

[0006] Natural killer (NK) cells are a component of the innate immune system and make up approximately 15% of circulating lymphocytes. NK cells infiltrate virtually all tissues and were originally characterized by their ability to kill tumor cells effectively without the need for prior sensitization. Activated NK cells kill target cells by means similar to cytotoxic T cells – *i.e.*, via cytolytic granules that contain perforin and granzymes as well as via death receptor pathways. Activated NK cells also secrete inflammatory cytokines such as IFN- γ and chemokines that promote the recruitment of other leukocytes to the target tissue.

[0007] NK cells respond to signals through a variety of activating and inhibitory 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 foreign cells or 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 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. NKG2D is also be found on T cells where it acts as a costimulatory receptor. The ability to modulate NK cell function via NKG2D is useful in various therapeutic contexts including malignancy.

SUMMARY

[0008] In one aspect, the present disclosure refers to a polypeptide comprising a single-chain variable fragment (scFv) linked to an antibody constant domain via a hinge comprising Ala-Ser, wherein the scFv comprises a heavy chain variable domain and a light chain variable domain. In another aspect, the present disclosure refers to a protein comprising (a) the polypeptide as described herein, wherein the scFv of the polypeptide comprises a first antigen-binding site; (b) a second antigen-binding site; and (c) a second antibody constant domain.

[0008a] In another aspect, the present disclosure refers to a protein comprising: (a) a first antigen-binding site that binds a tumor-associated antigen; (b) a second antigen-binding site that binds the same tumor-associated antigen as the first antigen-binding site; (c) a third antigen-binding site that binds NKG2D; and (d) an antibody constant region or a portion thereof sufficient to bind CD16, or a fourth antigen-binding site that binds CD16.

[0008b] In yet another aspect, the present disclosure refers to a protein comprising: (a) an antigen-binding site that binds NKG2D; (b) an antigen-binding T cell receptor (TCR) fragment; and (c) an antibody constant region or a portion thereof sufficient to bind CD16, or an additional antigen-binding site that binds CD16. In yet another aspect, the present disclosure refers to a formulation comprising a protein as described herein and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure refers to a cell comprising one or more nucleic acids encoding a protein as described herein.

[0008c] In a further aspect, the present disclosure refers to the protein as described herein or the formulation as described herein for use in therapy. In a further aspect, the present disclosure refers to use of the protein as described herein or the formulation as described herein in the manufacture of a medicament for treating cancer.

[0008d] In one example, the current disclosure provides an improvement on a single-chain variable fragment (scFv) that is linked to an antibody constant domain via a hinge sequence. In some examples, the hinge comprises amino acids Ala-Ser. In some other examples, the hinge comprises amino acids Ala-Ser and Thr-Lys-Gly. The scFv may include a heavy chain variable domain and a light chain variable domain. In some examples, the scFv binds NKG2D or a tumor-associated antigen. The hinge sequence provides flexibility of binding to the target antigen.

[0009] In some examples of the scFv, the heavy chain variable domain forms a disulfide bridge with the light chain variable domain. For example, a disulfide bridge can be formed between the C44 residue of the heavy chain variable domain and the C100 residue of the light chain variable domain. In some examples, the heavy chain variable domain is linked to the light chain variable domain via a flexible linker, such as (G₄S)₄ (SEQ ID NO:427). In some examples of the scFv, the heavy chain variable domain is positioned at the N terminus of the light chain variable domain. In some examples of the scFv, the heavy chain variable domain is positioned at the C terminus of the light chain variable domain.

[0010] In some examples, the antibody constant domain linked to the scFv is able to bind to CD16. In some examples, the antibody constant domain comprises a CH2 domain and a CH3 domain of an IgG antibody, for example, a human IgG1 antibody. In some examples, mutations are introduced in the antibody constant domain to enable heterodimerization with another antibody constant domain. For example, if the antibody constant domain is derived

from the constant domain of a human IgG1, the antibody constant domain can comprise an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody, and differs at one or more positions selected from the group consisting of Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439. In some examples, the antibody constant domain can comprise an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody, and differs by one or more substitutions 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.

[0011] In another example, the current disclosure provides a protein that contains the scFv linked to an antibody constant region described above. In some examples, the protein includes a first antigen-binding site, which comprises the scFv linked to an antibody constant domain; a second antigen-binding site which may take the Fab or the scFv format described here; and a second antibody constant domain linked to the second antigen-binding site. In some examples, the protein is multi-specific, wherein the first antigen binding site binds NKG2D, the second antigen binding sites bind a tumor-associated antigen, and the antibody constant regions bind CD16.

