

[0010a] In one aspect, the present disclosure provides a protein comprising: (a) a first antigen-binding site that binds NKG2D; (b) a second antigen-binding site that binds fibroblast activation protein (FAP); and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16, wherein each of the antigen-binding sites comprises the part of an immunoglobulin molecule that participates in antigen binding.

~~[0010b] In another aspect, the present disclosure refers to a protein as described herein, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to an amino acid sequence selected from: SEQ ID NO:1, SEQ ID NO:41, SEQ ID NO:49, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:85, SEQ ID NO:167, SEQ ID NO:171, SEQ ID NO:175, SEQ ID NO:179, SEQ ID NO:183, SEQ ID NO:187, and SEQ ID NO:93.~~

~~[0010c0010b]~~ In ~~yet~~ another aspect, the present disclosure refers to a formulation comprising a protein as described herein and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure refers to a cell comprising one or more nucleic acids encoding a protein as described herein.

~~[0010d0010c]~~ In yet another aspect, the present disclosure refers to the use of a protein as described herein or a formulation as described herein in the manufacture of a medicament. In yet another aspect, the present disclosure refers to the use of a protein as described herein or a formulation as described herein in the manufacture of a medicament for enhancing tumor cell death, comprising exposing tumor cells and natural killer cells to an effective amount of the protein or formulation, wherein the tumor cell expresses FAP. In yet another aspect, the present disclosure refers to the use of a protein as described herein or the formulation as described herein in the manufacture of a medicament for treating a FAP-expressing cancer. In yet another aspect, the present disclosure refers to the use of a protein as described herein or a formulation as described herein in the manufacture of a medicament for treating an autoimmune disease. In yet another aspect, the present disclosure refers to the use of a protein as described herein or a formulation as described herein in the manufacture of a medicament for treating fibrosis.

[0011] Accordingly, in certain examples, the disclosure provides a protein that incorporates a first antigen-binding site that binds NKG2D; a second antigen-binding site that binds FAP; and an antibody fragment crystallizable (Fc) domain, a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.

[0012] The antigen-binding sites may each incorporate an antibody heavy chain variable domain and an antibody light chain variable domain (*e.g.*, arranged as in an antibody, or fused together to form an scFv), or one or more of the antigen-binding sites may be a single

WHAT IS CLAIMED IS:

1. A protein comprising:

- (a) a first antigen-binding site that binds NKG2D;
- (b) a second antigen-binding site that binds fibroblast activation protein (FAP); and
- (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.

wherein each of the antigen-binding sites comprises the part of an immunoglobulin molecule that participates in antigen binding.

2. The protein according to claim 1, wherein the first antigen-binding site binds to NKG2D in humans.

3. The protein according to claim 1 or 2, wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain present on the same polypeptide.

4. The protein according to claims 3, wherein the second antigen-binding site also comprises a heavy chain variable domain and a light chain variable domain present on the same polypeptide.

~~5. The protein according to claim 4, wherein the light chain variable domain of the first antigen-binding site has an amino acid sequence identical to the amino acid sequence of the light chain variable domain of the second antigen-binding site.~~ The protein according to any one of claims 1 to 4, wherein the first antigen-binding site comprises:

(a) a heavy chain CDR1 amino acid sequence of SEQ ID NO:163;

a heavy chain CDR2 amino acid sequence of SEQ ID NO:88;

a heavy chain CDR3 amino acid sequence of SEQ ID NO:164;

a light chain CDR1 amino acid sequence of SEQ ID NO:90;

a light chain CDR2 amino acid sequence of SEQ ID NO:91; and

a light chain CDR3 amino acid sequence of SEQ ID NO:92;

(b) a heavy chain CDR1 amino acid sequence of SEQ ID NO:151;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:106; and
a heavy chain CDR3 amino acid sequence of SEQ ID NO:152; and
a light chain variable domain sequence of SEQ ID NO: 2.

(c) a heavy chain CDR1 amino acid sequence of SEQ ID NO:153;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:44;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:154;
a light chain CDR1 amino acid sequence of SEQ ID NO:46;
a light chain CDR2 amino acid sequence of SEQ ID NO:47; and
a light chain CDR3 amino acid sequence of SEQ ID NO:48;

(d) a heavy chain CDR1 amino acid sequence of SEQ ID NO:155;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:52;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:156;
a light chain CDR1 amino acid sequence of SEQ ID NO:54;
a light chain CDR2 amino acid sequence of SEQ ID NO:55; and
a light chain CDR3 amino acid sequence of SEQ ID NO:56;

