

granzymes as well as via death receptor pathways. Activated NK cells also secrete inflammatory cytokines such as IFN-gamma and chemokines that promote the recruitment of other leukocytes to the target tissue.

[0006] NK cells respond to signals through a variety of activating and inhibitory

5 receptors on their surface. For example, when NK cells encounter healthy self-cells, their activity is inhibited through activation of the killer-cell immunoglobulin-like receptors (KIRs). Alternatively, when NK cells encounter cancer cells, they are activated via their activating receptors (*e.g.*, NKG2D, NCRs, DNAM1). NK cells are also activated by the constant region of some immunoglobulins through CD16 receptors on their surface. The
10 overall sensitivity of NK cells to activation depends on the sum of stimulatory and inhibitory signals. NKG2D is a type-II transmembrane protein that is expressed by essentially all natural killer cells where NKG2D serves as an activating receptor. The ability to modulate NK cell function via NKG2D is useful in various therapeutic contexts including malignancy.

SUMMARY

15 **[0007]** Antibodies to NKG2D have been identified that provide important advantages in the design of therapeutic agents. For example, some of these antibodies do not merely bind human NKG2D receptor, but have one or more further advantages such as the ability to agonize the receptor; the ability to compete with a natural ligand for binding to the receptor; and/or the ability to cross-react with NKG2D from other species such as cynomolgus
20 monkey. These advantages can be achieved across a range of affinities for NKG2D.

[0007a] In one aspect, the present disclosure relates to an antigen binding site that binds NKG2D comprising: a heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:7, the heavy chain variable domain comprising a heavy chain complementarity-determining region 1 (CDR1)
25 sequence of SEQ ID NO:48, a heavy chain complementarity-determining region 2 (CDR2) sequence of SEQ ID NO:30, a heavy chain complementarity-determining region 3 (CDR3) sequence of SEQ ID NO:44; and a light chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence SEQ ID NO:8, the light chain variable domain comprising a light chain CDR1 sequence of SEQ ID NO:32, a light chain
30 CDR2 sequence of SEQ ID NO:33, and a light chain CDR3 sequence of SEQ ID NO:34.

[0007b] In another aspect, the present disclosure relates to a protein comprising: (a) the antigen-binding site as described herein; and (b) an additional antigen-binding site. In another

aspect, the present disclosure relates to a protein comprising an antigen-binding site that competes for binding to human or cynomolgus NKG2D with an antibody comprising a heavy chain variable region having the amino acid sequence of SEQ ID NO:7 and a light chain variable region having the amino acid sequence of SEQ ID NO:8, wherein the competition is assessed by an epitope binning assay. In another aspect, the present disclosure relates to a formulation comprising the protein as described herein and a pharmaceutically acceptable carrier. In another aspect, the present disclosure relates to a cell comprising one or more nucleic acids encoding the protein as described herein.

[0007c] In another aspect, the present disclosure relates to an *in vitro* method of enhancing tumor cell death, the method comprising exposing a tumor cell and a natural killer cell to the protein as described herein. In a further aspect, the present disclosure relates to the protein as described herein for use in therapy. In a further aspect, the present disclosure relates to a use of the protein as described herein in the manufacture of a medicament for treating a cancer, optionally wherein the cancer is selected from the group consisting of acute myeloid leukemia, acute myelomonocytic leukemia, B cell lymphoma, bladder cancer, breast cancer, colorectal cancer, diffuse large B cell lymphoma esophageal cancer, Ewing's sarcoma, follicular lymphoma, gastric cancer, gastrointestinal cancer, gastrointestinal stromal tumors, glioblastoma, head and neck cancer, melanoma, mesothelioma, multiple myeloma, myelodysplastic syndrome, renal cell carcinoma, neuroblastoma, non-small cell lung cancer, neuroendocrine tumors, ovarian cancer, and pancreatic cancer, prostate cancer, sarcomas, small cell lung cancer, T cell lymphoma, testis cancer, thymic carcinoma, thyroid cancer, urothelial cancer, cancers infiltrated by myeloid-derived suppressor cells, cancers with extracellular matrix deposition, cancers with high levels of reactive stroma, and cancers with neoangiogenesis.

