

[0022] Solute carrier family 1 member 5 (SLC1A5) is a sodium-dependent amino acid transporter that has a broad substrate specificity, with a preference for zwitterionic amino acids. It accepts as substrates all neutral amino acids, including glutamine, asparagine, and branched-chain and aromatic amino acids, and excludes methylated, anionic, and cationic amino acids. It is expressed in many tissues, such as fat, prostate, lung, kidney, colon, and placenta. SLC1A5 can also act as a receptor for RD114/type D retrovirus.

SUMMARY

[0023] The disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and a tumor-associated antigen B7-H3, L1CAM, FLT1, KDR, TNC, TNN, CSPG4, BST1, SELP, CD200, INSR (HHF5), ITGA6, MELTF, PECAM1, or SLC1A5. Such proteins can engage more than one kind of NK-activating receptor, and may block the binding of natural ligands to NKG2D. In certain examples, the proteins can agonize NK cells in humans. In some examples, the proteins can agonize NK cells in humans and in other species such as rodents and cynomolgus monkeys. Various examples and examples of the disclosure are described in further detail below.

[0023a] 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 a tumor-associated antigen B7-H3; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16. ~~In another 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 a tumor-associated antigen L1CAM; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16. In another 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 a tumor-associated antigen selected from the group consisting of FLT1, KDR, TNC, TNN, CSPG4, BST1, SELP, CD200, INSR (HHF5), ITGA6, MELTF, PECAM1, and SLC1A5; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.~~

[0023b] In another aspect, the present disclosure provides a formulation comprising a protein as described herein and a pharmaceutically acceptable carrier. In another aspect, the present disclosure provides a cell comprising one or more nucleic acids expressing a protein as described herein. In another aspect, the present disclosure provides an *in vitro* method of enhancing tumor cell death, the method comprising exposing tumor cells and natural killer

cells to an effective amount of the protein as described herein, wherein the tumor cells express B7-H3. ~~In another aspect, the present disclosure provides an *in vitro* method of enhancing tumor cell death, the method comprising exposing tumor cells and natural killer cells to an effective amount of the protein as described herein, wherein the tumor cells express a tumor-associated antigen selected from B7-H3, L1CAM, FLT1, KDR, TNC, TNN, CSPG4, BST1, SELP, CD200, INSR (HHF5), ITGA6, MELTF, PECAM1, and SLC1A5.~~

[0023c] In a further aspect, the present disclosure provides use of the protein as described herein in the preparation of a medicament for treating cancer. In a further aspect, the present disclosure provides a protein comprising: (a) a first antigen-binding site comprising a Fab fragment that binds NKG2D; (b) a second antigen-binding site comprising a single-chain variable fragment (scFv) that binds B7-H3; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16. In a further aspect, the present disclosure provides use of the protein as described herein in the preparation of a medicament for treating cancer.

[0024] In certain examples, the present disclosure provides a protein (*e.g.*, a multi-specific binding protein) that binds to, for example, B7-H3 on a cancer cell, and the NKG2D receptor and CD16 receptor on natural killer cells to activate the natural killer cell. The binding protein (*e.g.*, a multi-specific binding protein) is useful in the pharmaceutical compositions and therapeutic methods described herein. Binding of the protein including an antigen-binding site that binds to, for example, B7-H3, and to NKG2D receptor and CD16 receptor on natural killer cell enhances the activity of the natural killer cell toward destruction of a cancer cell. Binding of the protein including an antigen-binding site that binds to, for example, B7-H3 (*e.g.*, a multi-specific binding protein) on a cancer cell brings the cancer cell into proximity to the natural killer cell, which facilitates direct and indirect destruction of the cancer cell by the natural killer cell. Further description of exemplary multi-specific binding proteins is provided below.

[0025] The first component of the multi-specific binding proteins of the present disclosure binds to, for example, B7-H3-expressing cells.