[0012] In some other examples, the protein is multi-specific, wherein the first antigen binding site binds a tumor-associated antigen, the second antigen binding site binds NKG2D, and the antibody constant regions bind CD16. The antibody constant region linked to the scFv can heterodimerize with a second antibody constant region. The multi-specific binding proteins in these examples bind to the NKG2D receptor and CD16 receptor on natural killer cells, and to a tumor-associated antigen on cancer cells. Such proteins can engage more than one kind of NK-activating receptor, and may block the binding of natural ligands to NKG2D. In certain examples, the proteins can agonize NK cells in humans. In some examples, the proteins can agonize NK cells in humans, and/or in other species such as rodents and/or cynomolgus monkeys.

[0013] In another example, the current disclosure provides a multi-specific binding protein, and the protein contains a first antigen-binding site that binds a tumor-associated antigen; a second antigen-binding site that binds the same tumor-associated antigen as the first antigen-binding site; a third antigen-binding site that binds NKG2D; and an antibody constant region or a portion thereof sufficient to bind CD16, or a fourth antigen-binding site that binds CD16. Any one of the antigen binding sites can either take the form of an Fab or an scFv, such as those described above. The multi-specific binding protein provided here provides bivalent engagement of tumor-associated antigen, thereby stabilizing the tumor-associated antigen on cancer cell surface, and enhance cytotoxicity towards the cancer cells by NK cells.

[0014] In some examples, bivalent engagement of tumor-associated antigens by the multi-specific binding proteins confer stronger binding of the multi-specific binding proteins to the cancer cells, thereby facilitating stronger cytotoxic response of NK cells towards the cancer cells, especially towards cancer cells expressing a low level of tumor-associated antigen.

[0015] In some examples, the multi-specific binding proteins incorporate a portion of an antibody Fc domain sufficient to bind CD16, wherein the antibody Fc domain comprises a hinge and a CH2 domain and/or amino acid sequences at least 90% identical to amino acid sequence 234-332 of a human IgG antibody. Mutations can be introduced into the antibody constant domain to enable heterodimerization with another antibody constant domain. For example, if the antibody constant domain is derived from the constant domain of a human IgG1, the antibody constant domain can comprise an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody, and differs at one or more positions selected from the group consisting of Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439.

[0016] In some examples, the antibody constant domain can comprise an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody, and differs by one or more substitutions 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.

[0017] The tumor-associated antigen binding site described above can be a site that bind to any tumor-associated antigen, for example, ANO1, BCMA, EpCAM, CAIX, CEA, CCR4, CD2, CD123, CD133, CD19, CD20, CD22, CD25, CD30, CD33, CD37, CD38, CD40, CD52, CD70, CLAUDIN-18.2, DLL3, EGFR/ERBB1, GD2, IGF1R, HER2, HER3/ERBB3, HER4/ERBB4, MUC1, cMET, SLAMF7, PSMA, mesothelin, MICA, MICB, TRAILR1, TRAILR2, TROP2, MAGE-A3, B7.1, B7.2, CTLA4, PD1, 5T4, GPNMB, FR-alpha, PAPP-A, FLT3, GPC3, CXCR4, ROR1, ROR2, HLA-E, PD-L1, VLA4, CD44, CD13, CD15, CD47, CLL1, CD81, CD23, CD79a, CD79b, CD80, CRLF2, SLAMF7, CD138, CA125, NaPi2b, Nectin4, ADAM8, ADAM9, SLC44A4, CA19-9, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LILRA1, LILRA2, LILRA3, LILRA4, LILRA5, and LILRA6, CCR8, CD7, CTLA4, CX3CR1, ENTPD1, HAVCR2, IL-1R2, PDCD1LG2, TIGIT, TNFRSF4, TNFRSF8, TNFRSF9, GEM, NT5E, TNFRSF18, MUC1, P-cadherin, Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, Axl, erbB-3, EDNRB, Tyrp1, CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4 (VTCN1), CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, CCR5, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, EDNRB, OLR1, and TYRP1.

[0018] In another example, the present disclosure provides a protein comprising: (a) an antigen-binding site that binds NKG2D; (b) an antigen-binding T cell receptor (TCR) fragment; and (c) an antibody constant region or a portion thereof sufficient to bind CD16, or an additional antigen-binding site that binds CD16. In certain examples, the protein is a multi-specific binding protein comprising an antigen-binding TCR fragment that binds a tumor-associated antigen (TAA).

[0019] In certain examples, the antigen-binding site is an Fab fragment, and the antigen-binding TCR fragment is a single-chain TCR (scTCR) fragment. In certain examples, the scTCR fragment is linked to a polypeptide chain of the antibody constant region via a hinge comprising Ala-Ser. In certain examples, the hinge further comprises amino acid sequence Thr-Lys-Gly.

