

WHAT IS CLAIMED IS:

1. A method of enhancing tumor cell death directly or indirectly, the method comprising exposing a tumor and natural killer cells to a protein comprising:

- 5 (a) a first antigen-binding site that binds NKG2D;
- (b) a second antigen-binding site that binds a tumor-associated antigen; and
- (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16;

 in combination with a second therapeutic agent selected from: a checkpoint blocker, a
10 cytokine, a TLR agonist, a STING agonist, a chemotherapeutic agent, a cancer targeted agent, an oncolytic virus, a vaccine, radiation, an adoptive NK therapy, a stem cell transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces cellular senescence,

 wherein the first antigen-binding site comprises a heavy chain variable domain and a
15 light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:47 and the light chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:48.

2. A method of treating cancer in a subject in need thereof, the method comprising
20 administering to the subject a protein comprising:

- (a) a first antigen-binding site that binds NKG2D;
- (b) a second antigen-binding site that binds a tumor-associated antigen; and
- (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16;
- 25 or a formulation comprising the protein;

 in combination with a second therapeutic agent selected from: a checkpoint blocker, a cytokine, a TLR agonist, a STING agonist, a chemotherapeutic agent, a cancer target agent,

an oncolytic virus, a vaccine, radiation, an adoptive NK therapy, a stem cell transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces cellular senescence,

5 wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:47 and the light chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:48.

3. The method of claim 1 or 2, wherein the heavy chain variable domain comprises a complementarity-determining region 1 (CDR1) sequence represented by the amino acid
10 sequence of SEQ ID NO:92, a complementarity-determining region 2 (CDR2) sequence represented by the amino acid sequence of SEQ ID NO:58, and a complementarity-determining region 3 (CDR3) sequence represented by the amino acid sequence of SEQ ID NO:113; and the light chain variable domain comprises a CDR1 sequence represented by the
15 amino acid sequence of SEQ ID NO:60, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:61, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:62.

4. The method of claim 3, wherein the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:92, a CDR2 sequence
20 represented by the amino acid sequence of SEQ ID NO:58, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:93; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:60, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:61, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:62.

25 5. The method of claim 3, wherein the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:57, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:58, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:59; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID
30 NO:60, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:61, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:62.

6. The method of claim 3, wherein the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:92, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:58, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:104; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:60, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:61, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:62.
7. The method of claim 3, wherein the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:57, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:58, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:103; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:60, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:61, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:62.
8. A method of enhancing tumor cell death directly or indirectly, the method comprising exposing a tumor and natural killer cells to a protein comprising:
- (a) a first antigen-binding site that binds NKG2D;
 - (b) a second antigen-binding site that binds a tumor-associated antigen; and
 - (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16;
- in combination with a second therapeutic agent selected from: a checkpoint blocker, a cytokine, a TLR agonist, a STING agonist, a chemotherapeutic agent, a cancer targeted agent, an oncolytic virus, a vaccine, radiation, an adoptive NK therapy, and a stem cell transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces cellular senescence,
- wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:45 and the light

chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:46.

9. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject a protein comprising:

- 5 (a) a first antigen-binding site that binds NKG2D;
- (b) a second antigen-binding site that binds a tumor-associated antigen; and
- (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16;

or a formulation comprising the protein;

10 in combination with a second therapeutic agent selected from: a checkpoint blocker, a cytokine, a TLR agonist, a STING agonist, a chemotherapeutic agent, a cancer target agent, an oncolytic virus, a vaccine, radiation, an adoptive NK therapy, and a stem cell transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces cellular senescence,

15 wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:45 and the light chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:46.

20 10. The method of claim 8 or 9, wherein the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:90, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:52, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:91; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID
25 NO:54, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:55, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:56.

11. The method of claim 8 or 9, wherein the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:51, a CDR2

sequence represented by the amino acid sequence of SEQ ID NO:52, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:53; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:54, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:55, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:56.

