

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) 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.

[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 ~~aspect~~example of the ~~invention~~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 ~~embodiment~~examples, the antibody heavy chain variable domain is at least 95% identical to SEQ ID NO:1. In some ~~embodiment~~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 ~~embodiment~~examples, the heavy chain variable domain includes amino acid

sequences SYYM (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 aspectexample of the inventiondisclosure relates to an antibody heavy chain variable domain at least 90% identical to the amino acid sequence

5 QVQLVQSGAEVKKPGASVKVSKASGYTFTGYMHVWRQAPGQGLEWMGWINPN  
SGGTNYAQKFQGRVTMTRDTSISTAYMELSRSDDTAVYYCARDTGEYYDTDDH  
GMDVWGQGTTVTVSS (SEQ ID NO: 3, ADI-29463). In some embodimentexamples, the  
antibody heavy chain variable domain is at least 95% identical to SEQ ID NO:3. In some  
embodimentexamples, the heavy chain variable domain includes amino acid sequences  
10 YTFTGYMH (SEQ ID 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 embodimentexamples, the heavy chain variable domain includes amino acid  
sequences GYYMH (SEQ ID NO: 92) as CDR1, WINPNSGGTNYAQKFQG (SEQ ID  
15 NO:18) as CDR2, and DTGEYYDTDDHGMDV (SEQ ID NO:69) as CDR3 of SEQ ID  
NO:3.

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

EVQLLESGLLVQPGSLRLSCAASGFTFSSYAMSVWRQAPGKGLEWVSAISGSGG  
20 STYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKDGGYYDSGAGDYW  
GQGTLLVTVSS (SEQ ID NO:5, ADI-27744). In some embodimentexamples, the antibody  
heavy chain variable domain is at least 95% identical to SEQ ID NO:5. In some  
embodimentexamples, the heavy chain variable domain includes amino acid sequences  
FTFSSYAMS (SEQ ID NO:23) as the first complementarity-determining region ("CDR1"),  
25 AISGSGGSTYYADSVKG (SEQ ID NO:24) as the second CDR ("CDR2"), and  
AKDGGYYDSGAGDY (SEQ ID NO:25) as the third CDR ("CDR3") of SEQ ID NO:5. In  
some embodimentexamples, 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.

30 [0011] Another aspectexample of the inventiondisclosure relates to an antibody heavy chain variable domain at least 90% identical to the amino acid sequence

EVQLVESGGGLVKPGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSSYIYY  
ADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARGAPMGAAAGWFDPPWGQGTLL  
VTVSS (SEQ ID NO:7, ADI-27749). In some embodimentexamples, the antibody heavy

chain variable domain is at least 95% identical to SEQ ID NO:7. In some

embodimentexamples, 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

- 5 ARGAPMGAAAGWFDP (SEQ ID NO:31) as the third CDR (“CDR3”) of SEQ ID NO:7. In some embodimentexamples, 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 aspectexample of the inventiondisclosure relates to an antibody heavy chain variable domain at least 90% identical to the amino acid sequence EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARGAPIGAAAGWFDPWGQGTLLTVSS (SEQ ID NO:85, A49MI). In some embodimentexamples, the antibody heavy chain variable domain is at least 95% identical to SEQ ID NO:85. In some

- 15 embodimentexamples, 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 embodimentexamples, 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 aspectexample of the inventiondisclosure relates to an antibody heavy chain variable domain at least 90% identical to the amino acid sequence QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRTMTRDTSTSTVYMELSSLRSEDVAVYYCAREGAGFAYGMDYY YMDVWGKGTITVTVSS (SEQ ID NO:9, ADI-29378). In some embodimentexamples, the antibody heavy chain variable domain is at least 95% identical to SEQ ID NO:9. In some embodimentexamples, 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
- 25 AREGAGFAYGMDYYYMDV (SEQ ID NO:37) as the third CDR (“CDR3”) of SEQ ID NO:9. In some embodimentexamples, the heavy chain variable domain includes amino acid sequences SYMH (SEQ ID NO:45) as CDR1, IINPSGGSTSYAQKFQG (SEQ ID NO: 36) as CDR2, and EGAGFAYGMDYYYMDV (SEQ ID NO:72) as CDR3 of SEQ ID NO:9.
- 30

[0014] An antibody heavy chain variable domain of the inventiondisclosure 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 embodimentexamples, the amino acid sequence of the constant region is at least 90% identical to a human antibody constant region, such as a human IgG1 constant region, an IgG2 constant region, IgG3 constant region, or IgG4 constant region. In some other embodimentexamples, 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 embodimentexamples, 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 embodimentexamples, mutations that can be included into the C $\kappa$  of a human IgG1 constant region may be at amino acid E123, F116, S176, V163, S174, and/or T164.

