiety, anti-IL-6 binding moiety, anti-IL-6R binding moiety, anti-Lag-3 binding moiety, anti-LAG3 binding moiety, anti-MAG binding moiety, anti-Met binding moiety, anti-NgR binding moiety, anti-NogoA binding moiety, anti-OMGp binding moiety, anti-OX40 binding moiety, anti-PDGFR binding moiety, anti-PSMA binding moiety, anti-RGMA binding moiety, anti-RGMB binding moiety, anti-SARS-CoV-2 binding moiety, anti-Te38 binding moiety, anti-TIM-3 binding moiety, anti-TNF binding moiety, anti-TNFa binding moiety, anti-TROP-2 binding moiety, anti-TWEAK binding moiety, or anti-TRAIL binding moiety.

55. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 54, wherein the bispecific antibody or antigen-binding fragment thereof exhibits a reduced binding affinity to HVEM relative to a comparator bispecific antibody or antigen-binding fragment thereof.

56. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 55, wherein the bispecific antibody or antigen-binding fragment thereof exhibits a reduced binding affinity to DcR3 relative to a comparator bispecific antibody or antigen-binding fragment thereof.

57. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 56, wherein the bispecific antibody or antigen-binding fragment thereof exhibits a reduced binding affinity to DPPIV relative to a comparator bispecific antibody or antigen-binding fragment thereof.

58. The bispecific antibody or antigen-binding fragment thereof of any one of claims 15 to 57, wherein upon contacting the bispecific antibody or antigen-binding fragment thereof to a FAP expressing cell, and the bispecific antibody or antigen-binding fragment thereof induces: the formation of secondary lymphoid organs (SLOs), the formation of tertiary lymphoid structures (TLSs), the stimulation of immune cells, the apoptosis of tumor cells, the treatment of cancer, or any combination thereof.

59. A fusion protein, wherein the fusion protein is comprised in the bispecific antibody or antigen-binding fragment thereof of any one of claims 15-58.

60. The fusion protein of claim 59, wherein the fusion protein comprises a heavy chain 1 (HC1) region comprising a VH, a heavy chain constant domain 1 (CH1), and a Fc fragment comprising a heavy chain constant domain 2 (CH2) and a heavy chain constant domain 3 (CH3).
61. The fusion protein of claim 60, wherein the HC1 region comprises an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in **SEQ ID NO: 1, 4, 5, 8, 10, 13, 14, and 15**.
62. The fusion protein of claim 60 or 61, wherein the HC1 region comprises an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in **SEQ ID NO: 5, 8, 10, 13, 14, and 15**.
63. The fusion protein of claim 60, wherein the Fc fragment of the HC1 region comprises one or more units of the second binding moiety fused to a C-terminus of the Fc fragment.
64. The fusion protein of claim 63, wherein the Fc fragment of the HC1 region comprises a first unit of the second binding moiety fused to the C-terminus of the HC1 Fc fragment.
65. The fusion protein of claim 64, wherein the first unit of the second binding moiety is fused to the Fc unit by a first linker.
66. The fusion protein of any one of claims 60 to 65, wherein the second binding moiety is a cytokine moiety, and wherein the cytokine moiety comprises a first cytokine unit.
67. The fusion protein of claim 46, wherein the first cytokine unit comprises a LIGHT unit or a lymphotoxin β unit.
68. The fusion protein of any one of claims 63 to 66, wherein the first unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to any one of the amino acid sequences set forth in **TABLE 10**.
69. The fusion protein of any one of claims 63 to 68, wherein the unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to SEQ ID NO: 17 or SEQ ID NO: 39.
70. The fusion protein of claim 59, wherein the fusion protein comprises a heavy chain 2 (HC2) region comprising a VH, a CH1, and a Fc fragment comprising a CH1 and a CH3.
71. The fusion protein of claim 70, wherein the HC2 region comprises an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence

identity or is identical to any one of the amino acid sequence as shown in **SEQ ID NO: 1, 4, 6, 9, 11, 12, 13, and 16**.

72. The fusion protein of claim 70 or 71, wherein the HC2 region comprises an amino acid sequence that comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity or is identical to any one of the amino acid sequence as shown in **SEQ ID NO: 6, 9, 11, 12, 13, and 16**.
73. The fusion protein of claim 70, wherein the Fc fragment of the HC2 region comprises one or more units of the second binding moiety fused to a C-terminus of the HC2 Fc fragment.
74. The fusion protein of claim 73, wherein the Fc fragment of the HC2 region comprises a second unit and a third unit of the second binding moiety.
75. The fusion protein of claim 74, wherein the second unit of the second binding moiety is fused to the Fc fragment of the HC2 region.
76. The fusion protein of claim 75, wherein the third unit of the second binding moiety is fused to the second unit of the second binding moiety.
77. The fusion protein of claim 76, wherein the second unit of the second binding moiety is fused to the Fc fragment of the HC2 region by a second linker and the third unit is fused to the second unit of the second binding moiety by a third linker.
78. The fusion protein of claim 77, wherein the second unit and third unit of the second binding moiety unit are tandem linked.
79. The fusion protein of claim 73-78, wherein the second binding moiety is a cytokine moiety, and wherein the cytokine moiety comprises a second cytokine unit and a third cytokine unit.
80. The fusion protein of claim 79, wherein the second cytokine unit comprises a LIGHT unit, a lymphotoxin α unit, or a lymphotoxin β unit.
81. The fusion protein of claim 79 or 80, wherein the third cytokine unit comprises a LIGHT unit, a lymphotoxin α unit, or a lymphotoxin β unit.
82. The fusion protein of any one of claims 79 to 81, wherein the second cytokine unit comprises a LIGHT unit, and the third cytokine unit comprises a LIGHT unit.
83. The fusion protein of any one of claims 79 to 81, wherein the second cytokine unit comprises a lymphotoxin α unit, and the third cytokine unit comprises a lymphotoxin β unit.
84. The fusion protein of any one of claims 74 to 83, wherein the second unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to any one of the amino acid sequences set forth in **TABLE 10**.

85. The fusion protein of claim 84, wherein the second unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to **SEQ ID NO: 17** or **SEQ ID NO: 40**.
86. The fusion protein of any one of claims 74 to 85, wherein the third unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to any one of the amino acid sequences set forth in **TABLE 10.**
87. The fusion protein of claim 86, wherein the third unit comprises an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or is identical to **SEQ ID NO: 17** or **SEQ ID NO: 39**.
88. The fusion protein of claim 65 or 77, wherein the fusion protein comprises one or more linkers.
89. The fusion protein of claim 88, wherein each of the one or more linker independently comprises a VH-CHl linker ASTKGPSVFPLAPS; VL-CL linker RTVAAPSVFIFPPS (SEQ ID NO: 91); CH2-CH3 linker ISKAKGQPREPQ (SEQ ID NO: 92); IgM tail linker KSTGKPTLYNVSLVMSDTAGTCY (SEQ ID NO: 93); GGGGSGGGGSGGGGSGGGGT (SEQ ID NO: 94); G; or $(GGGGS)_n$ (SEQ ID NO: 95), wherein n=l, 2, 3, 4, 5, 6, 7, 8, 9, or 10.
90. The fusion protein of any one of claims 63 to 69, wherein the HC1 region comprises one or more heterodimerization modification.
91. The fusion protein of claim 90, wherein the one or more heterodimerization modification is a knob modification or a hole modification.
92. The fusion protein of claim 90 or 91, wherein the one or more heterodimerization modification is a hole modification.
93. The fusion protein of any one of claims 70 to 87, wherein the HC2 region comprises one or more heterodimerization modification.
94. The fusion protein of claim 93, wherein the one or more heterodimerization modification is a knob modification or a hole modification.
95. The fusion protein of claim 93 or 94, wherein the one or more heterodimerization modification is a knob modification.
96. An isolated polynucleotide comprising one or more nucleotide sequences encoding:

a) any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment thereof of any one of claims 1-14,

b) any one of the bispecific antibodies of any one of claims 15-58, or

c) any one of the fusion proteins of any one of claims 59 to 95.

97. A construct comprising the polynucleotide of claim 96.

98. An antibody expressing system, comprising the construct of claim 97 or vector comprising the polynucleotide of claim 96.