[0008] Accordingly, one example of the disclosure relates to an antibody heavy chain variable domain at least 90% identical to the amino acid sequence
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYMHWRQAPGQGLEWMGIINPSG
GSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDVAVYYCARGAPNYGDTTHDYY
YMDVWGKGTITVTVSS (SEQ ID NO:1, ADI-29379). In some examples, the antibody heavy chain variable domain is at least 95% identical to SEQ ID NO:1. In some examples, the heavy chain variable domain includes amino acid sequences YTFTSYMH (SEQ ID NO:11) as the first complementarity-determining region 1 ("CDR1"),

IINPSGGSTSYAQKFQG (SEQ ID NO:12) as the second CDR (“CDR2”), and ARGAPNYGDTTHDYYYMDV (SEQ ID NO:13) as the third CDR (“CDR3”) of SEQ ID NO:1. In some examples, the heavy chain variable domain includes amino acid

sequences SYVMH (SEQ ID NO:45) as CDR1, IINPSGGSTSYAQKFQG (SEQ ID NO:12) as CDR2, and GAPNYGDTTHDYMDV (SEQ ID NO:68) as CDR3 of SEQ ID NO:1.

[0009] Another example of the disclosure relates to an antibody heavy chain variable domain at least 90% identical to the amino acid sequence

5 QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPN
SGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARDTGEYYDTDDH
GMDVWGQGTTVTVSS (SEQ ID NO: 3, ADI-29463). In some examples, the antibody
heavy chain variable domain is at least 95% identical to SEQ ID NO:3. In some examples,
the heavy chain variable domain includes amino acid sequences YTFTGYYMH (SEQ ID
10 NO: 17) as the first complementarity-determining region (“CDR1”),
WINPNSGGTNYAQKFQG (SEQ ID NO:18) as the second CDR (“CDR2”), and
ARDTGEYYDTDDHGMDV (SEQ ID NO:19) as the third CDR (“CDR3”) of SEQ ID
NO:3. In some examples, the heavy chain variable domain includes amino acid sequences
GYYMH (SEQ ID NO: 92) as CDR1, WINPNSGGTNYAQKFQG (SEQ ID NO:18) as
15 CDR2, and DTGEYYDTDDHGMDV (SEQ ID NO:69) as CDR3 of SEQ ID NO:3.

[0010] Another example of the disclosure relates to an antibody heavy chain variable domain at least 90% identical to the amino acid sequence

EVQLLES GGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGG
STYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDGGYYDSGAGDYW
20 GQGTLVTVSS (SEQ ID NO:5, ADI-27744). In some examples, the antibody heavy chain
variable domain is at least 95% identical to SEQ ID NO:5. In some examples, the heavy
chain variable domain includes amino acid sequences FTFSSYAMS (SEQ ID NO:23) as the
first complementarity-determining region (“CDR1”), AISGSGGSTYYADSVKG (SEQ ID
NO:24) as the second CDR (“CDR2”), and AKDGGYYDSGAGDY (SEQ ID NO:25) as the
25 third CDR (“CDR3”) of SEQ ID NO:5. In some examples, the heavy chain variable domain
includes amino acid sequences SYAMS (SEQ ID NO:47) as CDR1,
AISGSGGSTYYADSVKG (SEQ ID NO:24) as CDR2, and DGGYYDSGAGDY (SEQ ID
NO:70) as CDR3 of SEQ ID NO:5.

[0011] Another example of the disclosure relates to an antibody heavy chain variable domain at least 90% identical to the amino acid sequence

30 EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSSYI
YYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYCARGAPMGAAAGWFDPW
GQGTLVTVSS (SEQ ID NO:7, ADI-27749). In some examples, the antibody heavy chain
variable domain is at least 95% identical to SEQ ID NO:7. In some examples, the heavy

chain variable domain includes amino acid sequences FTFSSYSMN (SEQ ID NO:29) as the first complementarity-determining region (“CDR1”), SISSSSSYIYYADSVKG (SEQ ID NO: 30) as the second CDR (“CDR2”), and ARGAPMGAAAGWFDP (SEQ ID NO:31) as the third CDR (“CDR3”) of SEQ ID NO:7. In some examples, the heavy chain variable domain includes amino acid sequences SYSMN (SEQ ID NO:48) as CDR1, SISSSSSYIYYADSVKG (SEQ ID NO: 30) as CDR2, and GAPMGAAAGWFDP (SEQ ID NO:71) as CDR3 of SEQ ID NO:7.