[0026] The second component of the multi-specific binding proteins of the present disclosure binds to NKG2D receptor-expressing cells, which can include but are not limited to NK cells, $\gamma\delta$ T cells and CD8⁺ $\alpha\beta$ T cells. Upon NKG2D binding, the multi-specific binding proteins may block natural ligands, such as ULBP6 and MICA, from binding to NKG2D and activating NKG2D receptors.

CLAIMS

1. A protein comprising:
 - (a) a first antigen-binding site that binds NKG2D;
 - (b) a second antigen-binding site that binds a tumor-associated antigen B7-H3;and
 - (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.
2. The protein according to claim 1, wherein the first antigen-binding site that binds NKG2D comprises:
 - (i) a heavy chain variable domain comprising complementarity-determining region 1 (CDR1), complementarity-determining region 2 (CDR2), and complementarity-determining region 3 (CDR3) sequences represented by the amino acid sequences of SEQ ID NOs: 347, 88, and 352, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 90, 91, and 92, respectively
 - (ii) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 347, 88, and 348, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 90, 91, and 92, respectively;
 - (iii) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 341, 64, and 342, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 66, 67, and 68, respectively;
 - (iv) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 343, 72, and 344, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 74, 75, and 76, respectively;
 - (v) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 345, 80, and 346, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 82, 83, and 84, respectively;

(vi) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 87, 88, and 89, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 90, 91, and 92, respectively;

(vii) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 349, 96, and 350, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 98, 99, and 100, respectively;

(viii) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 347, 88, and 355, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 90, 91, and 92, respectively;

(ix) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 347, 88, and 358, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 90, 91, and 92, respectively;

(x) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 347, 88, and 361, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 90, 91, and 92, respectively;

(xi) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 347, 88, and 364, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 90, 91, and 92, respectively;

(xii) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 347, 88, and 367, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 90, 91, and 92, respectively; or

(xiii) a heavy chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 87, 88, and 354, respectively; and a light chain variable domain comprising CDR1, CDR2, and CDR3 sequences represented by the amino acid sequences of SEQ ID NOs: 90, 91, and 92, respectively.

3. The protein according to claim 24, wherein the first antigen-binding site that binds NKG2D comprises:

(i) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:351 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:86;

(ii) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:85 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:86;

(iii) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:77 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:78;

(iv) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:69 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:70;

(v) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:61 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:62;

(vi) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:93 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:94;

(vii) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:353 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:86;

(viii) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:356 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:86;

(ix) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:359 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:86;

(x) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:362 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:86; or

(xi) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:365 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:86.

4. The protein according to any one of claims 1-3, wherein the second antigen-binding site that binds B7-H3 comprises a heavy chain variable domain comprising heavy chain CDR1 (CDRH1), heavy chain CDR2 (CDRH2), and heavy chain CDR3 (CDRH3), and a light chain variable domain comprising light chain CDR1 (CDRL1), light chain CDR2 (CDRL2), and light chain CDR3 (CDRL3), wherein the amino acid sequences of CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 are set forth in SEQ ID NOs: 110, 111, 112, 114, 115, and 116; 118, 119, 120, 122, 123, and 124; 371, 372, 373, 374, 375, and 376; or 379, 380, 381, 382, 383, and 384, respectively.

5. The protein according to ~~any one of~~ claims ~~41-3~~, wherein the second antigen-binding site that binds B7-H3 comprises:

(a) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:109 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:113;

(b) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:117 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:121;

(c) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:369 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:370;

(d) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:377 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:378;

(e) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:386 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:387;

(f) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:388 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:389; or

(g) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:390 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:391.

~~6. A protein comprising:~~

~~(a) a first antigen-binding site that binds NKG2D;~~

~~(b) a second antigen-binding site that binds a tumor-associated antigen L1CAM;~~
~~and~~

~~(c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.~~

~~7. A protein comprising:~~

~~(a) a first antigen-binding site that binds NKG2D;~~

~~(b) a second antigen-binding site that binds a tumor-associated antigen selected from the group consisting of FLT1, KDR, TNC, TNN, CSPG4, BST1, SELP, CD200, INSR (HHF5), ITGA6, MELTF, PECAM1, and SLC1A5; and~~

~~(c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.~~

68. The protein of ~~any one of claims 1, 6, or 7,~~ further comprising an additional antigen-binding site that binds the same tumor-associated antigen as the second antigen-binding site.