[0016] In some embodimentexamples, 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  
EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGI  
PARFSGSGSGTEFTLTISLQSEDAVYYCQQYDDWPFTFGGGTKVEIK (SEQ ID  
NO:2, ADI-29379). In some embodimentexamples, the antibody light chain variable domain is at least 95% identical to SEQ ID NO:2. In some embodimentexamples, 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

EIVLTQSPGTLSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISLQSEDFAVYYCQQDDYWPPTFGGGTKVEIK (SEQ ID NO:4, ADI-29463). In some [embodimentexamples](#), the antibody light chain variable domain is at least 95% identical to SEQ ID NO:4. In some [embodimentexamples](#), 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

DIQMTQSPSSVSASVGDRVTITCRASQGIDSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQGVSYPRTFGGGTKVEIK (SEQ ID NO:6, ADI-27744). In some [embodimentexamples](#), the antibody light chain variable domain is at least 95% identical to SEQ ID NO:6. In some [embodimentexamples](#), 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

DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQGVSPRTPFGGGTKVEIK (SEQ ID NO:8, ADI-27749). In some [embodimentexamples](#), the antibody light chain variable domain is at least 95% identical to SEQ ID NO:8. In some [embodimentexamples](#), 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 QQGVSPRTP (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  
PARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSDNWPFTFGGGTKVEIK (SEQ ID

NO:10, ADI-29378). In some [embodimentexamples](#), the antibody light chain variable domain  
is at least 95% identical to SEQ ID NO:10. In some [embodimentexamples](#), 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 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 linked to the light  
chain variable domain).

**[0022]** In some [embodimentexamples](#), any NKG2D antigen-binding site disclosed in the  
instant [inventiondisclosure](#) 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, 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, ED-B  
fibronectin, PSA, and IL-6, MAGE-A3, B7.1, B7.2, CTLA4 or PD1.

**[0023]** In some [embodimentexamples](#), any NKG2D antigen-binding site disclosed in the  
instant [inventiondisclosure](#) 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 include one or more mutations affecting, for example, effector activity or CD16  
binding affinity).

**[0024]** Another [aspectexample](#) of the [inventiondisclosure](#) 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 antibody 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;

5 ~~2. The the antibody heavy chain variable domain according to claim 1, wherein the amino acid sequence comprises~~comprising :

a heavy chain complementarity-determining region 1 (CDR1) sequence ~~represented by the amino acid sequence~~ of SEQ ID NO:48;

10 a heavy chain complementarity-determining region 2 (CDR2) sequence ~~represented by the amino acid sequence~~ of SEQ ID NO:30; ~~and,~~

a heavy chain complementarity-determining region 3 (CDR3) sequence ~~represented by the amino acid sequence~~ of SEQ ID NO:44; and

~~3. The antibody heavy chain variable domain according to claim 1, wherein the amino acid sequence comprises:~~

15 ~~a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:29;~~

~~— a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:30; and~~

~~— a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:31.~~

~~4. The antibody heavy chain variable domain according to claim 1, wherein the amino acid sequence comprises:~~

20 ~~a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:48;~~

~~— a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:30; and~~

~~— a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:71.~~

~~5. The antibody heavy chain variable domain according to claim 1, wherein the amino acid sequence comprises:~~

25 ~~a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:29;~~

~~—— a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:30; and~~

~~—— a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:77.~~

~~6. The antibody heavy chain variable domain according to claim 1, wherein the amino acid sequence comprises:~~

5       ~~a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:48;~~

~~—— a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:30; and~~

~~—— a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:78.~~

~~7. An antibody heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:1.~~

10   ~~8. The antibody heavy chain variable domain according to claim 7, wherein the amino acid sequence comprises:~~