99. The antibody expressing system of claim 98, wherein the antibody expressing system is a cell expression system.

100. A method for producing the anti-FAP antibody or FAP antigen-binding fragment thereof, bispecific antibody or antigen-binding fragment thereof or fusion protein, the method comprising: under conditions suitable for expressing the the anti-FAP antibody or FAP antigen-binding fragment thereof, bispecific antibody or antigen-binding fragment thereof or fusion protein using the antibody expressing system of claim 98 or 99.

101. A pharmaceutical composition, wherein the pharmaceutical composition comprises:

a) any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment thereof of any one of claims 1-14,

b) any one of the bispecific antibodies of any one of claims 15-58, or

c) any one of the fusion proteins of any one of claims 59 to 95; and

a pharmaceutically acceptable carrier.

102. A kit, wherein the kit comprises:

a) any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment thereof of any one of claims 1-14,

b) any one of the bispecific antibodies of any one of claims 15-58, or

c) any one of the fusion proteins of any one of claims 59 to 95,

d) the isolated polynucleotide of claim 96, or

e) the construct of claim 97.

103. Use of the anti-FAP antibody or FAP antigen-binding fragment thereof, bispecific antibody or antigen-binding fragment thereof, or fusion protein in the manufacture of a therapeutic agent for preventing, diagnosing, or treating a disease, disorder, or condition, wherein:

a) the anti-FAP antibody or FAP antigen-binding fragment thereof is any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment thereof of any one of claims 1-14,

b) the bispecific antibody or antigen-binding fragment thereof is any one of the bispecific antibodies of any one of claims 15-58, or

c) the fusion protein is any one of the fusion proteins of any one of claims 59 to 95.

104. The use of claim 103, wherein the disease, disorder, or condition comprises tumor diseases.

105. The use of claim 103 or 104, wherein at least a tumor cell expresses FAP.

106. The use of any one of claims 104-105, wherein the tumor diseases are solid tumors.

107. The use of any one of claims 104-106, wherein the tumor diseases comprises gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, skin cancer, bone cancer, multiple myeloma, glioma, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer and lymphoma.

108. A method for treating a subject in need, comprising administrating to the subject a therapeutically effective amount of:

a) any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment thereof of any one of claims 1 to 14,

b) any one of the bispecific antibodies of any one of claims 15 to 58,

c) any one of the fusion proteins of any one of claims 59 to 95, or

d) the pharmaceutical composition of claim 101.

109. A method for decreasing the rate of tumor growth or the number of tumor cells, comprising contacting a tumor cell with an effective amount of:

a) any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment thereof of any one of claims 1 to 14,

b) any one of the bispecific antibodies of any one of claims 15 to 58,

c) any one of the fusion proteins of any one of claims 59 to 95, or

d) the pharmaceutical composition of claim 101.

110. A method of killing a tumor cell, comprising contacting a tumor cell with an effective amount of:

a) any one of the isolated anti-FAP antibodies or FAP antigen-binding fragment thereof of any one of claims 1 to 14,

b) any one of the bispecific antibodies of any one of claims 15 to 58,

c) any one of the fusion proteins of any one of claims 59 to 95, or

d) the pharmaceutical composition of claim 101.