79. The protein of ~~any one of claims 1 or 6, 7, or 8,~~ wherein

a) the first antigen-binding site that binds NKG2D is a single-chain variable fragment (scFv), and the second and/or the additional antigen-binding site that binds a tumor-associated antigen is a Fab fragment; or

b) the first antigen-binding site that binds NKG2D is an scFv, and the second and/or the additional antigen-binding site that binds a tumor-associated antigen is an scFv.

810. The protein of ~~any one of claims 1, 6, or 7,~~ wherein

- a) the first antigen-binding site that binds NKG2D is a Fab fragment, and the second antigen-binding site that binds a tumor-associated antigen is an scFv; or
- b) the first antigen-binding site that binds NKG2D is an scFv, and the second antigen-binding site that binds a tumor-associated antigen is a Fab fragment.

941. The protein of ~~any one of claims 79 or 840~~, wherein the scFv that binds the tumor-associated antigen and/or the scFv that binds NKG2D is linked to an antibody constant domain or a portion thereof sufficient to bind CD16, via a hinge comprising Ala-Ser or Gly-Ala-Ser, wherein the scFv comprises a heavy chain variable domain and a light chain variable domain.

1042. The protein according to claim 941, wherein a disulfide bridge is formed between C44 from the heavy chain variable domain and C100 from the light chain variable domain.

1143. The protein according to claim 941 or 1042, wherein

- a) within the scFv the heavy chain variable domain is linked to the light chain variable domain via a flexible linker comprising (GlyGlyGlyGlySer)₄ ((G4S)₄) (SEQ ID NO:126); and/or
- b) within the scFv the heavy chain variable domain is positioned at the N-terminus or the C-terminus of the light chain variable domain.

1244. The protein according to any one of claims 941-1143, wherein within the scFv the hinge further comprises amino acid sequence Thr-Lys-Gly.

1345. The protein according to claim ~~12~~~~or according to any one of claims 6-14~~, wherein the first antigen-binding site that binds NKG2D 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:351, SEQ ID NO:353, SEQ ID NO:356, SEQ ID NO:359, SEQ ID NO:362, SEQ ID NO:365, and SEQ ID NO:93.

1446. The protein according to claim ~~21~~~~or according to any one of claims 6-14~~, wherein:

- a) the first antigen-binding site that binds NKG2D comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:351 and a light chain variable domain at least 90% identical to SEQ ID NO:86;
- b) the first antigen-binding site that binds NKG2D comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:365 and a light chain variable domain at least 90% identical to SEQ ID NO:86;
- c) the first antigen-binding site that binds NKG2D comprises 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;
- d) the first antigen-binding site that binds NKG2D comprises 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;
- e) the first antigen-binding site that binds NKG2D comprises 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;
- f) the first antigen-binding site that binds NKG2D comprises 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;
- g) the first antigen-binding site that binds NKG2D comprises 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;
- h) the first antigen-binding site that binds NKG2D comprises 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;
- i) the first antigen-binding site that binds NKG2D comprises 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;

- j) the first antigen-binding site that binds NKG2D comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:85 and a light chain variable domain at least 90% identical to SEQ ID NO:86;
- k) the first antigen-binding site that binds NKG2D comprises 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;
- l) the first antigen-binding site that binds NKG2D comprises 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
- m) the first antigen-binding site that binds NKG2D comprises 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.