~~—— a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:11;~~

~~—— a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:12; and~~

~~—— a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:13.~~

15   ~~9. The antibody heavy chain variable domain according to claim 7, wherein the amino acid sequence comprises:~~

~~—— a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:45;~~

~~—— a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:12; and~~

~~—— a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:68.~~

20   ~~10. An antibody heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:3.~~

~~11. The antibody heavy chain variable domain according to claim 10, wherein the amino acid sequence comprises:~~

~~a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:17;~~

~~—— a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:18; and~~

~~—— a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:19.~~

~~12. The antibody heavy chain variable domain according to claim 10, wherein the amino acid sequence comprises:~~

5       ~~a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:46;~~

~~—— a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:18; and~~

~~—— a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:69.~~

~~13. An antibody heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:5.~~

10   ~~14. The antibody heavy chain variable domain according to claim 13, wherein the amino acid sequence comprises:~~

~~a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:23;~~

~~—— a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:24; and~~

~~—— a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:25.~~

15   ~~15. The antibody heavy chain variable domain according to claim 13, wherein the amino acid sequence comprises:~~

~~a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:47;~~

~~—— a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:24; and~~

~~—— a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:70.~~

20   ~~16. An antibody heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:9.~~

~~17. The antibody heavy chain variable domain according to claim 16, wherein the amino acid sequence comprises:~~

~~a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:35;~~

~~—— a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:36; and~~

~~—— a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:37.~~

~~18. The antibody heavy chain variable domain according to claim 16, wherein the amino acid sequence comprises:~~

5       ~~a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:45;~~

~~—— a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:36; and~~

~~—— a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:72.~~

10       ~~19. An antibody heavy chain comprising an antibody heavy chain variable domain according to any one of claims 1-18 and an amino acid sequence at least 90% identical to an antibody constant region.~~

~~20. The antibody heavy chain according to claim 19, wherein the antibody constant region is a human IgG constant region comprising hinge, CH2, and CH3 domains.~~

~~21. The antibody heavy chain according to claim 20, wherein the antibody constant region is a human IgG constant region further comprising a CH1 domain.~~

15       ~~22. The antibody heavy chain according to any one of claims 19-21, wherein the antibody constant region is 90% identical to a IgG1 constant region.~~

20       ~~23. The antibody heavy chain according to claim 22, wherein the amino acid sequence at least 90% identical to an 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, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, or K439, or any combination thereof.~~

25       ~~24. The antibody heavy chain according to claim 23, wherein the amino acid sequence at least 90% identical to an antibody constant region differs from the amino acid sequence of an IgG1 constant region by a substitution selected from the group consisting of Q347E, Q347R, Y349S, Y349K, Y349T, Y349D, Y349E, Y349C, 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, D399R, D399K, D399V, S400K, S400R, D401K, F405A, F405T, Y407A, Y407I, Y407V, K409F, K409W, K409D, T411D, T411E, K439D, and K439E, or any combination thereof.

25. ~~An antigen-binding site comprising the antibody heavy chain variable domain~~

5 ~~according to any one of claims 1-6, and an antibody~~ light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:8.

26. ~~The antigen-binding site according to claim 25, wherein the light chain variable domain comprises a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:32, a CDR2 sequence identical to the amino acid sequence of SEQ ID NO:33, and a CDR3 sequence identical to the amino acid sequence of SEQ ID NO:34.~~

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27. ~~The antigen-binding site according to claim 25 or 26 having a  $K_D$  of between 2—120 nM, as measured by surface plasmon resonance.~~

28. ~~An antigen-binding site comprising the antibody heavy chain variable domain according to any one of claims 7-9, and an antibody light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:2.~~

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29. ~~The antigen-binding site according to claim 28, wherein the light chain variable domain comprises a CDR1 sequence identical to the amino acid sequence of SEQ ID NO:14, a CDR2 sequence identical to the amino acid sequence of SEQ ID NO:15, and a CDR3 sequence identical to the amino acid sequence of SEQ ID NO:16.~~

30. ~~The antigen-binding site according to claim 28 or 29 having a  $K_D$  of between 5—500 nM, as measured by surface plasmon resonance.~~