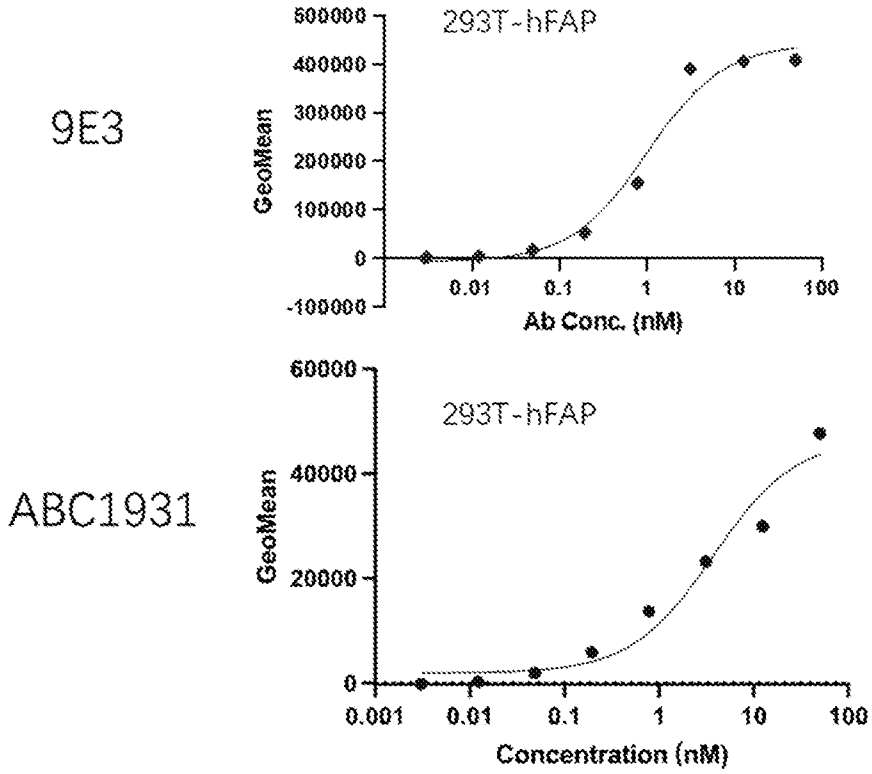


Figure 1A

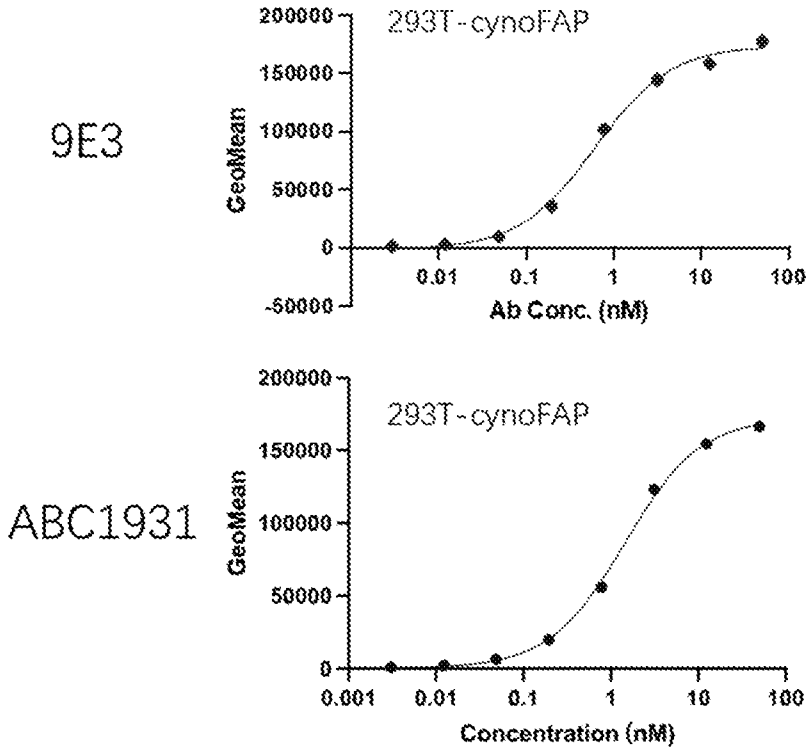


Figure 1B

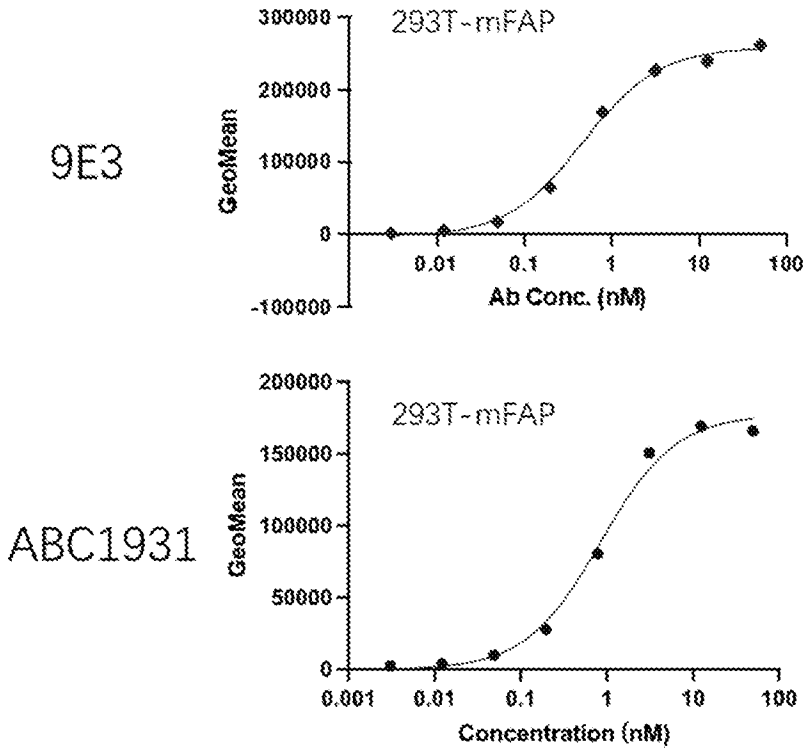


Figure 1C

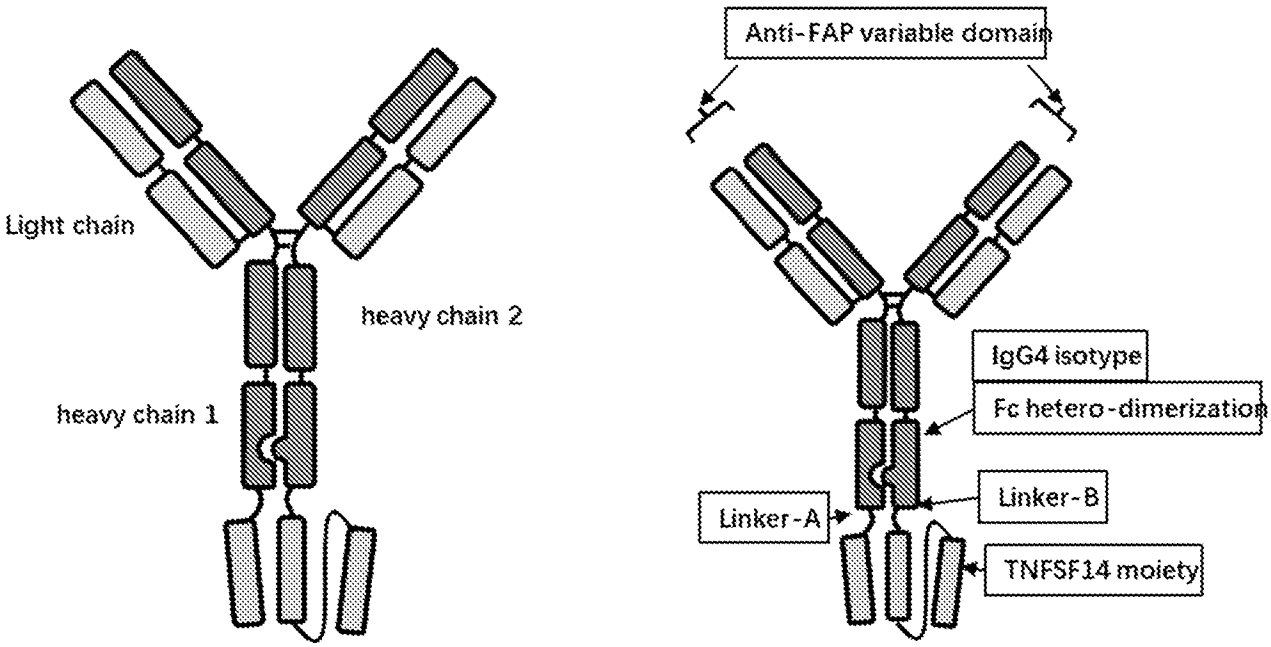


Figure 2

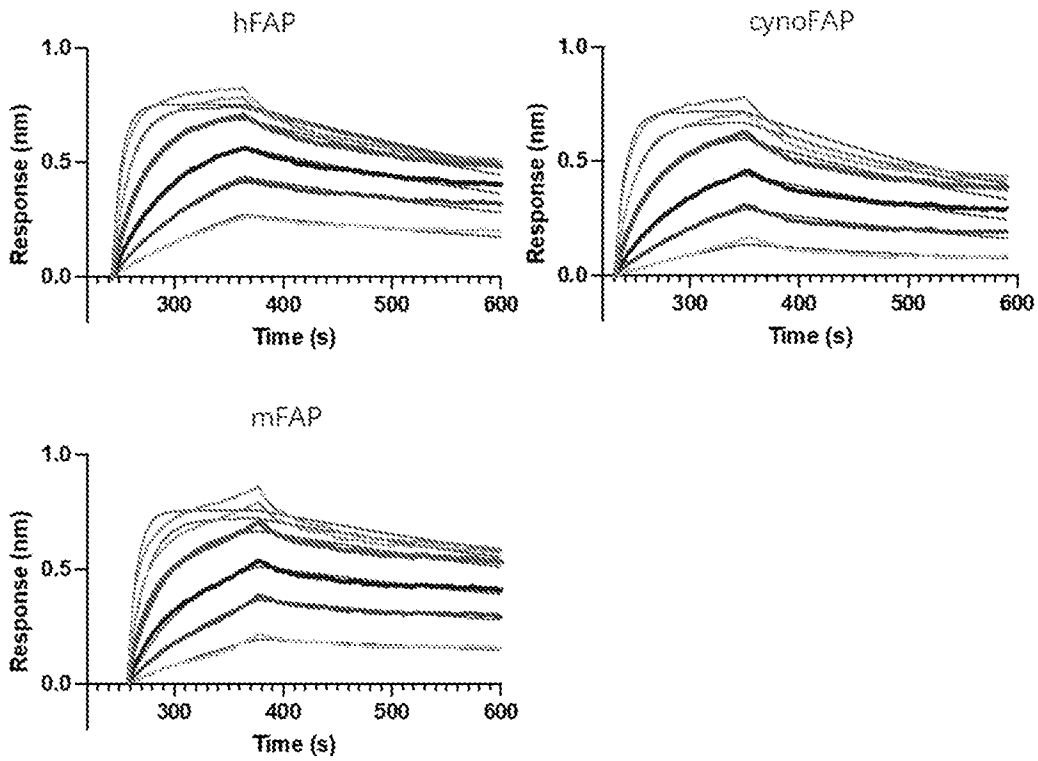


Figure 3A