1517. The protein according to claim ~~41~~ or according to any one of claims 8-16, wherein:

- a) the second antigen-binding site binds B7-H3, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO: 109 or 386 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO: 113 or 387;
- b) the second antigen-binding site binds B7-H3, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:117 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:121;
- c) the second antigen-binding site binds B7-H3, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:369 or 388 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:370 or 389; or
- d) the second antigen-binding site binds B7-H3, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical

to SEQ ID NO:377 or 390 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:378 or 391.

~~18. The protein according to claim 6 or according to any one of claims 8-16, wherein:~~

- ~~a) the second antigen-binding site binds L1CAM, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:133 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:137; or~~
- ~~b) the second antigen-binding site binds L1CAM, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:141 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:145.~~

~~19. The protein according to claim 7 or according to any one of claims 8-16, wherein:~~

- ~~a) the second antigen-binding site binds FLT1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:150 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:154;~~
- ~~b) the second antigen-binding site binds FLT1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:158 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:162;~~
- ~~c) the second antigen-binding site binds KDR, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:166 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:170;~~
- ~~d) the second antigen-binding site binds KDR, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:174 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:178;~~

- e) ~~the second antigen-binding site binds TNC, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:182 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:186;~~
- f) ~~the second antigen-binding site binds TNC, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:190 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:194;~~
- g) ~~the second antigen-binding site binds CSPG4, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:198 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:202;~~
- h) ~~the second antigen-binding site binds CSPG4, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:206 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:210;~~
- i) ~~the second antigen-binding site binds BST1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:214 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:218;~~
- j) ~~the second antigen-binding site binds BST1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:222 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:226;~~
- k) ~~the second antigen-binding site binds SELP, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:230 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:234;~~

- ~~l) the second antigen-binding site binds SELP, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:238 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:242;~~
- ~~m) the second antigen-binding site binds CD200, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:246 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:250;~~
- ~~n) the second antigen-binding site binds INSR, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:254 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:258;~~
- ~~o) the second antigen-binding site binds INSR, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:262 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:266;~~
- ~~p) the second antigen-binding site binds ITGA6, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:270 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:274;~~
- ~~q) the second antigen-binding site binds MELTF, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:284 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:288;~~
- ~~r) the second antigen-binding site binds MELTF, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:292 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:296;~~

- s) ~~the second antigen-binding site binds SLC1A5, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:300 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:304; or~~
- t) ~~the second antigen-binding site binds SLC1A5, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:308 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:312.~~
20. ~~The protein according to claim 6 or according to any one of claims 8-16, wherein the second antigen-binding site binds L1CAM, and wherein:~~
- i. ~~the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
- ~~—— (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:134;~~
 - ~~—— (b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:135; and~~
 - ~~—— (c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:136,~~
 - ~~—— and wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
 - ~~—— (d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:138;~~
 - ~~—— (e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:139; and~~
 - ~~—— (f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:140; or~~
- ii. ~~the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
- ~~—— (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ~~

ID NO:142;

~~——(b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:143; and~~

~~——(c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:144;~~

~~——and wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

~~——(d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:146;~~

~~——(e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:147; and~~

~~——(f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:148.~~

21. ~~The protein according to claim 7 or according to any one of claims 8-16, wherein:~~

~~i. the second antigen-binding site binds FLT1, and wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

~~——(a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:151;~~

~~——(b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:152; and~~

~~——(c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:153;~~

~~——and wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

~~——(d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:155;~~

~~——(e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:156; and~~

~~——(f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:157;~~

- ii. ~~the second antigen-binding site binds FLT1, and wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
- ~~—— (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:159;~~
 - ~~—— (b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:160; and~~
 - ~~—— (c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:161,~~
 - ~~—— and wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
 - ~~—— (d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:163;~~
 - ~~—— (e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:164; and~~
 - ~~—— (f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:165;~~
- iii. ~~the second antigen-binding site binds KDR, and wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
- ~~—— (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:167;~~
 - ~~—— (b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:168; and~~
 - ~~—— (c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:169,~~
 - ~~—— and wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
 - ~~—— (d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:171;~~
 - ~~—— (e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:172; and~~