[0020] In certain examples, the antigen-binding site is an scFv, and the antigen-binding TCR fragment is an extracellular TCR fragment. In certain examples, the scFv is linked to a

polypeptide chain of the antibody constant region via a hinge comprising Ala-Ser. In certain examples, the hinge further comprises amino acid sequence Thr-Lys-Gly.

[0021] In certain examples, the antigen-binding site is an Fab fragment, and the antigen-binding TCR fragment is an extracellular TCR fragment.

5 **[0022]** In certain examples, the multi-specific binding protein further comprises an additional antigen-binding TCR fragment that binds the same antigen as the antigen-binding TCR fragment. In certain examples, the antigen-binding site is an scFv, and the antigen-binding TCR fragment and the additional antigen-binding TCR fragment are extracellular TCR fragments. In certain examples, the antigen-binding site is an scFv, and the antigen-binding TCR fragment and the additional antigen-binding TCR fragment are scTCR
10 fragments.

[0023] In certain examples of the multi-specific binding protein that contains an scFv, the scFv comprises a heavy chain variable domain linked to a light chain variable domain via a flexible linker. In certain examples, the flexible linker comprises (G₄S)₄. In certain
15 examples, the scFv comprises a heavy chain variable domain positioned at the N-terminus or the C-terminus of a light chain variable domain.

[0024] In certain examples, the antigen-binding site comprises a heavy chain variable domain and a light chain variable domain, and wherein the heavy chain variable domain forms a disulfide bridge with the light chain variable domain. In certain examples, the
20 disulfide bridge is formed between Cys at position 44 in the heavy chain variable domain and Cys at position 100 in the light chain variable domain, the positions defined under the Kabat numbering. In certain examples, the antigen-binding site that contains such a disulfide bridge is an scFv.

[0025] In certain examples, the antigen-binding site binds to NKG2D in humans.

25 **[0026]** In certain examples, the antigen-binding TCR fragment binds a peptide from a tumor-associated antigen presented by a major histocompatibility complex (MHC).

[0027] In certain examples, the antigen-binding TCR fragment binds an ELAVL4 peptide having the amino acid sequence of SEQ ID NO:425 presented by HLA-A*02:01:48. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable

domain related to SEQ ID NO:351 and a beta chain variable domain related to SEQ ID NO:352. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:351 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:353) of the alpha chain variable domain. Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:352 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:354) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:349 and a beta chain amino acid sequence set forth in SEQ ID NO:350.

[0028] In certain examples, the antigen-binding TCR fragment binds an Insulin peptide having the amino acid sequence of SEQ ID NO:426 presented by HLA-A*02:01:48. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:357 and a beta chain variable domain related to SEQ ID NO:358. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:357 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:359) of the alpha chain variable domain. Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:358 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:360) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:355 and a beta chain amino acid sequence set forth in SEQ ID NO:356.

[0029] In certain examples, the antigen-binding TCR fragment binds a TERT peptide having the amino acid sequence of SEQ ID NO:340 presented by HLA-A*02:01:48. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:363 and a beta chain variable domain related to SEQ ID NO:364. For example, in certain examples, the antigen-binding TCR fragment comprises an

alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:363 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:365) of the alpha chain variable domain.

Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:364 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:366) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:361 and a beta chain amino acid sequence set forth in SEQ ID NO:362.

[0030] In certain examples, the antigen-binding TCR fragment binds an ERBB2 peptide having the amino acid sequence of SEQ ID NO:341 presented by HLA-A*02. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:430 and a beta chain variable domain related to SEQ ID NO:431. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:430 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:367) of the alpha chain variable domain. Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:431 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:368) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:428 and a beta chain amino acid sequence set forth in SEQ ID NO:429.

[0031] In certain examples, the antigen-binding TCR fragment binds a WT1 peptide having the amino acid sequence of SEQ ID NO:342 presented by HLA-A*02. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:434 and a beta chain variable domain related to SEQ ID NO:435. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:434 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:369) of the alpha chain variable domain. Similarly, in

certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:435 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:370) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:432 and a beta chain amino acid sequence set forth in SEQ ID NO:433.

[0032] In certain examples, the antigen-binding TCR fragment binds a WT1 peptide having the amino acid sequence of SEQ ID NO:342 presented by HLA-A*02. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:438 and a beta chain variable domain related to SEQ ID NO:439. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:438 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:371) of the alpha chain variable domain. Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:439 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:372) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:436 and a beta chain amino acid sequence set forth in SEQ ID NO:437.