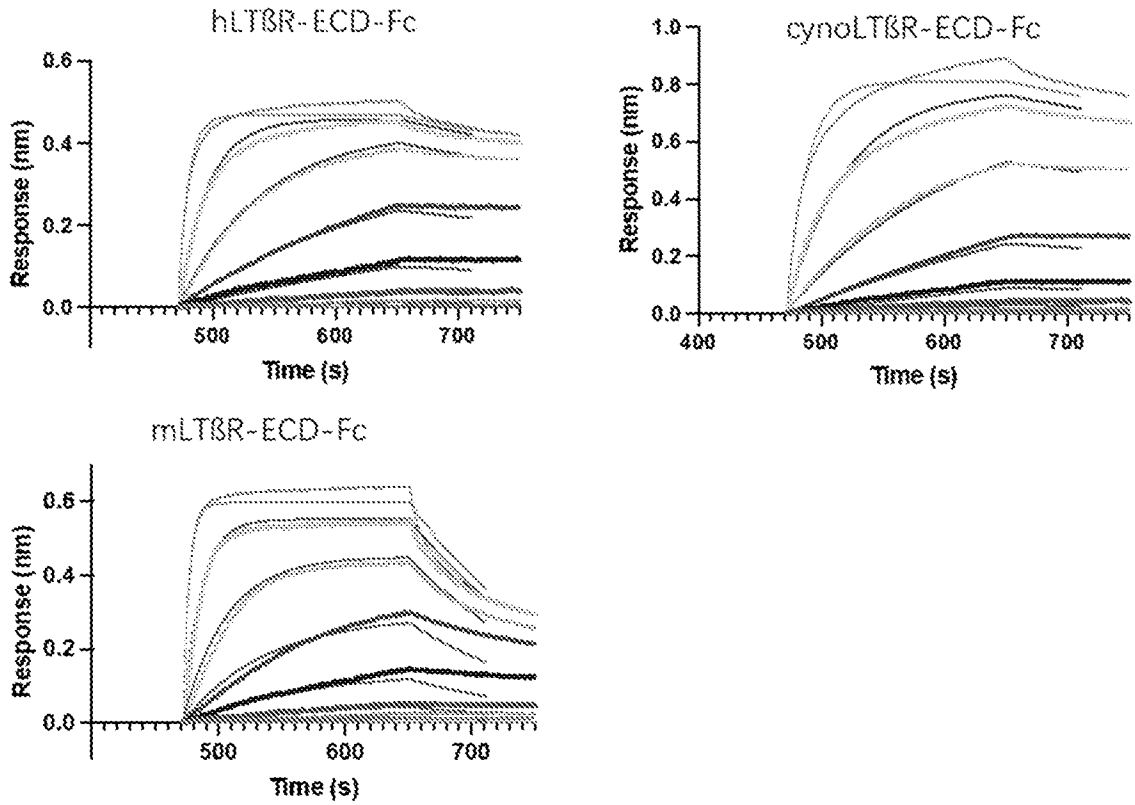


Figure 3B

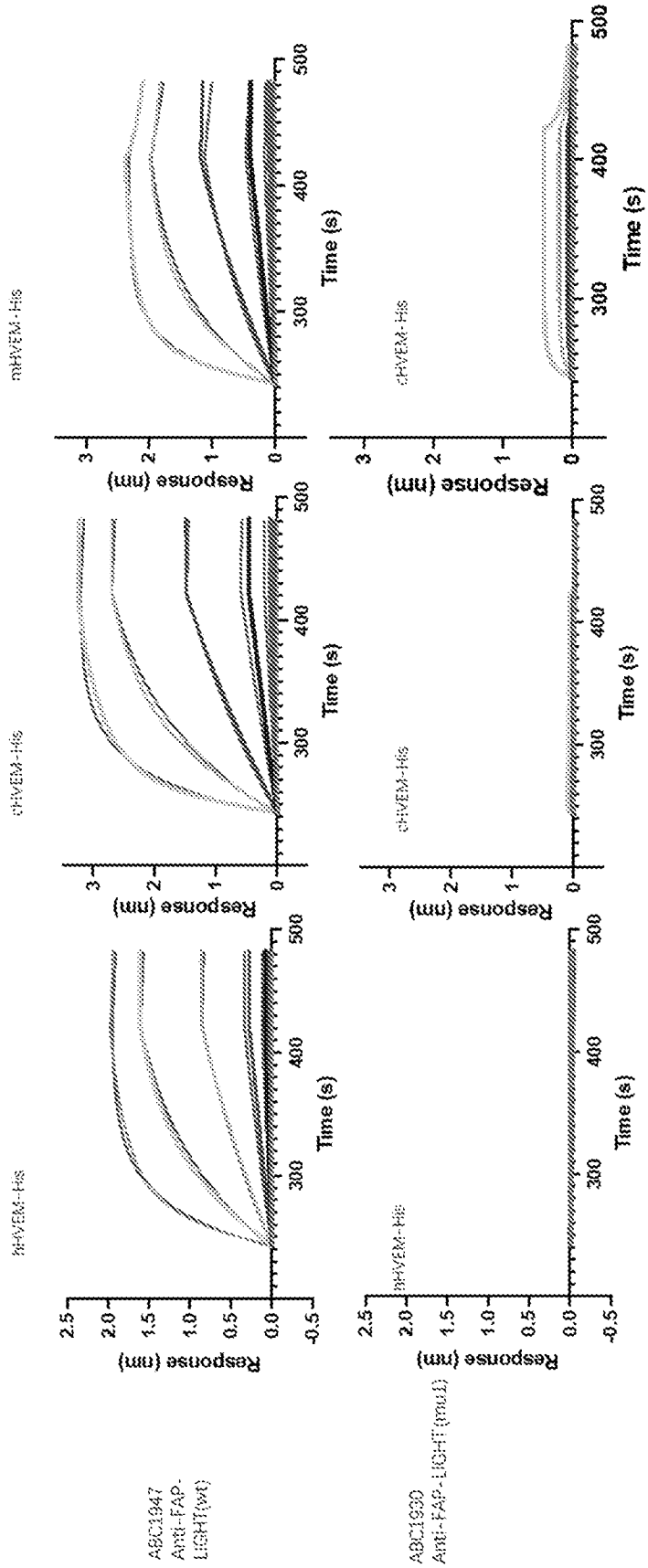
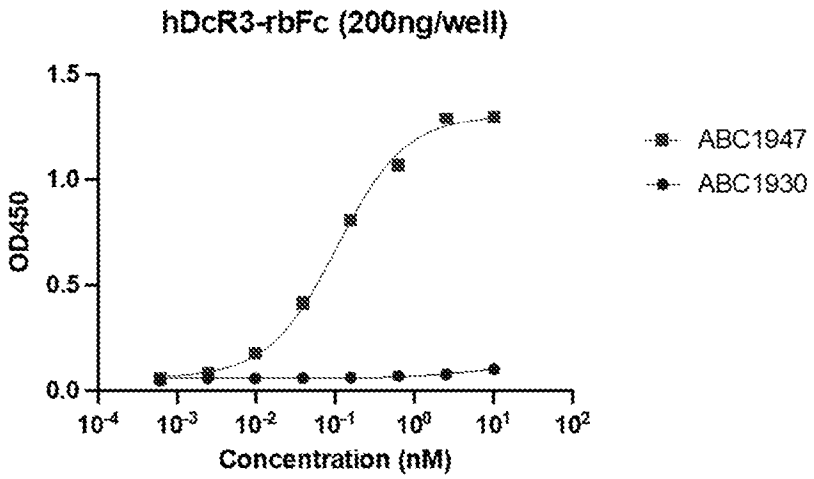
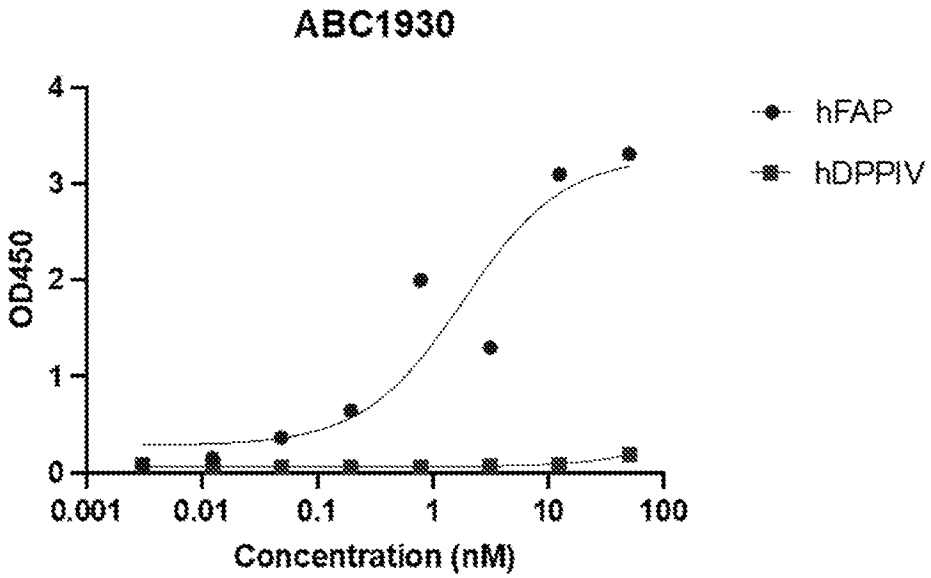

ABC1947
Anti-FAP-LIGHT(wt)
ABC1930
Anti-FAP-LIGHT(mut)
mHVEM-His
cHVEM-His
Response (nm)
Time (s)