~~——(f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:173;~~

~~iv. the second antigen binding site binds KDR, and wherein the heavy chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~

~~——(a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:175;~~

~~——(b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:176; and~~

~~——(c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:177;~~

~~——and wherein the light chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~

~~——(d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:179;~~

~~——(e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:180; and~~

~~——(f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:181;~~

~~v. the second antigen binding site binds TNC, and wherein the heavy chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~

~~——(a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:183;~~

~~——(b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:184; and~~

~~——(c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:185;~~

~~——and wherein the light chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~

~~——(d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:187;~~

- ~~——(e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:188; and~~
 - ~~——(f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:189;~~
- ~~vi. the second antigen-binding site binds TNC, and wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
 - ~~——(a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:191;~~
 - ~~——(b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:192; and~~
 - ~~——(c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:193,~~
 - ~~——and wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
 - ~~——(d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:195;~~
 - ~~——(e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:196; and~~
 - ~~——(f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:197;~~
- ~~vii. the second antigen-binding site binds CSPG4, and wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
 - ~~——(a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:199;~~
 - ~~——(b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:200; and~~
 - ~~——(c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:201,~~
 - ~~——and wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

- ~~—— (d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:203;~~
 - ~~—— (e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:204; and~~
 - ~~—— (f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:205;~~
- ~~viii. the second antigen binding site binds CSPG4, and wherein the heavy chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
 - ~~—— (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:207;~~
 - ~~—— (b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:208; and~~
 - ~~—— (c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:209;~~
 - ~~—— and wherein the light chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
 - ~~—— (d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:211;~~
 - ~~—— (e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:212; and~~
 - ~~—— (f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:213;~~
- ~~ix. the second antigen binding site binds BST1, and wherein the heavy chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
 - ~~—— (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:215;~~
 - ~~—— (b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:216; and~~
 - ~~—— (c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:217;~~

~~——and wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

~~——(d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:219;~~

~~——(e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:220; and~~

~~——(f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:221;~~

x. ~~the second antigen-binding site binds BST1, and wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

~~——(a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:223;~~

~~——(b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:224; and~~

~~——(c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:225;~~

~~——and wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

~~——(d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:227;~~

~~——(e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:228; and~~

~~——(f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:229;~~

xi. ~~the second antigen-binding site binds SELP, and wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

~~——(a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:231;~~

~~——(b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:232; and~~

- ~~——(c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:233;~~
 - ~~——and wherein the light chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
 - ~~——(d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:235;~~
 - ~~——(e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:236; and~~
 - ~~——(f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:237;~~
- xii. ~~the second antigen binding site binds SELP, and wherein the heavy chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
 - ~~——(a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:239;~~
 - ~~——(b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:240; and~~
 - ~~——(c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:241;~~
 - ~~——and wherein the light chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
 - ~~——(d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:243;~~
 - ~~——(e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:244; and~~
 - ~~——(f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:245;~~
- xiii. ~~the second antigen binding site binds CD200, and wherein the heavy chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
 - ~~——(a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:247;~~

- ~~—— (b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:248; and~~
 - ~~—— (c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:249,~~
 - ~~—— and wherein the light chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
 - ~~—— (d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:251;~~
 - ~~—— (e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:252; and~~
 - ~~—— (f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:253;~~
- xiv. ~~the second antigen binding site binds INSR, and wherein the heavy chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
 - ~~—— (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:255;~~
 - ~~—— (b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:256; and~~
 - ~~—— (c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:257,~~
 - ~~—— and wherein the light chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
 - ~~—— (d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:259;~~
 - ~~—— (e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:260; and~~
 - ~~—— (f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:261;~~
- xv. ~~the second antigen binding site binds INSR, and wherein the heavy chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~