[0033] In certain examples, the antigen-binding TCR fragment binds a MAGE-A3 peptide having the amino acid sequence of SEQ ID NO:343 presented by HLA-A1. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:375 and a beta chain variable domain related to SEQ ID NO:376. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:375 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:377) of the alpha chain variable domain. Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:376 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:378) of the beta chain variable domain. In certain examples,

the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:373 and a beta chain amino acid sequence set forth in SEQ ID NO:374.

[0034] In certain examples, the antigen-binding TCR fragment binds a MART1 peptide having the amino acid sequence of SEQ ID NO:344 presented by HLA-A2. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:381 and a beta chain variable domain related to SEQ ID NO:382. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:381 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:383) of the alpha chain variable domain. Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:382 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:384) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:379 and a beta chain amino acid sequence set forth in SEQ ID NO:380.

[0035] In certain examples, the antigen-binding TCR fragment binds a BIRC5 peptide having the amino acid sequence of SEQ ID NO:346 presented by HLA-A2. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:442 and a beta chain variable domain related to SEQ ID NO:443. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:442 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:389) of the alpha chain variable domain. Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:443 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:390) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:440 and a beta chain amino acid sequence set forth in SEQ ID NO:441.

[0036] In certain examples, the antigen-binding TCR fragment binds a BIRC5 peptide having the amino acid sequence of SEQ ID NO:346 presented by HLA-A2. In certain

examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:444 and a beta chain variable domain related to SEQ ID NO:445. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:444 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:391) of the alpha chain variable domain. Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:445 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:392) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:385 and a beta chain amino acid sequence set forth in SEQ ID NO:386.

[0037] In certain examples, the antigen-binding TCR fragment binds a PRAME peptide having the amino acid sequence of SEQ ID NO:347 presented by HLA-A2. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:395 and a beta chain variable domain related to SEQ ID NO:396. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:395 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:397) of the alpha chain variable domain. Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:396 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:398) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:393 and a beta chain amino acid sequence set forth in SEQ ID NO:394.

[0038] In certain examples, the antigen-binding TCR fragment binds a PRAME peptide having the amino acid sequence of SEQ ID NO:347 presented by HLA-A2. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:401 and a beta chain variable domain related to SEQ ID NO:402. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%,

or 100%) identical to SEQ ID NO:401 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:403) of the alpha chain variable domain. Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%)

5 identical to SEQ ID NO:402 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:404) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:399 and a beta chain amino acid sequence set forth in SEQ ID NO:400.

[0039] In certain examples, the antigen-binding TCR fragment binds a PRAME peptide

10 having the amino acid sequence of SEQ ID NO:347 presented by HLA-A2. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:407 and a beta chain variable domain related to SEQ ID NO:408. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%,
15 or 100%) identical to SEQ ID NO:407 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:409) of the alpha chain variable domain. Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:408 and/or incorporate an amino acid sequence identical to the

20 CDR3 β sequence (SEQ ID NO:410) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:405 and a beta chain amino acid sequence set forth in SEQ ID NO:406.

[0040] In certain examples, the antigen-binding TCR fragment binds an NY-ESO-1 peptide having the amino acid sequence of SEQ ID NO:348 presented by HLA-A2. In

25 certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:413 and a beta chain variable domain related to SEQ ID NO:414. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:413 and/or incorporate an amino acid sequence
30 identical to the CDR3 α sequence (SEQ ID NO:415) of the alpha chain variable domain. Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%,

or 100%) identical to SEQ ID NO:414 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:416) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:411 and a beta chain amino acid sequence set forth in SEQ ID NO:412.

[0041] In certain examples, the antigen-binding TCR fragment binds an NY-ESO-1 peptide having the amino acid sequence of SEQ ID NO:348 presented by HLA-A2. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:418 and a beta chain variable domain related to SEQ ID

NO:414. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:418 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:415) of the alpha chain variable domain.

Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:414 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:416) of the beta chain variable domain. In certain examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:417 and a beta chain amino acid sequence set forth in SEQ ID

NO:412.

[0042] In certain examples, the antigen-binding TCR fragment binds an NY-ESO-1 peptide having the amino acid sequence of SEQ ID NO:348 presented by HLA-A2. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain related to SEQ ID NO:421 and a beta chain variable domain related to SEQ ID

NO:422. For example, in certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:421 and/or incorporate an amino acid sequence identical to the CDR3 α sequence (SEQ ID NO:423) of the alpha chain variable domain.