Figure 3C



	ABC1930	ABC1947
EC50	3.973	0.1056

Figure 4



	hFAP	hDPP4
EC50	1.831	~ 7.285e+015

Figure 5

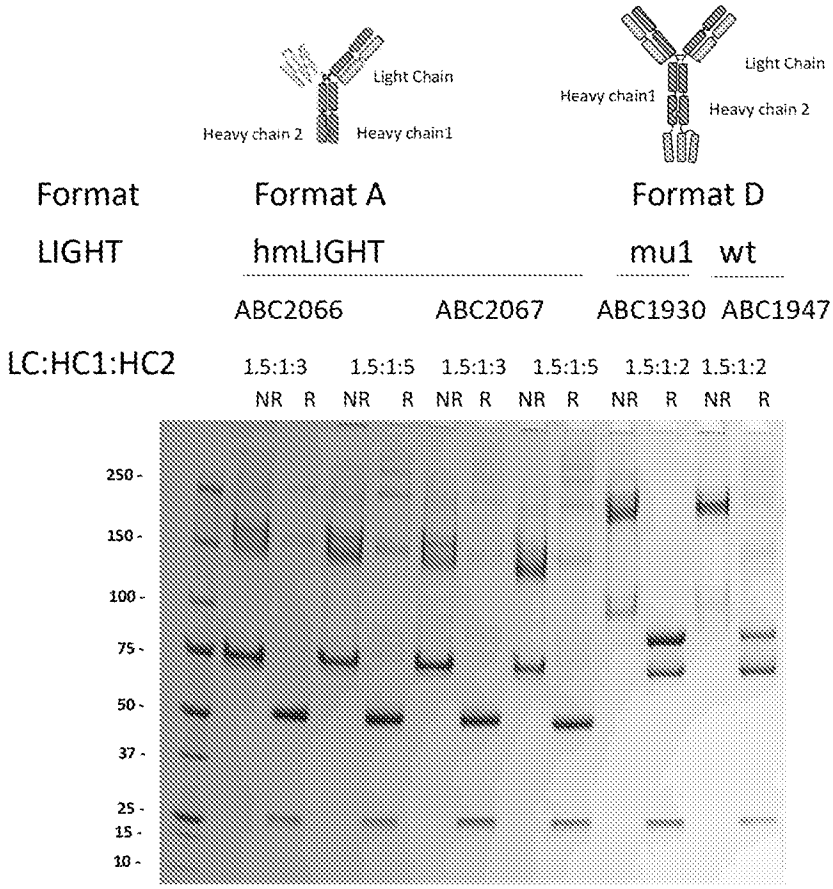


Figure 6

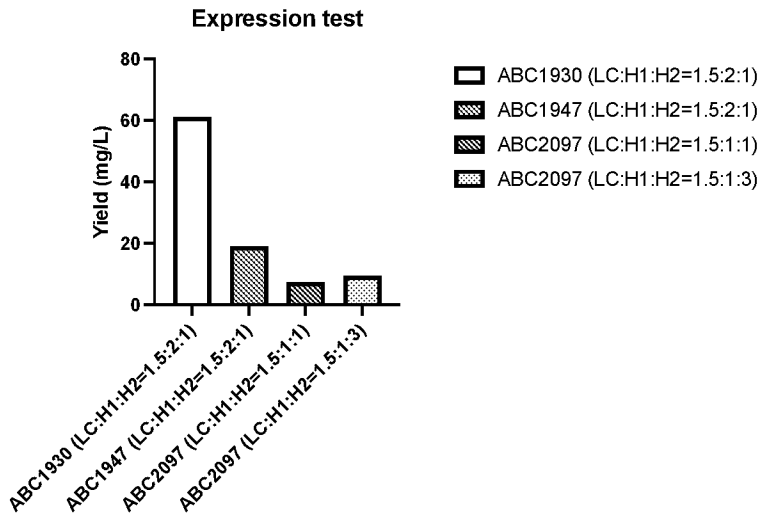


Figure 7A

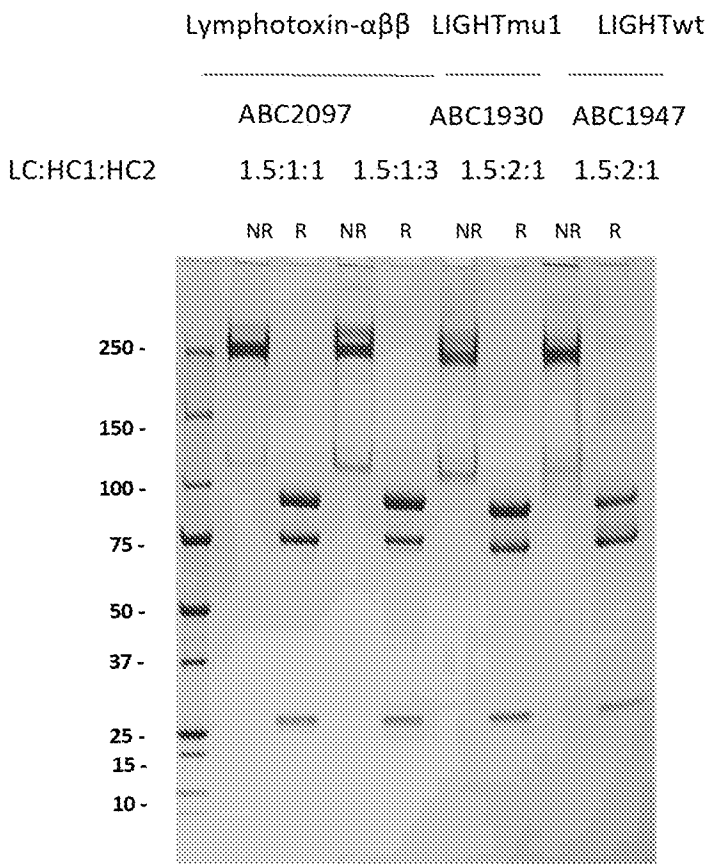


Figure 7B

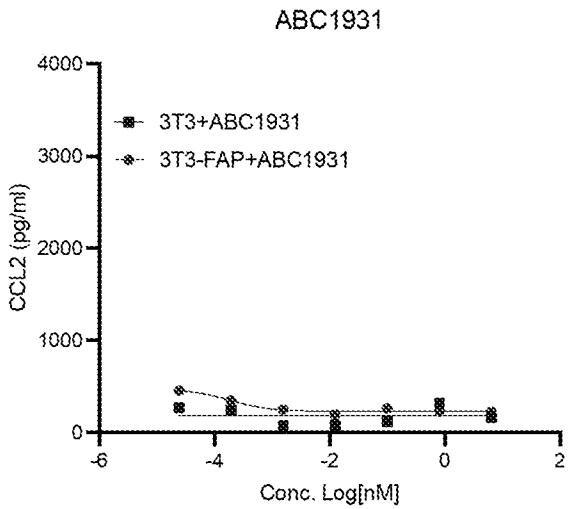
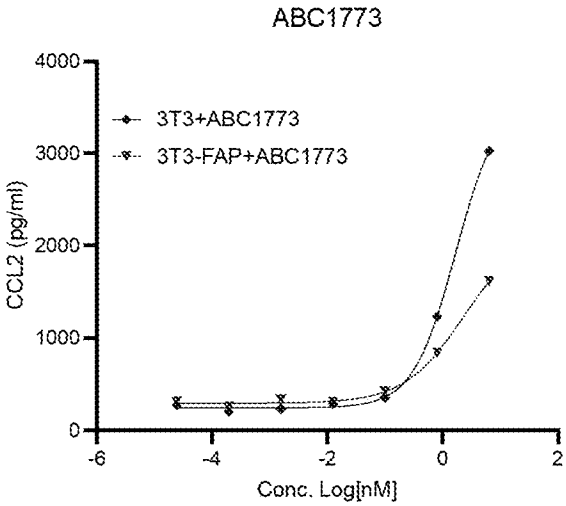
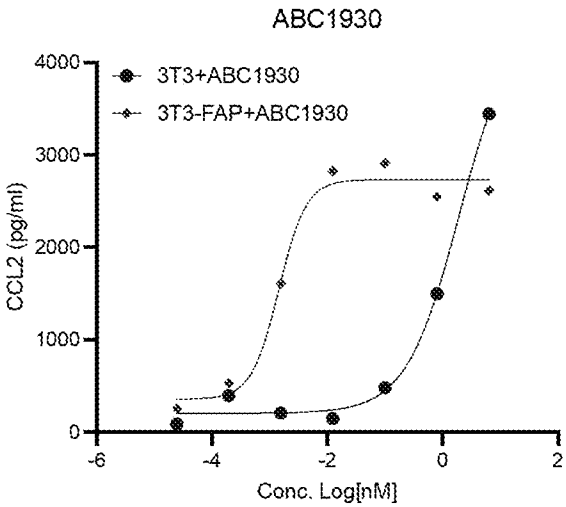