- ~~—— (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:263;~~
 - ~~—— (b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:264; and~~
 - ~~—— (c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:265;~~
 - ~~—— and wherein the light chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
 - ~~—— (d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:267;~~
 - ~~—— (e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:268; and~~
 - ~~—— (f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:269;~~
- xvi. ~~the second antigen binding site binds ITGA6, and wherein the heavy chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
- ~~—— (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:271;~~
 - ~~—— (b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:272; and~~
 - ~~—— (c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:273;~~
 - ~~—— and wherein the light chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
 - ~~—— (d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:275;~~
 - ~~—— (e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:276; and~~
 - ~~—— (f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:277;~~

- xvii. ~~the second antigen-binding site binds ITGA6, and wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
- ~~—— (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:278;~~
 - ~~—— (b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:279; and~~
 - ~~—— (c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:280;~~
- ~~and wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
- ~~—— (d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:281;~~
 - ~~—— (e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:282; and~~
 - ~~—— (f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:283;~~
- xviii. ~~the second antigen-binding site binds MELTF, and wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
- ~~—— (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:285;~~
 - ~~—— (b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:286; and~~
 - ~~—— (c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:287;~~
- ~~and wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~
- ~~—— (d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:289;~~
 - ~~—— (e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:290; and~~

- ~~—— (f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:291;~~
- xix. ~~the second antigen binding site binds MELTF, and wherein the heavy chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
- ~~—— (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:293;~~
- ~~—— (b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:294; and~~
- ~~—— (c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:295,~~
- ~~—— and wherein the light chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
- ~~—— (d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:297;~~
- ~~—— (e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:298; and~~
- ~~—— (f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:299;~~
- xx. ~~the second antigen binding site binds SLC1A5, and wherein the heavy chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
- ~~—— (a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:301;~~
- ~~—— (b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:302; and~~
- ~~—— (c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:303,~~
- ~~—— and wherein the light chain variable domain of the second antigen binding site comprises an amino acid sequence including:~~
- ~~—— (d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:305;~~

~~——(e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:306; and~~

~~——(f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:307; or~~

~~xxi. the second antigen-binding site binds SLC1A5, and wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

~~——(a) a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:309;~~

~~——(b) a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:310; and~~

~~——(c) a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:311,~~

~~——and wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:~~

~~——(d) a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:313;~~

~~——(e) a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:314; and~~

~~——(f) a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:315.~~

1622. The protein according to claim 1 or according to any one of claims 6-1521, wherein the protein comprises an antibody Fc domain or a portion thereof sufficient to bind CD16, wherein the antibody Fc domain comprises hinge and CH2 domains.

1723. The protein according to claim 1622, wherein:

- a) the antibody Fc domain comprises hinge and CH2 domains of a human IgG1 antibody;
- b) the antibody Fc domain comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody; and/or

- c) the antibody Fc domain comprises 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, Q352, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439.

1824. A formulation comprising a protein according to any one of the claims 1-1723 and a pharmaceutically acceptable carrier.

1925. A cell comprising one or more nucleic acids expressing a protein according to any one of claims 1-1723.

2026. An *in vitro* method of enhancing tumor cell death, the method comprising exposing tumor cells and natural killer cells to an effective amount of the protein according to any one of claims 1-~~5, 8-17, 22, or 23~~17, wherein the tumor cells express B7-H3.

~~27. —An *in vitro* method of enhancing tumor cell death, the method comprising exposing tumor cells and natural killer cells to an effective amount of the protein according to any one of claims 1-23, wherein the tumor cells express a tumor associated antigen selected from B7-H3, L1CAM, FLT1, KDR, TNC, TNN, CSPG4, BST1, SELP, CD200, INSR (HHF5), ITGA6, MELTF, PECAM1, and SLC1A5.~~