12. A method of enhancing tumor cell death directly or indirectly, the method comprising exposing a tumor and natural killer cells to a protein comprising:

- (a) a first antigen-binding site that binds NKG2D;
- (b) a second antigen-binding site that binds a tumor-associated antigen; and
- 10 (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16;

in combination with a second therapeutic agent selected from: a checkpoint blocker, a cytokine, a TLR agonist, a STING agonist, a chemotherapeutic agent, a cancer targeted agent, an oncolytic virus, a vaccine, radiation, an adoptive NK therapy, and a stem cell transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces cellular senescence,

wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:49 and the light chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:50.

13. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject a protein comprising:

- (a) a first antigen-binding site that binds NKG2D;
- 25 (b) a second antigen-binding site that binds a tumor-associated antigen; and
- (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16;

or a formulation comprising the protein;

in combination with a second therapeutic agent selected from: a checkpoint blocker, a cytokine, a TLR agonist, a STING agonist, a chemotherapeutic agent, a cancer target agent, an oncolytic virus, a vaccine, radiation, an adoptive NK therapy, and a stem cell transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces cellular senescence,

wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:49 and the light chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:50.

14. The method of claim 12 or 13, wherein the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:94, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:64, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:95; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:66, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:67, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:68.

15. The method of claim 12 or 13, wherein the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:63, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:64, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:65; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:66, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:67, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:68.

16. A method of enhancing tumor cell death directly or indirectly, the method comprising exposing a tumor and natural killer cells to a protein comprising:

- (a) a first antigen-binding site that binds NKG2D;
- (b) a second antigen-binding site that binds a tumor-associated antigen; and

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

in combination with a second therapeutic agent selected from: a checkpoint blocker, a cytokine, a TLR agonist, a STING agonist, a chemotherapeutic agent, a cancer targeted agent, an oncolytic virus, a vaccine, radiation, an adoptive NK therapy, and a stem cell transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces cellular senescence,

wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:114 and the light chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:115.

17. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject a protein comprising:

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

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

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

or a formulation comprising the protein;

in combination with a second therapeutic agent selected from: a checkpoint blocker, a cytokine, a TLR agonist, a STING agonist, a chemotherapeutic agent, a cancer target agent, an oncolytic virus, a vaccine, radiation, an adoptive NK therapy, and a stem cell transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces cellular senescence,

wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:114 and the light

chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:115.

18. The method of claim 16 or 17, wherein the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:122, a CDR2
5 sequence represented by the amino acid sequence of SEQ ID NO:117, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:123; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:119, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:120, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:121.
- 10 19. The method of claim 16 or 17, wherein the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:116, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:117, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:118; and the light chain variable
15 domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:119, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:120, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:121.
20. A method of enhancing tumor cell death directly or indirectly, the method comprising exposing a tumor and natural killer cells to a protein comprising:
 - (a) a first antigen-binding site that binds NKG2D;
 - 20 (b) a second antigen-binding site that binds a tumor-associated antigen; and
 - (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16;

in combination with a second therapeutic agent selected from: a checkpoint blocker, a cytokine, a TLR agonist, a STING agonist, a chemotherapeutic agent, a cancer targeted
25 agent, an oncolytic virus, a vaccine, radiation, an adoptive NK therapy, and a stem cell transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces cellular senescence,

wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid

sequence at least 90% identical to the amino acid sequence of SEQ ID NO:124 and the light chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:125.

21. A method of treating cancer in a subject in need thereof, the method comprising
5 administering to the subject a protein comprising:

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

10 or a formulation comprising the protein;

in combination with a second therapeutic agent selected from: a checkpoint blocker, a cytokine, a TLR agonist, a STING agonist, a chemotherapeutic agent, a cancer target agent, an oncolytic virus, a vaccine, radiation, an adoptive NK therapy, and a stem cell transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces
15 cellular senescence,

wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:124 and the light chain variable domain comprising an amino acid sequence at least 90% identical to the
20 amino acid sequence of SEQ ID NO:125.

22. The method of claim 20 or 21, wherein the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:122, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:117, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:130; and the light chain variable
25 domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:127, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:128, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:129.