[0012] Another example of the disclosure relates to an antibody heavy chain variable domain at least 90% identical to the amino acid sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSYIYYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARGAPIGAAAGWFDPWGQGTLLTVSS (SEQ ID NO:85, A49MI). In some examples, the antibody heavy chain variable domain is at least 95% identical to SEQ ID NO:85. In some examples, the heavy chain variable domain includes amino acid sequences FTFSSYSMN (SEQ ID NO:29) as CDR1, SISSSSSYIYYADSVKG (SEQ ID NO: 30) as CDR2, and ARGAPIGAAAGWFDP (SEQ ID NO:77) as CDR3 of SEQ ID NO:85. In some examples, the heavy chain variable domain includes amino acid sequences SYSMN (SEQ ID NO:48) as CDR1, SISSSSSYIYYADSVKG (SEQ ID NO: 30) as CDR2, and GAPIGAAAGWFDP (SEQ ID NO:78) as CDR3 of SEQ ID NO:85.

[0013] Another example of the disclosure relates to an antibody heavy chain variable domain at least 90% identical to the amino acid sequence QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYMHWVRQAPGQGLEWMGIINPSG GSTSYAQKFQGRVTMTRDTSTSTVYMESSLRSEDTAVYYCAREGAGFAYGMDYY YMDVWGKGTITVTVSS (SEQ ID NO:9, ADI-29378). In some examples, the antibody heavy chain variable domain is at least 95% identical to SEQ ID NO:9. In some examples, the heavy chain variable domain includes amino acid sequences YTFTSYMH (SEQ ID NO:35) as the first complementarity-determining region (“CDR1”), IINPSGGSTSYAQKFQG (SEQ ID NO: 36) as the second CDR (“CDR2”), and AREGAGFAYGMDYYMDV (SEQ ID NO:37) as the third CDR (“CDR3”) of SEQ ID NO:9. In some examples, the heavy chain variable domain includes amino acid sequences SYMH (SEQ ID NO:45) as CDR1, IINPSGGSTSYAQKFQG (SEQ ID NO: 36) as CDR2, and EGAGFAYGMDYYMDV (SEQ ID NO:72) as CDR3 of SEQ ID NO:9.

[0014] An antibody heavy chain variable domain of the disclosure can optionally be coupled to an amino acid sequence at least 90% identical to an antibody constant region, such as an IgG constant region including hinge, CH2 and CH3 domains with or without CH1 domain. In some examples, the amino acid sequence of the constant region is at least 90% identical to a human antibody constant region, such as an human IgG1 constant region, an IgG2 constant region, IgG3 constant region, or IgG4 constant region. In some other examples, the amino acid sequence of the constant region is at least 90% identical to an antibody constant region from another mammal, such as rabbit, dog, cat, mouse, or horse. One or more mutations can be included into the constant region as compared to human IgG1 constant region, for example at Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411 and/or K439. Exemplary substitutions include, for example, Q347E, Q347R, Y349S, Y349K, Y349T, Y349D, Y349E, Y349C, T350V, L351K, L351D, L351Y, S354C, E356K, E357Q, E357L, E357W, K360E, K360W, Q362E, S364K, S364E, S364H, S364D, T366V, T366I, T366L, T366M, T366K, T366W, T366S, L368E, L368A, L368D, K370S, N390D, N390E, K392L, K392M, K392V, K392F, K392D, K392E, T394F, T394W, D399R, D399K, D399V, S400K, S400R, D401K, F405A, F405T, Y407A, Y407I, Y407V, K409F, K409W, K409D, T411D, T411E, K439D, and K439E.

[0015] In certain examples, mutations that can be included into the CH1 of a human IgG1 constant region may be at amino acid V125, F126, P127, T135, T139, A140, F170, P171, and/or V173. In certain examples, mutations that can be included into the C_κ of a human IgG1 constant region may be at amino acid E123, F116, S176, V163, S174, and/or T164.