Figure 8A



	3T3+ABC1773	3T3-FAP+ABC1773
EC50	1.680	2.319

Figure 8B



	3T3+ABC1930	3T3-FAP+ABC1930
EC50	1.928	0.001444

Figure 8C

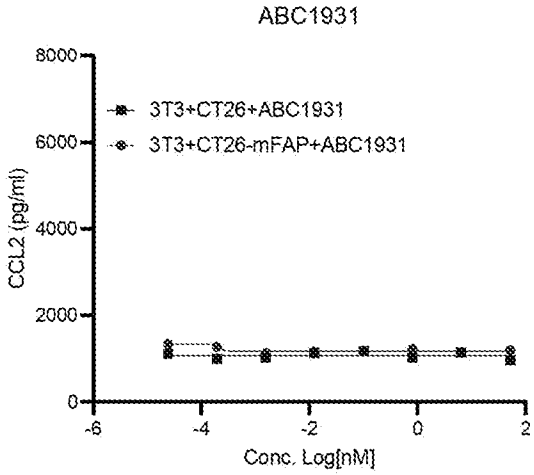
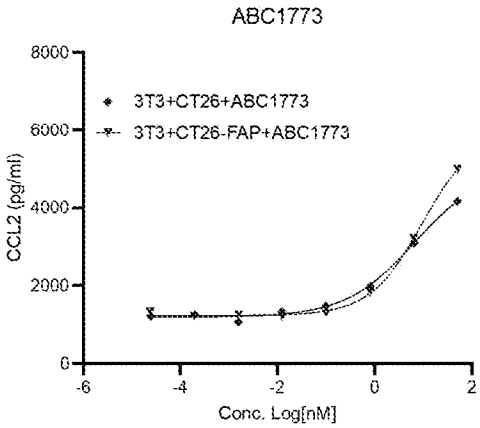
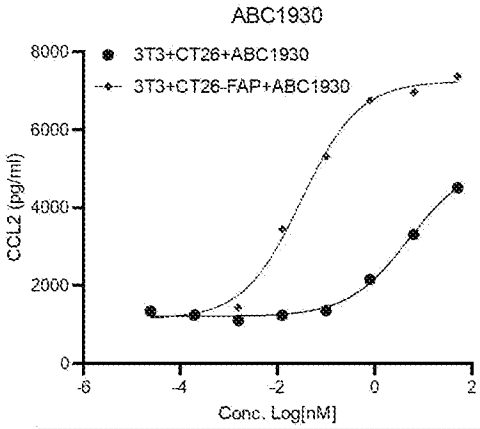


Figure 9A



	3T3+CT26+ABC1773	3T3+CT26-FAP+ABC1773
EC50	6.694	9.592

Figure 9B



	3T3+CT26+ABC1930	3T3+CT26-FAP+ABC1930
EC50	4.973	0.03098

Figure 9C

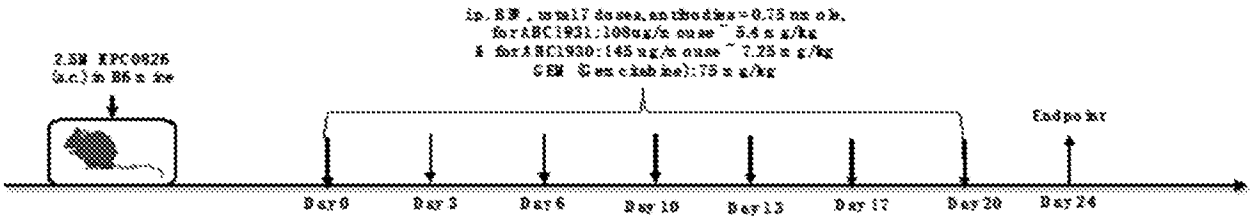
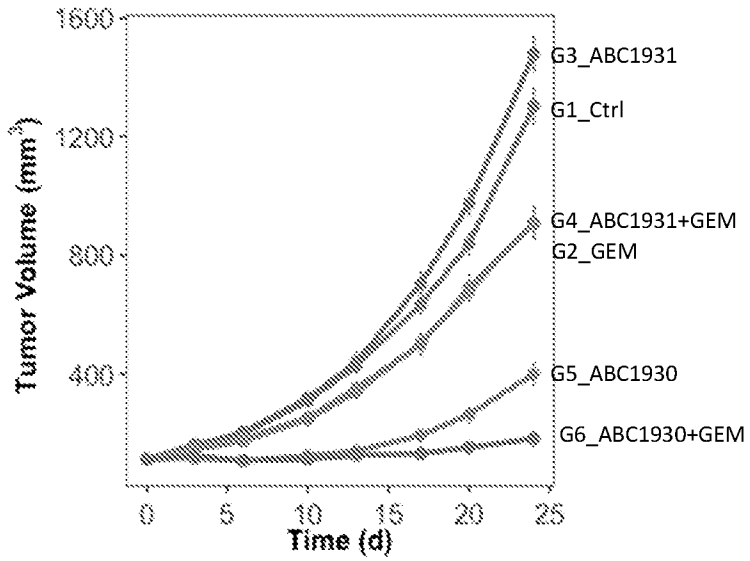