23. The method of claim 20 or 21, wherein the heavy chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:116, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:117, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:126; and the light chain variable domain comprises a CDR1 sequence represented by the amino acid sequence of SEQ ID NO:127, a CDR2 sequence represented by the amino acid sequence of SEQ ID NO:128, and a CDR3 sequence represented by the amino acid sequence of SEQ ID NO:129.

24. A method of enhancing tumor cell death directly or indirectly, the method comprising exposing a tumor and natural killer cells to a protein comprising:

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

in combination with a second therapeutic agent selected from: a TLR agonist, a STING agonist, an oncolytic virus, a vaccine, an adoptive NK therapy, a stem cell transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces cellular senescence.

25. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject a protein comprising:

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

or a formulation comprising the protein;

in combination with a second therapeutic agent selected from: a TLR agonist, a STING agonist, an oncolytic virus, a vaccine, an adoptive NK therapy, and a stem cell

transplant (SCT) therapy, a chimeric antigen receptor (CAR) T cell therapy, and an agent that induces cellular senescence.

26. The method of any one of claims 1-23, wherein the checkpoint blocker is selected from: an anti-PD1 antibody, an anti-PD-L1 antibody, an anti-CTLA4 antibody, an anti-KIR
5 antibody, an anti-NKG2A antibody, an anti-LAG3 antibody, and an anti-TIM3 antibody.
27. The method of any one of claims 1-23, wherein the cytokine is selected from: IL-2, IL-15, IL-12, INF α , IL-21, PEG-IL-2 and IL15/IL15R heterodimers.
28. The method of any one of claims 1-25, wherein the TLR agonist is selected from a TLR7 agonist, a TLR8 agonist, a TLR7/8 agonist, a TLR9 agonist, a TLR4 agonist, and a
10 TLR3 agonist.
29. The method of any one of claims 1-25, wherein the STING agonist is ADU-S100.
30. The method of any one of claims 1-23, wherein the chemotherapeutic agent is paclitaxel, nab-paclitaxel or cyclophosphamide.
31. The method of any one of claims 1-23, wherein the checkpoint blocker is selected
15 from: nivolumab, pembrolizumab, atezolizumab, durvalumab, avelumab, ipilimumab, tremelimumab, lirilumab, and monalizumab.
32. The method of any one of claims 1-25, wherein the TLR agonist is selected from: R848/resiquimod, VTX-2337, imiquimod, and CpG oligodeoxynucleotide.
33. The method of any one of claims 2-7, 9-11, 13-15, 17-19, and 21-32, wherein the
20 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,
25 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.

34. The method of any one of claims 1-33, wherein the first antigen-binding site of the protein binds to NKG2D in humans, non-human primates, and rodents.
- 5 35. The method of any one of claims 24-34, wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.
36. The method of any one of claims 1-35, wherein the heavy chain variable domain and the light chain variable domain are present on the same polypeptide.
37. The method of any one of claims 1-36, wherein the second antigen-binding site also
10 comprises a heavy chain variable domain and a light chain variable domain.
38. The method of claim 37, wherein the light chain variable domain of the first antigen-binding site has an amino acid sequence identical to the amino acid sequence of the light chain variable domain of the second antigen-binding site.
39. The method of any one of claims 24-34, wherein the first antigen-binding site is a
15 single-domain antibody.
40. The method of claim 39, wherein the single-domain antibody is a V_HH fragment or a V_{NAR} fragment.
41. The method of any one of claims 1-36, 39, and 40, wherein the second antigen-binding site is a single-domain antibody.
- 20 42. The method of claim 41, wherein the second antigen-binding site is a V_HH fragment or a V_{NAR} fragment.
43. The method of any one of claims 1-40, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.
44. The method of any one of claims 1-43, wherein the tumor-associated antigen is
25 selected from the group consisting of EpCAM, CD2, 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 PD-L1.

45. The method of any one of claims 1-44, wherein the protein comprises a portion of an antibody Fc domain sufficient to bind CD16, wherein the antibody Fc domain comprises
5 hinge and CH2 domains.
46. The method of any one of claims 1-44, wherein the protein comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody.
47. The method of any one of claims 2-7, 9-11, 13-15, 17-19, and 21-46, wherein the formulation further comprises a pharmaceutically acceptable carrier.