(e) a heavy chain CDR1 amino acid sequence of SEQ ID NO:108;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:109;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:110;
a light chain CDR1 amino acid sequence of SEQ ID NO:111;
a light chain CDR2 amino acid sequence of SEQ ID NO:112; and
a light chain CDR3 amino acid sequence of SEQ ID NO:113;

(f) a heavy chain CDR1 amino acid sequence of SEQ ID NO:157;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:64;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:158;
a light chain CDR1 amino acid sequence of SEQ ID NO:66;
a light chain CDR2 amino acid sequence of SEQ ID NO:67; and
a light chain CDR3 amino acid sequence of SEQ ID NO:68;

(g) a heavy chain CDR1 amino acid sequence of SEQ ID NO:159;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:72;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:160;

a light chain CDR1 amino acid sequence of SEQ ID NO:74;
a light chain CDR2 amino acid sequence of SEQ ID NO:75; and
a light chain CDR3 amino acid sequence of SEQ ID NO:76;

(h) a heavy chain CDR1 amino acid sequence of SEQ ID NO:161;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:80;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:162;
a light chain CDR1 amino acid sequence of SEQ ID NO:82;
a light chain CDR2 amino acid sequence of SEQ ID NO:83; and
a light chain CDR3 amino acid sequence of SEQ ID NO:84;

(i) a heavy chain CDR1 amino acid sequence of SEQ ID NO:168;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:88;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:170;
a light chain CDR1 amino acid sequence of SEQ ID NO:90;
a light chain CDR2 amino acid sequence of SEQ ID NO:91; and
a light chain CDR3 amino acid sequence of SEQ ID NO:92;

(j) a heavy chain CDR1 amino acid sequence of SEQ ID NO:172;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:88;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:174;
a light chain CDR1 amino acid sequence of SEQ ID NO:90;
a light chain CDR2 amino acid sequence of SEQ ID NO:91; and
a light chain CDR3 amino acid sequence of SEQ ID NO:92;

(k) a heavy chain CDR1 amino acid sequence of SEQ ID NO:176;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:88;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:178;
a light chain CDR1 amino acid sequence of SEQ ID NO:90;
a light chain CDR2 amino acid sequence of SEQ ID NO:91; and
a light chain CDR3 amino acid sequence of SEQ ID NO:92;

(l) a heavy chain CDR1 amino acid sequence of SEQ ID NO:180;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:88;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:182;
a light chain CDR1 amino acid sequence of SEQ ID NO:90;
a light chain CDR2 amino acid sequence of SEQ ID NO:91; and

a light chain CDR3 amino acid sequence of SEQ ID NO:92;

(m) a heavy chain CDR1 amino acid sequence of SEQ ID NO:184;

a heavy chain CDR2 amino acid sequence of SEQ ID NO:88;

a heavy chain CDR3 amino acid sequence of SEQ ID NO:186;

a light chain CDR1 amino acid sequence of SEQ ID NO:90;

a light chain CDR2 amino acid sequence of SEQ ID NO:91; and

a light chain CDR3 amino acid sequence of SEQ ID NO:92;

(n) a heavy chain CDR1 amino acid sequence of SEQ ID NO:188;

a heavy chain CDR2 amino acid sequence of SEQ ID NO:88;

a heavy chain CDR3 amino acid sequence of SEQ ID NO:190;

a light chain CDR1 amino acid sequence of SEQ ID NO:90;

a light chain CDR2 amino acid sequence of SEQ ID NO:91; and

a light chain CDR3 amino acid sequence of SEQ ID NO:92; or

(o) a heavy chain CDR1 amino acid sequence of SEQ ID NO:165;

a heavy chain CDR2 amino acid sequence of SEQ ID NO:96;

a heavy chain CDR3 amino acid sequence of SEQ ID NO:166;

a light chain CDR1 amino acid sequence of SEQ ID NO:98;

a light chain CDR2 amino acid sequence of SEQ ID NO:99; and

a light chain CDR3 amino acid sequence of SEQ ID NO:100.

6. ~~A-The~~ protein according to ~~any one of claims 1 to~~ 5, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to an amino acid sequence selected from: SEQ ID NO:1, SEQ ID NO:41, SEQ ID NO:49, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:85, SEQ ID NO:167, SEQ ID NO:171, SEQ ID NO: 175, SEQ ID NO:179, SEQ ID NO:183, SEQ ID NO:187, and SEQ ID NO:93.