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31. ~~An antigen-binding site comprising the antibody heavy chain variable domain according to any one of claims 10-12, and an antibody light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:4.~~

32. ~~The antigen-binding site according to claim 31, wherein the light chain variable domain comprises a CDR1 sequence identical to the amino acid sequence of SEQ ID NO:20, a CDR2 sequence identical to the amino acid sequence of SEQ ID NO:21, and a CDR3 sequence identical to the amino acid sequence of SEQ ID NO:22.~~

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33. ~~The antigen-binding site according to claim 31 or 32 having a  $K_D$  of between 6—600 nM, as measured by surface plasmon resonance.~~

34. ~~An antigen-binding site comprising the antibody heavy chain variable domain according to any one of claims 13–15, and an antibody light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:6.~~

35. ~~The antigen-binding site according to claim 34, wherein the light chain variable domain comprises a CDR1 sequence identical to the amino acid sequence of SEQ ID NO:26, a CDR2 sequence identical to the amino acid sequence of SEQ ID NO:27, and a CDR3 sequence identical to the amino acid sequence of SEQ ID NO:28.~~

36. ~~The antigen-binding site according to claim 34 or 35 having a  $K_D$  of between 1—100 nM, as measured by surface plasmon resonance.~~

37. ~~An antigen-binding site comprising the antibody heavy chain variable domain according to any one of claims 16–18, and an antibody light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:10.~~

38. ~~The antigen-binding site according to claim 37, wherein the light chain variable domain comprises a CDR1 sequence identical to the amino acid sequence of SEQ ID NO:38, a CDR2 sequence identical to the amino acid sequence of SEQ ID NO:39, and a CDR3 sequence identical to the amino acid sequence of SEQ ID NO:40.~~

39. ~~The antigen-binding site according to claim 37 or 38 having a  $K_D$  of between 6—600 nM, as measured by surface plasmon resonance.~~

402. A protein comprising:

(a) an antigen-binding site according to any one of claims 25–391, wherein the antigen-binding site binds to NKG2D; and (b) an additional antigen-binding site.

413. The protein according to claim 402, wherein the additional antigen-binding site binds to a tumor-associated antigen, optionally:-

~~42. The protein according to claim 41, wherein the tumor-associated antigen is selected from the group consisting of CD33, HER2, EpCAM, CD2<sub>L</sub>, CD19, CD20, CD30, CD38, CD40, CD52, CD70, EGFR/ERBB1, IGF1R, HER3/ERBB3, HER4/ERBB4, MUC1, cMET,~~

SLAMF7, PSCA, MICA, MICB, TRAILR1, TRAILR2, MAGE-A3, B7.1, B7.2, CTLA4, and PD1.

434. The protein according to ~~any one of~~ claims ~~40-422 or 3~~, wherein the additional antigen-binding site ~~binding NKG2D~~ comprises an first antibody heavy chain variable domain, and ~~the additional antigen-binding site comprises a second antibody heavy chain variable domain; and~~

wherein the ~~protein further comprises: (a) first antibody heavy chain variable domain is present on a first polypeptide further comprising~~ 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) the second antibody heavy chain variable domain is present on a second polypeptide further comprising~~ a second antibody constant region on the same polypeptide as the antibody heavy chain variable domain of the additional antigen-binding site.

445. The protein ~~according to~~ claim 434, wherein the first antibody constant region and the second antibody constant region form a complex capable of binding CD16.

456. The protein according to claim ~~43-4~~ or 445, wherein the first antibody constant region and the second antibody constant region each comprise hinge, CH2, and CH3 domains.

467. The protein according to any one of claims ~~434-456~~, wherein the first antibody constant region and the second antibody constant region each ~~further~~ comprise a CH1 domain.