[0016] In some examples, one of the heavy chain variable domains described herein is combined with a light chain variable domain to form an antigen-binding site capable of binding NKG2D. For example, an antibody heavy chain variable domain at least 90% identical to the amino acid sequence of SEQ ID NO:1 can be paired with an antibody light chain variable domain at least 90% identical to the amino acid sequence EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYDDWPFTFGGGTKVEIK (SEQ ID NO:2, ADI-29379). In some examples, the antibody light chain variable domain is at least 95% identical to SEQ ID NO:2. In some examples, the light chain variable domain includes amino acid sequences RASQSVSSNLA (SEQ ID NO:14) as the first

complementarity-determining region (“CDR”), GASTRAT (SEQ ID NO:15) as the second CDR, and QQYDDWPFT (SEQ ID NO:16) as the third CDR.

[0017] For example, an antibody heavy chain variable domain at least 90% identical to the amino acid sequence of SEQ ID NO:3 can be paired with an antibody light chain variable domain at least 90% identical to the amino acid sequence

EIVLTQSPGTLSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISLQSEDAVYYCQQDDYWPPTFGGGTKVEIK (SEQ ID NO:4, ADI-29463). In some examples, the antibody light chain variable domain is at least 95% identical to SEQ ID NO:4. In some examples, the light chain variable domain includes amino acid sequences RASQSVSSNLA (SEQ ID NO:20) as the first complementarity-determining region (“CDR”), GASTRAT (SEQ ID NO:21) as the second CDR, and QQDDYWPPT (SEQ ID NO:22) as the third CDR.

[0018] For example, an antibody heavy chain variable domain at least 90% identical to the amino acid sequence of SEQ ID NO:5 can be paired with an antibody light chain variable domain at least 90% identical to the amino acid sequence

DIQMTQSPSSVSASVGDRVTITCRASQGIDSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQGVSYPRFTFGGGTKVEIK (SEQ ID NO:6, ADI-27744). In some examples, the antibody light chain variable domain is at least 95% identical to SEQ ID NO:6. In some examples, the light chain variable domain includes amino acid sequences RASQGIDSWLA (SEQ ID NO:26) as the first complementarity-determining region (“CDR”), AASSLQS (SEQ ID NO:27) as the second CDR, and QQGVSYPRT (SEQ ID NO:28) as the third CDR.

[0019] For example, an antibody heavy chain variable domain at least 90% identical to the amino acid sequence of SEQ ID NO:7 or 85 can be paired with an antibody light chain variable domain at least 90% identical to the amino acid sequence

DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQGVSPRFTFGGGTKVEIK (SEQ ID NO:8, ADI-27749). In some examples, the antibody light chain variable domain is at least 95% identical to SEQ ID NO:8. In some examples, the light chain variable domain includes amino acid sequences RASQGISSWLA (SEQ ID NO:32) as the first complementarity-determining region (“CDR”), AASSLQS (SEQ ID NO:33) as the second CDR, and QQGVSPRFT (SEQ ID NO:34) as the third CDR.

[0020] For example, an antibody heavy chain variable domain at least 90% identical to the amino acid sequence of SEQ ID NO:9 can be paired with an antibody light chain variable

domain at least 90% identical to the amino acid sequence

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGI
PARFSGSGSGTDFTLTISSELPEDFAVYYCQQSDNWPFTFGGGTKVEIK (SEQ ID
NO:10, ADI-29378). In some examples, the antibody light chain variable domain is at least
5 95% identical to SEQ ID NO:10. In some examples, the light chain variable domain includes
amino acid sequences RASQSVSSYLA (SEQ ID NO:38) as the first complementarity-
determining region (“CDR”), DASNRAT (SEQ ID NO:39) as the second CDR, and
QQSDNWPFT (SEQ ID NO:40) as the third CDR.

[0021] When a heavy chain variable domain is combined with a light chain variable
10 domain to form an antigen-binding site capable of binding NKG2D, the antigen-binding site
can be included into a variety of structures, for example, a typical antibody structure with two
identical heavy chains and two identical light chains, forming a pair of antigen-binding sites
capable of binding NKG2D; a bi-specific, tri-specific, tetra-specific or other multi-specific
antibody; or a smaller structure such as an scFv (in which the heavy chain variable domain is
15 linked to the light chain variable domain).