2128. Use of the protein according to any one of claims 1-1723 in the preparation of a medicament for treating cancer.

2229. The use of claim 2128, wherein:

a) ~~the second antigen binding site of the protein binds B7-H3, and~~ wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, glioblastoma, head and neck cancer, lung cancer, liver cancer, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, sarcoma, renal cancer, colorectal cancer, gastric cancer, neuroblastoma, squamous cell carcinoma, and acute myeloid leukemia (AML).;

b) ~~the second antigen binding site of the protein binds L1CAM, and wherein the cancer is selected from the group consisting of bladder cancer, renal cancer, breast cancer, cervical cancer, sarcoma, lung cancer, head and neck cancer, glioblastoma, neuroblastoma, melanoma, ovarian cancer, endometrial cancer, esophageal cancer, gastric cancer,~~

gastrointestinal stromal tumor (GIST), cholangiocarcinoma, colorectal cancer, pancreatic cancer, and prostate cancer;

e) the second antigen binding site of the protein binds FLT1, and wherein the cancer to be treated is selected from the group consisting of renal cancer, gastric cancer, glioma, colorectal cancer, biliary tract cancer, prostate cancer, sarcoma, and breast cancer;

d) the second antigen binding site of the protein binds KDR, and wherein the cancer to be treated is selected from the group consisting of renal cancer, gastric cancer, glioma, colorectal cancer, biliary tract cancer, lung cancer, melanoma, liver cancer, sarcoma, breast cancer, mesothelioma, and thyroid cancer;

e) the second antigen binding site of the protein binds TNC, and wherein the cancer to be treated is selected from the group consisting of cervical cancer, breast cancer, pancreatic cancer, lung cancer, non-Hodgkin lymphoma, head and neck cancer, colorectal cancer, esophageal cancer, glioma, and prostate cancer;

f) the second antigen binding site of the protein binds TNN, and wherein the cancer to be treated is selected from the group consisting of cervical cancer, breast cancer, pancreatic cancer, lung cancer, non-Hodgkin lymphoma, head and neck cancer, colorectal cancer, esophageal cancer, glioma, and prostate cancer;

g) the second antigen binding site of the protein binds CSPG4, and wherein the cancer to be treated is selected from the group consisting of melanoma, renal cancer, sarcoma, glioma, head and neck cancer, breast cancer, bladder cancer, lung cancer, and cervical cancer;

h) the second antigen binding site of the protein binds BST1, and wherein the cancer to be treated is selected from the group consisting of acute myeloid leukemia, mesothelioma, bladder cancer, and sarcoma;

i) the second antigen binding site of the protein binds SELP, and wherein the cancer to be treated is selected from the group consisting of myeloproliferative neoplasms, acute myeloid leukemia, breast cancer, bladder cancer, thyroid cancer, renal cancer, and pancreatic cancer;

j) the second antigen binding site of the protein binds CD200, and wherein the cancer to be treated is selected from the group consisting of breast cancer, colorectal cancer, B-cell malignancies, multiple myeloma, acute myeloid leukemia, lymphoma, and mesothelioma;

- ~~k) the second antigen-binding site of the protein binds INSR, and wherein the cancer to be treated is selected from the group consisting of prostate cancer, gastric cancer, colorectal cancer, glioblastoma, breast cancer, endometrial cancer, liver cancer, and renal cancer;~~
- ~~l) the second antigen-binding site of the protein binds ITGA6, and wherein the cancer to be treated is selected from the group consisting of breast cancer, leukemia, prostate cancer, colorectal cancer, renal cancer, head and neck cancer, ovarian cancer, gastric cancer, and lung cancer;~~
- ~~m) the second antigen-binding site of the protein binds MELTF, and wherein the cancer to be treated is selected from the group consisting of breast cancer, lung cancer, melanoma, bladder cancer, renal cancer, sarcoma, head and neck cancer, mesothelioma, pancreatic cancer;~~
- ~~n) the second antigen-binding site of the protein binds PECAM1, and wherein the cancer to be treated is a solid tumor, wherein the solid tumor has significant neovasculature; or~~
- ~~o) the second antigen-binding site of the protein binds SLC1A5, and wherein the cancer to be treated is selected from the group consisting of lung cancer, colorectal cancer, breast cancer, prostate cancer, renal cancer, head and neck cancer, neuroblastoma, gastric cancer, and ovarian cancer.~~

2330. A protein comprising:

- (a) a first antigen-binding site comprising a Fab fragment that binds NKG2D;
- (b) a second antigen-binding site comprising a single-chain variable fragment (scFv) that binds B7-H3; and
- (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.