~~47. The protein according to any one of claims 43-46, wherein the amino acid sequences of the first antibody constant region and the second antibody constant region are each at least 90% identical to human IgG1 constant region.~~

488. The protein according to any one of claims ~~474-7~~, 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

5 ~~49. — The protein according to claim 47, 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 ~~wherein~~ 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.;

10 ~~50. — The protein according to claim 47, wherein (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 ~~wherein~~ the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region at position T366.;

15 ~~51. — The protein according to claim 47, wherein (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 ~~wherein~~ 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.;

20 ~~52. — The protein according to claim 47, wherein (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 ~~wherein~~ 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.;

25 ~~53. — The protein according to claim 47, wherein (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 ~~wherein~~ 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.;

54. ~~The protein according to claim 47, wherein (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 ~~wherein~~ 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.;

55. ~~The protein according to claim 47, wherein (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 ~~wherein~~ 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.;

56. ~~The protein according to claim 47, wherein (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 ~~wherein~~ 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.;

57. ~~The protein according to claim 47, wherein (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 ~~wherein~~ 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.;

58. ~~The protein according to claim 47, wherein (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 ~~wherein~~ 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.;

59. ~~The protein according to claim 47, wherein (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 ~~wherein~~ 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.;

60. ~~The protein according to claim 47, wherein (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 ~~wherein~~ 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.

619. The protein according to claim ~~47~~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 ~~wherein~~ 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;

62. ~~The protein according to claim 47, wherein (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 ~~wherein~~ 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;

63. ~~The protein according to claim 47, wherein (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 ~~wherein~~ the amino acid sequence of the second antibody constant region differs from the amino acid sequence of an IgG1 constant region by ~~Q347R~~Q347R, D399V and F405T substitutions;

64. ~~The protein according to claim 47, wherein (d)~~ the amino acid sequence of the first antibody constant region differs from the amino acid sequence of an IgG1 constant region by ~~Q347R~~Q347R, D399V and F405T substitutions, and ~~wherein~~ 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;

65. ~~The protein according to claim 47, wherein (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 ~~wherein~~ 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;

~~66. The protein according to claim 47, wherein (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 wherein 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;~~

~~67. The protein according to claim 47, wherein (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 wherein 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~~

~~68. The protein according to claim 47, wherein (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 wherein 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.~~

~~69~~10. The protein according to ~~any one of claims 40-422~~ or 3, wherein the protein further comprises an antigen-binding site capable of binding CD16.

~~70~~11. A protein comprising an antigen-binding site that competes for binding to human and/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.

~~71. A protein comprising an antigen-binding site that competes for binding to human and/or cynomolgus NKG2D with an antibody comprising a heavy chain variable region having the amino acid sequence of SEQ ID NO:85 and a light chain variable region having the amino acid sequence of SEQ ID NO:8.~~

~~72. A protein comprising an antigen-binding site that competes for binding to human and/or cynomolgus NKG2D with an antibody comprising a heavy chain variable region having the amino acid sequence of SEQ ID NO:1 and a light chain variable region having the amino acid sequence of SEQ ID NO:2.~~

~~73. — A protein comprising an antigen-binding site that competes for binding to human and/or cynomolgus NKG2D with an antibody comprising a heavy chain variable region having the amino acid sequence of SEQ ID NO:3 and a light chain variable region having the amino acid sequence of SEQ ID NO:4.~~

5 ~~74. — A protein comprising an antigen-binding site that competes for binding to human and/or cynomolgus NKG2D with an antibody comprising a heavy chain variable region having the amino acid sequence of SEQ ID NO:5 and a light chain variable region having the amino acid sequence of SEQ ID NO:6.~~

10 ~~75. — A protein comprising an antigen-binding site that competes for binding to human and/or cynomolgus NKG2D with an antibody comprising a heavy chain variable region having the amino acid sequence of SEQ ID NO:9 and a light chain variable region having the amino acid sequence of SEQ ID NO:20.~~

~~76~~12. A formulation comprising the protein according to any one of claims ~~40~~2-7511 and a pharmaceutically acceptable carrier.

15 ~~77~~13. A cell comprising one or more nucleic acids encoding the protein according to any one of claims ~~40~~2-7511.

~~78~~14. An *in vitro* method of enhancing tumor cell death, the method comprising exposing a tumor and natural killer cells to the protein according to any one of claims ~~40~~2-7511.

20 ~~79~~15. ~~A method of treating cancer, wherein the method comprises administering T~~the protein according to ~~any one of claims 40-75-11 or the formulation according to claim 76 to a patient for use in therapy.~~

~~80~~16. Use of the protein ~~The method~~ according to claim ~~79~~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

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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.