7. The protein according to ~~any one of claims 1 to~~ 5, wherein the first antigen-binding site comprises:

a. a heavy chain variable domain at least 90% identical to SEQ ID NO:41 and a light chain variable domain at least 90% identical to SEQ ID NO:42;

- b. a heavy chain variable domain at least 90% identical to SEQ ID NO:49 and a light chain variable domain at least 90% identical to SEQ ID NO:50;
 - c. a heavy chain variable domain at least 90% identical to SEQ ID NO:57 and a light chain variable domain at least 90% identical to SEQ ID NO:58;
 - d. a heavy chain variable domain at least 90% identical to SEQ ID NO:59 and a light chain variable domain at least 90% identical to SEQ ID NO:60;
 - e. a heavy chain variable domain at least 90% identical to SEQ ID NO:61 and a light chain variable domain at least 90% identical to SEQ ID NO:62;
 - f. a heavy chain variable domain at least 90% identical to SEQ ID NO:69 and a light chain variable domain at least 90% identical to SEQ ID NO:70;
 - g. a heavy chain variable domain at least 90% identical to SEQ ID NO:77 and a light chain variable domain at least 90% identical to SEQ ID NO:78;
 - h. a heavy chain variable domain at least 90% identical to SEQ ID NO:85, SEQ ID NO:167, SEQ ID NO:171, SEQ ID NO: 175, SEQ ID NO:179, SEQ ID NO:183, or SEQ ID NO:187, and a light chain variable domain at least 90% identical to SEQ ID NO:86;
 - i. a heavy chain variable domain at least 90% identical to SEQ ID NO:93 and a light chain variable domain at least 90% identical to SEQ ID NO:94;
 - j. a heavy chain variable domain at least 90% identical to SEQ ID NO:101 and a light chain variable domain at least 90% identical to SEQ ID NO:102; or
 - k. a heavy chain variable domain at least 90% identical to SEQ ID NO:103 and a light chain variable domain at least 90% identical to SEQ ID NO:104.
8. The protein according to claim 1 or 2, wherein the first antigen-binding site is a single-domain antibody.
9. The protein according to claim 8, wherein the single-domain antibody is a V_HH fragment or a V_{NAR} fragment.

10. The protein according to any one of claims 1, 2, 8, or 9, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain present on the same polypeptide.

1142. The protein according to any one of claims 1 to 10, wherein the second antigen-binding site comprises:

a. CDR1, CDR2, and CDR3 sequences of a heavy chain variable domain and a light chain variable domain selected from the group consisting of SEQ ID NO:114 and 118, 131 and 135, 139 and 143, and 122 and 126, respectively;

b. a heavy chain CDR1 amino acid sequence of SEQ ID NO:147;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:148;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:117;
a light chain CDR1 amino acid sequence of SEQ ID NO:149;
a light chain CDR2 amino acid sequence of SEQ ID NO:120; and
a light chain CDR3 amino acid sequence of SEQ ID NO:121;

c. a heavy chain CDR1 amino acid sequence of SEQ ID NO:123;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:124;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:125;
a light chain CDR1 amino acid sequence of SEQ ID NO:127;
a light chain CDR2 amino acid sequence of SEQ ID NO:128; and
a light chain CDR3 amino acid sequence of SEQ ID NO:129;

d. a heavy chain CDR1 amino acid sequence of SEQ ID NO:132;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:133;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:134;
a light chain CDR1 amino acid sequence of SEQ ID NO:136;
a light chain CDR2 amino acid sequence of SEQ ID NO:137; and
a light chain CDR3 amino acid sequence of SEQ ID NO:138; or

e. a heavy chain CDR1 amino acid sequence of SEQ ID NO:140;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:141;

a heavy chain CDR3 amino acid sequence of SEQ ID NO:142;
a light chain CDR1 amino acid sequence of SEQ ID NO:144;
a light chain CDR2 amino acid sequence of SEQ ID NO:145; and
a light chain CDR3 amino acid sequence of SEQ ID NO:146.

1211. The protein according to ~~any one of claims 11 to 10~~, wherein the second antigen-binding site comprises:

a. a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:114 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:118;

b. a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:131 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:135;

c. a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:139 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:143; or

d. a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:122 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:126.

~~12. The protein according to any one of claims 1 to 10, wherein the second antigen-binding site comprises:~~

~~a. CDR1, CDR2, and CDR3 sequences of a heavy chain variable domain and a light chain variable domain selected from the group consisting of SEQ ID NO:114 and 118, 131 and 135, 139 and 143, and 122 and 126, respectively;~~