[0022] In some examples, any NKG2D antigen-binding site disclosed in the instant
disclosure is included into a protein that also includes a separate antigen-binding site that
binds a tumor-associated antigen, which may permit the protein to simultaneously interact
with an NK cell and a tumor cell. The tumor-associated antigen, for example, can be CD33,
20 HER2, EpCAM, CD2, CD3, CD8, CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD25,
CD30, CD37, CD38, CD40, CD45RO, CD48, CD52, CD55, CD59, CD70, CD74, CD80,
CD86, CD138, CD147, HLA-DR, CSAp, CA-125, TAG-72, EFGR/ERBB1, IGF1R, HER2,
HER3, HER4, IGF-1R, c-Met, PDGFR, MUC1, MUC2, MUC3, MUC4, TNFR1, TNFR2,
NGFR, TRAILR1, TRAILR2, Fas (CD95), DR3, DR4, DR5, DR6, VEGF, PIGF, tenascin,
25 ED-B fibronectin, PSA, and IL-6, MAGE-A3, B7.1, B7.2, CTLA4 or PD1.

[0023] In some examples, any NKG2D antigen-binding site disclosed in the instant
disclosure is included into a protein that also contain a tumor-associated antigen site and CD16
binding site. The CD16 binding site can be an additional antigen-binding site or an antibody
constant region or a portion thereof, such as an IgG1 constant region (which may optionally
30 include one or more mutations affecting, for example, effector activity or CD16 binding affinity).

[0024] Another example of the disclosure provides a method of enhancing tumor cell death
and treating cancer in a patient. The method comprises administering to a patient in need thereof
a therapeutically effective amount of a protein described herein to treat the cancer.

WHAT IS CLAIMED IS:

1. An antigen binding site that binds NKG2D comprising:

a heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:7, the heavy chain variable domain comprising a heavy chain complementarity-determining region 1 (CDR1) sequence of SEQ ID NO:48, a heavy chain complementarity-determining region 2 (CDR2) sequence of SEQ ID NO:30, a heavy chain complementarity-determining region 3 (CDR3) sequence of SEQ ID NO:44; and

a light chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence SEQ ID NO:8, the light chain variable domain comprising a light chain CDR1 sequence of SEQ ID NO:32, a light chain CDR2 sequence of SEQ ID NO:33, and a light chain CDR3 sequence of SEQ ID NO:34.

2. A protein comprising:

- (a) the antigen-binding site according to claim 1; and
- (b) an additional antigen-binding site.

3. The protein according to claim 2, wherein the additional antigen-binding site binds to a tumor-associated antigen, optionally wherein the tumor-associated antigen is selected from the group consisting of HER2, EpCAM, CD2, CD20, CD30, CD38, CD40, CD52, CD70, EGFR/ERBB1, IGF1R, HER3/ERBB3, HER4/ERBB4, MUC1, SLAMF7, PSCA, MICA, MICB, TRAILR1, TRAILR2, MAGE-A3, B7.1, B7.2, CTLA4, and PD1.

4. The protein according to claim 2 or 3, wherein the additional antigen-binding site comprises an antibody heavy chain variable domain, and wherein the protein further comprises:
(a) a first antibody constant region on the same polypeptide as the antibody heavy chain variable domain of the antigen-binding site that binds NKG2D, and (b) a second antibody constant region on the same polypeptide as the antibody heavy chain variable domain of the additional antigen-binding site.

5. The protein of claim 4, wherein the first antibody constant region and the second antibody constant region form a complex capable of binding CD16.
6. The protein according to claim 4 or 5, wherein the first antibody constant region and the second antibody constant region each comprise hinge, CH2, and CH3 domains.
7. The protein according to any one of claims 4-6, wherein the first antibody constant region and the second antibody constant region each comprise a CH1 domain.
8. The protein according to any one of claims 4-7, wherein:

the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region at Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, K392, T394, D399, S400, D401, F405, Y407, K409, T411, or K439, or any combination thereof; and

the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region at Q347, Y349, L351, S354, E356, E357, S364, T366, L368, K370, N390, K392, T394, D399, D401, F405, Y407, K409, T411, or K439, or any combination thereof,

optionally wherein:

- (a) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region at position T366, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region at T366, L368, or Y407, or any combination thereof;
- (b) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region at T366, L368, or Y407, or any combination thereof, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region at position T366;
- (c) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region at E357, K360, Q362, S364, L368,