Figure 10A



Figures 10B

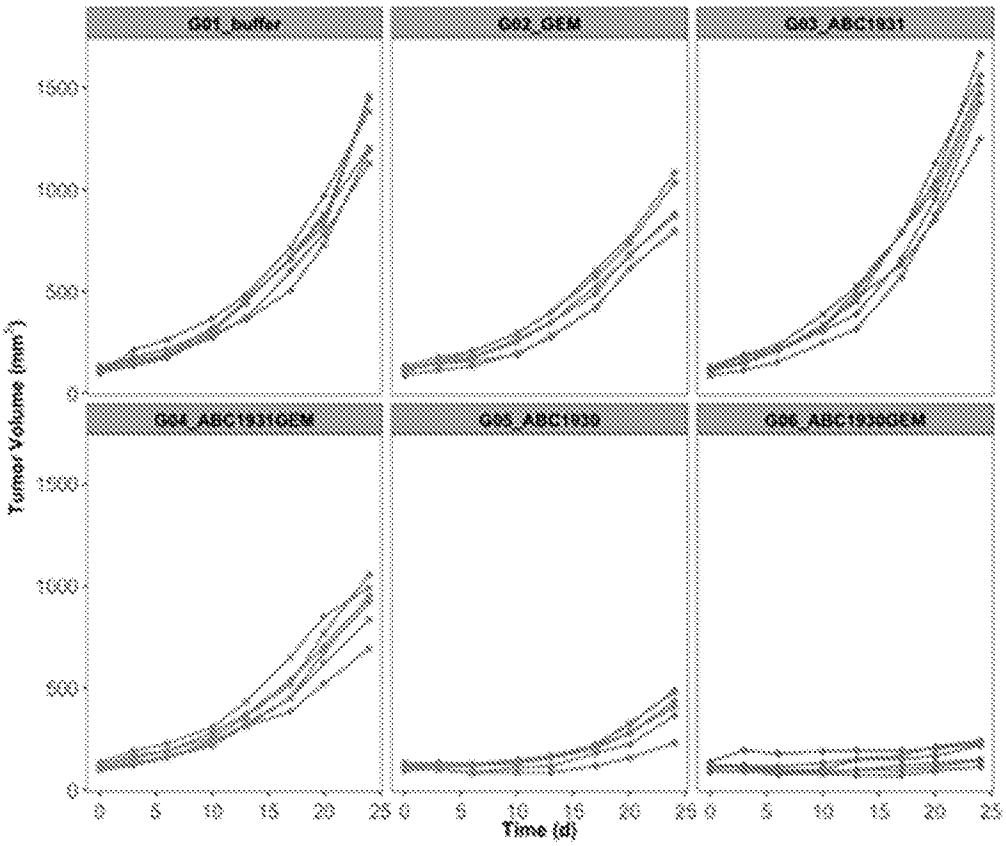


Figure 10C

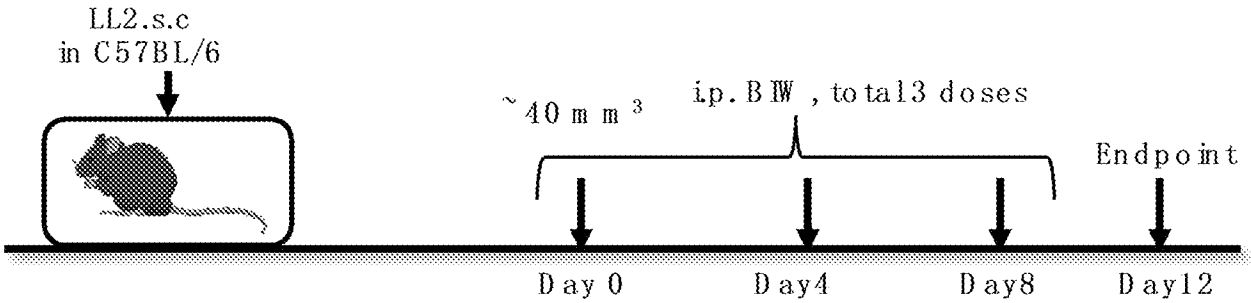


Figure 11A

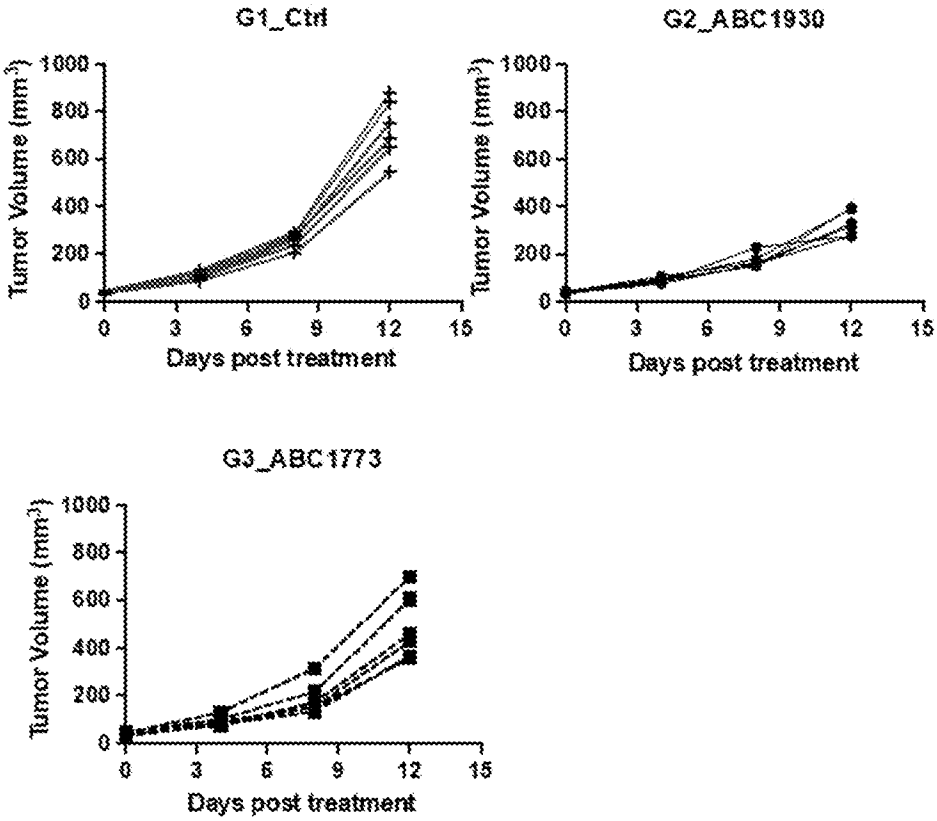


Figure 11B

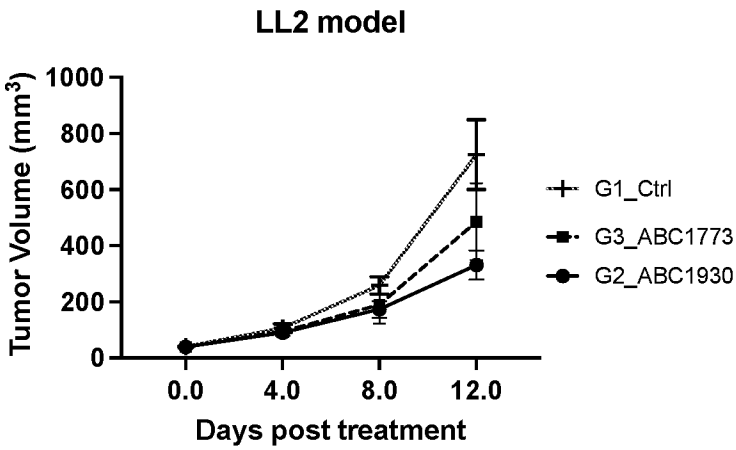
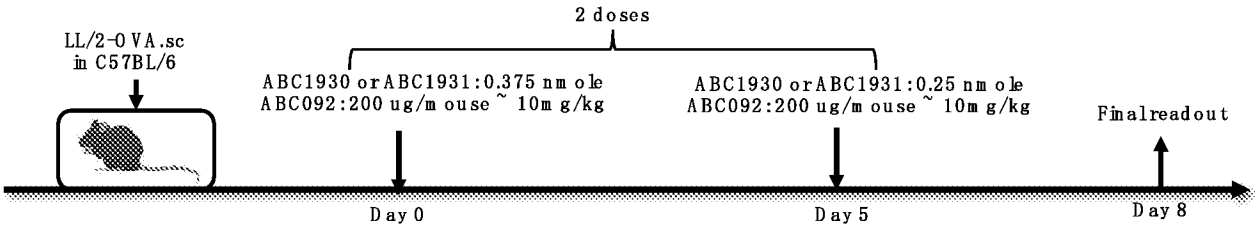


Figure 11C



Treatment		nmole/mouse	mg/kg
ABC092	anti-PD1 (RMP1-14) mIgG2A-LALAPG		10
ABC1930	anti-FAP-LIGHTmu1	1st dose: 0.375	3.625
		2nd dose: 0.25	2.417

Figure 12A

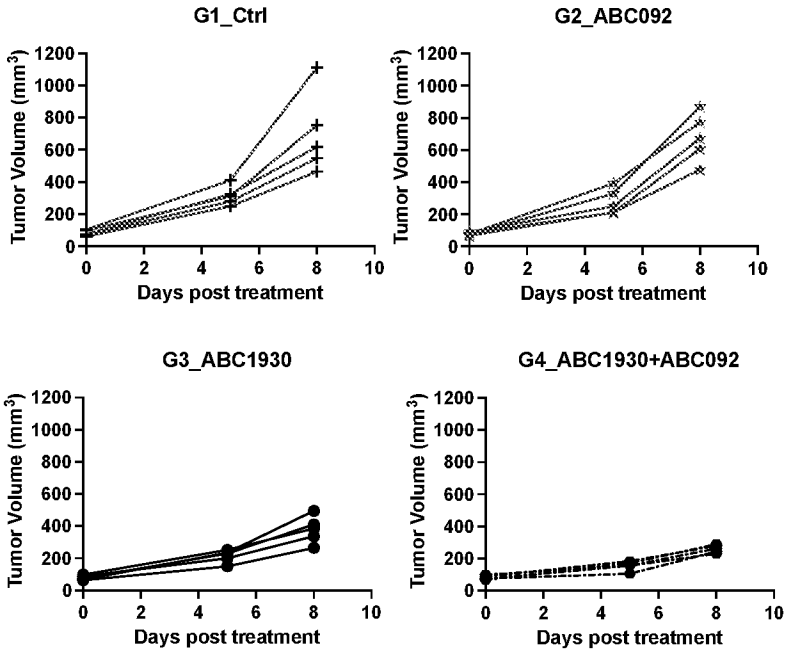


Figure 12B

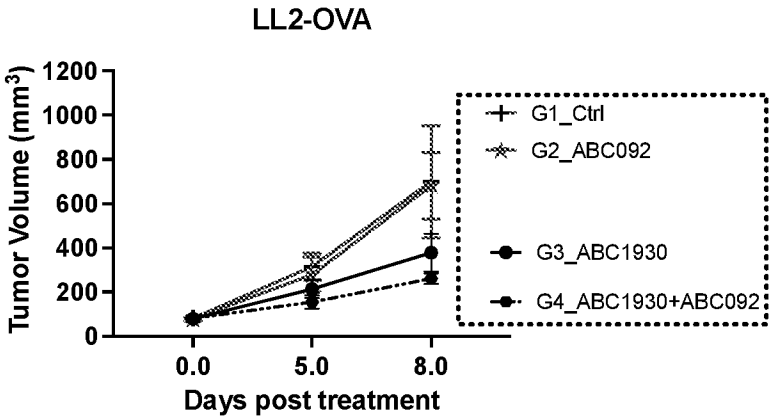
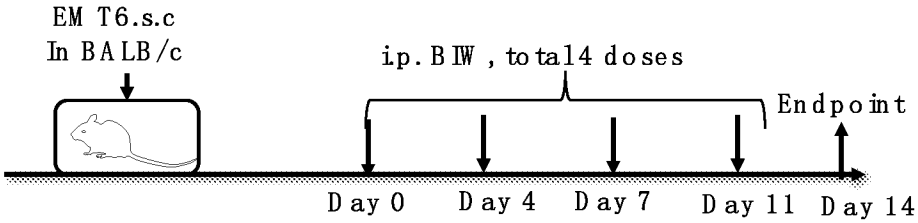


Figure 12C

A.