~~b. a heavy chain CDR1 amino acid sequence of SEQ ID NO:147;~~

~~a heavy chain CDR2 amino acid sequence of SEQ ID NO:148;~~

~~a heavy chain CDR3 amino acid sequence of SEQ ID NO:117;~~

~~a light chain CDR1 amino acid sequence of SEQ ID NO:149;~~

~~a light chain CDR2 amino acid sequence of SEQ ID NO:120; and~~
~~a light chain CDR3 amino acid sequence of SEQ ID NO:121;~~
 e. ~~a heavy chain CDR1 amino acid sequence of SEQ ID NO:123;~~
~~a heavy chain CDR2 amino acid sequence of SEQ ID NO:124;~~
~~a heavy chain CDR3 amino acid sequence of SEQ ID NO:125;~~
~~a light chain CDR1 amino acid sequence of SEQ ID NO:127;~~
~~a light chain CDR2 amino acid sequence of SEQ ID NO:128; and~~
~~a light chain CDR3 amino acid sequence of SEQ ID NO:129;~~
 d. ~~a heavy chain CDR1 amino acid sequence of SEQ ID NO:132;~~
~~a heavy chain CDR2 amino acid sequence of SEQ ID NO:133;~~
~~a heavy chain CDR3 amino acid sequence of SEQ ID NO:134;~~
~~a light chain CDR1 amino acid sequence of SEQ ID NO:136;~~
~~a light chain CDR2 amino acid sequence of SEQ ID NO:137; and~~
~~a light chain CDR3 amino acid sequence of SEQ ID NO:138; or~~
 e. ~~a heavy chain CDR1 amino acid sequence of SEQ ID NO:140;~~
~~a heavy chain CDR2 amino acid sequence of SEQ ID NO:141;~~
~~a heavy chain CDR3 amino acid sequence of SEQ ID NO:142;~~
~~a light chain CDR1 amino acid sequence of SEQ ID NO:144;~~
~~a light chain CDR2 amino acid sequence of SEQ ID NO:145; and~~
~~a light chain CDR3 amino acid sequence of SEQ ID NO:146.~~

13. The protein according to any one of claims 1 to 3 or 56 to 9, wherein the second antigen-binding site is a single-domain antibody.
14. The protein according to claim 13, wherein the second antigen-binding site is a V_HH fragment or a V_{NAR} fragment.
15. The protein according to any one of claims 1 to 14, wherein the protein comprises a portion of an antibody Fc domain sufficient to bind CD16, wherein the antibody Fc domain comprises a hinge and CH2 domains of a human IgG1 antibody.

16. The protein according to claim 15, wherein ~~(i) the antibody Fc domain comprises a hinge and CH2 domains of a human IgG1 antibody or (ii) the~~ antibody Fc domain or portion thereof sufficient to bind CD16 comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody.

17. The protein according to claim 16, wherein the Fc domain comprises an amino acid sequence at least 90% identical to the Fc domain of human IgG1 and differs at one or more positions selected from the group consisting of Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439, numbered according to the EU index as in Kabat.

18. A formulation comprising a protein according to any one of claims 1 to ~~19~~17 and a pharmaceutically acceptable carrier.

19. A cell comprising one or more nucleic acids encoding a protein according to any one of claims 1 to 17.

~~20. Use of a protein according to any one of claims 1 to 17 or a formulation according to claim 18 in the manufacture of a medicament.~~

~~21~~20. Use of a protein according to any one of claims 1 to 17 or a formulation according to claim 18 in the manufacture of a medicament for enhancing tumor cell death, comprising exposing a tumor cells and a natural killer cells to an effective amount of the protein or formulation, wherein the tumor cell expresses FAP.

~~21~~22. Use of a protein according to any one of claims 1 to 17 or the formulation according to claim 18 in the manufacture of a medicament for treating a FAP-expressing cancer.

~~22~~23. The use according to claim ~~22~~21, wherein the cancer to be treated is selected from the group consisting of infiltrating ductal carcinoma, pancreatic ductal adenocarcinoma, stomach cancer, uterine cancer, cervical cancer, colorectal cancer, breast cancer, ovarian cancer, bladder cancer, lung cancer, mesothelioma, gastric cancer, pancreatic cancer, head and neck cancer, liver cancer, endometrial cancer, neuroendocrine cancer, fibrosarcoma, malignant fibrous

histiocytoma, leiomyosarcoma, osteosarcoma, chondrosarcoma, liposarcoma, synovial sarcoma, schwannoma, melanoma, and glioma.

2423. Use of a protein according to any one of claims 1 to 17 or a formulation according to claim 18 in the manufacture of a medicament for treating an autoimmune disease.

2524. The use according to claim 2423, wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis, Grave's disease, Sjögren's syndrome, primary biliary cirrhosis, primary sclerosis cholangitis, and inflammatory destructive arthritis.

2625. Use of a protein according to any one of claims 1 to 17 or a formulation according to claim 18 in the manufacture of a medicament for treating fibrosis.

2726. The use according to claim 2625, wherein the fibrosis is selected from the group consisting of idiopathic pulmonary fibrosis, renal fibrosis, hepatic fibrosis, and cardiac fibrosis.