K370, T394, D401, F405, or T411, or any combination thereof, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region at Y349, E357, S364, L368, K370, T394, D401, F405, or T411, or any combination thereof;

(d) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region at Y349, E357, S364, L368, K370, T394, D401, F405, or T411, or any combination thereof, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region at E357, K360, Q362, S364, L368, K370, T394, D401, F405, or T411, or any combination thereof;

(e) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region at L351, D399, S400, or Y407, or any combination thereof, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region at T366, N390, K392, K409, or T411, or any combination thereof;

(f) the amino acid sequence of the a first antibody constant region differs from the amino acid sequence of an IgG1 constant region at T366, N390, K392, K409, or T411, or any combination thereof, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region at L351, D399, S400, or Y407, or any combination thereof;

(g) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region at Q347, Y349, K360, or K409, or any combination thereof, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region at Q347, E357, D399, or F405, or any combination thereof;

(h) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region at Q347, E357, D399, or F405, or any combination thereof, and the amino acid sequence of the second antibody constant region

differs from the amino acid sequence of an IgG1 constant region at Y349, K360, Q347, or K409, or any combination thereof;

(i) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region at K370, K392, K409, or K439, or any combination thereof, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region at D356, E357, or D399, or any combination thereof;

(j) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region at D356, E357, or D399, or any combination thereof, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region at K370, K392, K409, or K439, or any combination thereof;

(k) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region at L351, E356, T366, or D399, or any combination thereof, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region at Y349, L351, L368, K392, or K409, or any combination thereof; or

(l) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region at Y349, L351, L368, K392, or K409, or any combination thereof, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region at L351, E356, T366, or D399, or any combination thereof.

9. The protein according to claim 8, wherein:

(a) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region by an S354C substitution, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region by a Y349C substitution;

- (b) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region by a Y349C substitution, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region by an S354C substitution;
- (c) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region by K360E and K409W substitutions, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region by Q347R, D399V and F405T substitutions;
- (d) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region by Q347R, D399V and F405T substitutions, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region by K360E and K409W substitutions;
- (e) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region by a T366W substitution, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region by T366S, T368A, and Y407V substitutions;
- (f) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region by T366S, T368A, and Y407V substitutions, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region by a T366W substitution;
- (g) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, L351Y, F405A, and Y407V substitutions, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, T366L, K392L, and T394W substitutions; or
- (h) the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, T366L, K392L, and T394W

substitutions, and the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, L351Y, F405A, and Y407V substitutions.

10. The protein according to claim 2 or 3, wherein the protein further comprises an antigen-binding site that binds CD16.
11. A protein comprising an antigen-binding site that competes for binding to human or cynomolgus NKG2D with an antibody comprising a heavy chain variable region having the amino acid sequence of SEQ ID NO:7 and a light chain variable region having the amino acid sequence of SEQ ID NO:8, wherein the competition is assessed by an epitope binning assay.
12. A formulation comprising the protein according to any one of claims 2-11 and a pharmaceutically acceptable carrier.
13. A cell comprising one or more nucleic acids encoding the protein according to any one of claims 2-11.
14. An *in vitro* method of enhancing tumor cell death, the method comprising exposing a tumor cell and a natural killer cell to the protein according to any one of claims 2-11.
15. The protein according to claim 11 for use in therapy.
16. Use of the protein according to claim 11 in the manufacture of a medicament for treating a cancer, optionally wherein the cancer is selected from the group consisting of acute myeloid leukemia, acute myelomonocytic leukemia, B cell lymphoma, bladder cancer, breast cancer, colorectal cancer, diffuse large B cell lymphoma esophageal cancer, Ewing's sarcoma, follicular lymphoma, gastric cancer, gastrointestinal cancer, gastrointestinal stromal tumors, glioblastoma, head and neck cancer, melanoma, mesothelioma, multiple myeloma, myelodysplastic syndrome, renal cell carcinoma, neuroblastoma, non-small cell lung cancer, neuroendocrine tumors, ovarian cancer, and pancreatic cancer, prostate cancer, sarcomas, small cell lung cancer, T cell lymphoma, testis cancer, thymic carcinoma, thyroid cancer, urothelial cancer, cancers infiltrated by myeloid-derived suppressor cells, cancers with extracellular matrix deposition, cancers with high levels of reactive stroma, and cancers with neoangiogenesis.