Group	Treatment	nmole /mouse	mg/kg
G1_Ctrl	Vehicle		
G2_ABC1930	anti-FAP-LIGHTmu1	0.75	7.25
G3_ABC727	anti-PDL1		10
G4_ABC1930 + ABC727	anti-FAP-LIGHTmu1	0.75	7.25
	anti-PDL1		10

Figure 13A

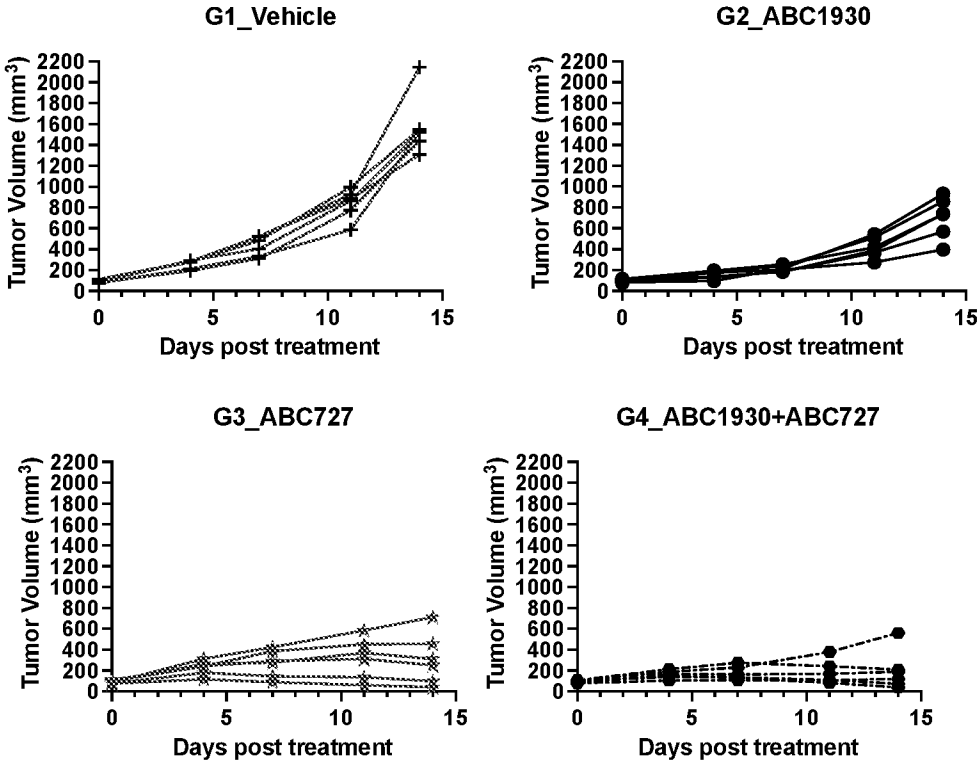


Figure 13B

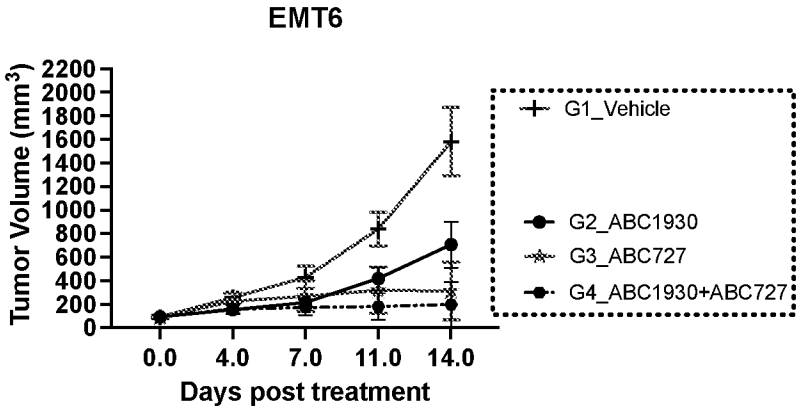

EMT6
Tumor Volume (mm3)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0.0
4.0
7.0
11.0
14.0
Days post treatment
G1_Vehicle
G2_ABC1930
G3_ABC727
G4_ABC1930+ABC727

Figure 13C

INTERNATIONAL SEARCH REPORT

International application No
PCT/CN2024/135694

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61P35/00 C07K14/52 C07K16/40
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61P C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2022/389098 A1 (ZACK DEBRA [US] ET AL) 8 December 2022 (2022-12-08) sequences 11,12 -----	1-14, 96-103, 108
X	WO 2023/224429 A1 (KANAPH THERAPEUTICS INC [KR]) 23 November 2023 (2023-11-23) claim 28; examples 1.1-1.4,1,2 the whole document -----	1-110
X	WO 2022/262496 A1 (SUZHOU FUSE BIOSCIENCES LTD [CN]) 22 December 2022 (2022-12-22) examples 1-3,6.3.2, 6; tables 13,14 -----	1-110

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 March 2025	18/03/2025

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Siaterli, Maria

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2024/135694

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/CN2024/135694

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/218244 A1 (EMRICH THOMAS [DE] ET AL) 6 August 2015 (2015-08-06) paragraph [0152] - paragraph [0157]; tables 1,2 the whole document -----	1-110
X	SINA FELLERMEIER ET AL: "Advancing targeted co-stimulation with antibody-fusion proteins by introducing TNF superfamily members in a single-chain format", ONCOIMMUNOLOGY, vol. 5, no. 11, 27 September 2016 (2016-09-27), page e1238540, XP055556219, DOI: 10.1080/2162402X.2016.1238540 the whole document figure 1 -----	1-110
X	WALDHAUER INJA ET AL: "Simlukafusp alfa (FAP-IL2v) immunocytokine is a versatile combination partner for cancer immunotherapy", MABS, vol. 13, no. 1, 1 January 2021 (2021-01-01), page 1913791, XP055839709, US ISSN: 1942-0862, DOI: 10.1080/19420862.2021.1913791 the whole document -----	1-110

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/CN2024/135694

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2022389098 A1	08-12-2022	CA 3026880 A1	14-12-2017
		DK 3468997 T5	09-09-2024
		EP 3468997 A1	17-04-2019
		EP 4371570 A2	22-05-2024
		ES 2963807 T3	02-04-2024
		FI 3468997 T3	31-10-2023
		HU E064454 T2	28-03-2024
		JP 7148504 B2	05-10-2022
		JP 2019525949 A	12-09-2019
		JP 2022177224 A	30-11-2022
		JP 2023144078 A	06-10-2023
		PL 3468997 T3	16-12-2024
		PT 3468997 T	07-12-2023
		US 2017355766 A1	14-12-2017
		US 2022389098 A1	08-12-2022
		WO 2017214452 A1	14-12-2017
WO 2023224429 A1	23-11-2023	AU 2023273527 A1	12-12-2024
		KR 20230162310 A	28-11-2023
		WO 2023224429 A1	23-11-2023
WO 2022262496 A1	22-12-2022	AU 2022292592 A1	25-01-2024
		CA 3224183 A1	22-12-2022
		CN 118043079 A	14-05-2024
		EP 4355372 A1	24-04-2024
		IL 309286 A	01-02-2024
		JP 2024526128 A	17-07-2024
		KR 20240075802 A	29-05-2024
		TW 202317201 A	01-05-2023
		US 2024358849 A1	31-10-2024
		WO 2022262496 A1	22-12-2022
US 2015218244 A1	06-08-2015	AR 099288 A1	13-07-2016
		BR 112016016658 A2	23-01-2018
		CA 2935665 A1	13-08-2015
		CN 106061997 A	26-10-2016
		EP 3102594 A1	14-12-2016
		JP 2017506075 A	02-03-2017
		JP 2020089371 A	11-06-2020
		JP 2022095643 A	28-06-2022
		KR 20160117463 A	10-10-2016
		RU 2016135788 A	07-03-2018
		TW 201613954 A	16-04-2016
		US 2015218244 A1	06-08-2015
		WO 2015117930 A1	13-08-2015