2431. The protein of claim 2330, wherein the scFv is linked to the antibody Fc domain or a portion thereof sufficient to bind CD16, or the third antigen-binding site that binds CD16, via a hinge comprising Ala-Ser or Gly-Ala-Ser, wherein the scFv comprises a heavy chain variable domain and a light chain variable domain.

2532. The protein according to claim 2431, wherein the scFv is linked to the antibody Fc domain.

2633. The protein according to claim 2431 or 2532, wherein the heavy chain variable domain of the scFv forms a disulfide bridge with the light chain variable domain of the scFv.

2734. The protein according to claim 2633, wherein the disulfide bridge is formed between C44 from the heavy chain variable domain and C100 from the light chain variable domain.

2835. The protein according to claim 2734, wherein the scFv is linked to the antibody Fc domain, wherein the light chain variable domain of the scFv is positioned at the N-terminus of the heavy chain variable domain of the scFv, and is linked to the heavy chain variable domain of the scFv via a flexible linker (GlyGlyGlyGlySer)₄ ((G4S)₄) (SEQ ID NO:126), and the Fab is linked to the antibody Fc domain.

2936. The protein according to any one of claims 2431-2835, wherein the heavy chain variable domain of the scFv is linked to the light chain variable domain of the scFv via a flexible linker comprising (GlyGlyGlyGlySer)₄ ((G4S)₄) (SEQ ID NO:126).

3037. The protein according to any one of claims 2431-2936, wherein the heavy chain variable domain of the scFv is positioned at the N-terminus or the C-terminus of the light chain variable domain of the scFv and/or the light chain variable domain of the scFv is positioned at the N-terminus of the heavy chain variable domain of the scFv.

3138. The protein according to any one of claims 2330-3037, wherein the Fab fragment is linked to the antibody Fc domain or a portion thereof sufficient to bind CD16 or the third antigen-binding site that binds CD16.

3239. The protein according to claim 3138, wherein the heavy chain portion of the Fab fragment comprises a heavy chain variable domain and a CH1 domain, and wherein the heavy chain variable domain is linked to the CH1 domain.

3340. The protein according to claim 3138 or 3239, wherein the Fab is linked to the antibody Fc domain.

3441. The protein according to any one of claims 2330-3340 comprising a sequence selected from SEQ ID NOs: 329, 330, 333, 334, 335, and 336.

3542. The protein according to any one of claims 2330-3340 comprising an scFv linked to an antibody Fc domain, wherein the scFv linked to the antibody Fc domain is represented by a sequence selected from SEQ ID NO:330, SEQ ID NO:334, and SEQ ID NO:336.

3643. The protein according to any one of claims 2330-3340 comprising a sequence of SEQ ID NO:329, SEQ ID NO:333, or SEQ ID NO:335.

3744. The protein according to any one of claims 2330-3340 comprising:

- a) a sequence at least 90%, at least 95%, or at least 99% identical to an amino acid sequence of SEQ ID NO:329, SEQ ID NO:333, or SEQ ID NO:335 or
- b) a sequence at least 90%, at least 95%, or at least 99% identical to an amino acid sequence selected from SEQ ID NO:330, SEQ ID NO:334, or SEQ ID NO:336.

3845. Use of the protein according to any one of claims 2330-3744 in the preparation of a medicament for treating cancer.

3946. The use of the protein according to claim 3845, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, glioblastoma, head and neck cancer, lung cancer, liver cancer, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, sarcoma, renal cancer, colorectal cancer, gastric cancer, neuroblastoma, squamous cell carcinoma, and acute myeloid leukemia (AML).