Similarly, in certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:422 and/or incorporate an amino acid sequence identical to the CDR3 β sequence (SEQ ID NO:424) of the beta chain variable domain. In certain

examples, the antigen-binding TCR fragment comprises an alpha chain amino acid sequence set forth in SEQ ID NO:419 and a beta chain amino acid sequence set forth in SEQ ID NO:420.

[0043] In certain examples, the antigen-binding TCR fragment binds an SSX2 peptide having the amino acid sequence of SEQ ID NO:345 presented by HLA-A2. In certain examples, the antigen-binding TCR fragment comprises an alpha chain variable domain incorporating a CDR3 α sequence set forth in SEQ ID NO:387. In certain examples, the antigen-binding TCR fragment comprises a beta chain variable domain incorporating a CDR3 β sequence set forth in SEQ ID NO:388.

[0044] In certain examples, the multi-specific binding protein comprises an antibody constant region or a portion thereof sufficient to bind CD16, wherein the antibody constant region or the portion thereof sufficient to bind CD16 comprises hinge and CH2 domain of a human IgG1 antibody. In certain examples, the antibody constant region or the portion thereof sufficient to bind CD16 comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody. Mutations can be introduced into the antibody constant domain to enable heterodimerization with another antibody constant domain. For example, if the antibody constant domain is derived from the constant domain of a human IgG1, the antibody constant domain can comprise an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody, and differs at one or more positions selected from the group consisting of Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439. In some examples, the antibody constant domain can comprise an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody, and differs by one or more substitutions 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.

[0045] In certain examples of any one of the foregoing polypeptides or proteins that contain an antigen-binding site that binds NKG2D (also called “NKG2D binding site”), the

NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:1, such as by having an amino acid sequence at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:1, and/or incorporating amino acid sequences identical to the CDR1 (SEQ ID NO:3 or SEQ ID NO:307), CDR2 (SEQ ID NO:4), and CDR3 (SEQ ID NO:5 or SEQ ID NO:308) sequences of SEQ ID NO:1. The heavy chain variable domain related to SEQ ID NO:1 can be coupled with a variety of light chain variable domains to form a NKG2D binding site. For example, the NKG2D binding site that incorporates a heavy chain variable domain related to SEQ ID NO:1 can further incorporate a light chain variable domain selected from any one of the sequences related to SEQ ID NOs:2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, and 43. For example, the NKG2D binding site incorporates a heavy chain variable domain with amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:1 and a light chain variable domain with amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to any one of the sequences selected from SEQ ID NOs: 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, and 43.

[0046] Alternatively, the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:44 and a light chain variable domain related to SEQ ID NO:48. For example, the heavy chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:44, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:45 or SEQ ID NO:309), CDR2 (SEQ ID NO:46), and CDR3 (SEQ ID NO:47 or SEQ ID NO:310) sequences of SEQ ID NO:44. Similarly, the light chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:48, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:49), CDR2 (SEQ ID NO:50), and CDR3 (SEQ ID NO:51) sequences of SEQ ID NO:48.

[0047] In other examples, the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:52 and a light chain variable domain related to SEQ ID NO:56. For example, the heavy chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:52, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID

NO:53 or SEQ ID NO:311), CDR2 (SEQ ID NO:54), and CDR3 (SEQ ID NO:55 or SEQ ID NO:312) sequences of SEQ ID NO:52. Similarly, the light chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:56, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:57), CDR2 (SEQ ID NO:58), and CDR3 (SEQ ID NO:59) sequences of SEQ ID NO:56.

[0048] Alternatively, the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:60 and a light chain variable domain related to SEQ ID NO:61, such as by having amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:60 and at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:61 respectively.

[0049] In another example, the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:62 and a light chain variable domain related to SEQ ID NO:66. For example, the heavy chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:62, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:63), CDR2 (SEQ ID NO:64), and CDR3 (SEQ ID NO:65) sequences of SEQ ID NO:62. Similarly, the light chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:66, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:67), CDR2 (SEQ ID NO:68), and CDR3 (SEQ ID NO:69) sequences of SEQ ID NO:66.

[0050] The NKG2D binding site, in some examples, can incorporate a heavy chain variable domain related to SEQ ID NO:70 and a light chain variable domain related to SEQ ID NO:74. For example, the heavy chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:70, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:71 or SEQ ID NO:313), CDR2 (SEQ ID NO:72), and CDR3 (SEQ ID NO:73 or SEQ ID NO:314) sequences of SEQ ID NO:70. Similarly, the light chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:74, and/or incorporate amino acid

sequences identical to the CDR1 (SEQ ID NO:75), CDR2 (SEQ ID NO:76), and CDR3 (SEQ ID NO:77) sequences of SEQ ID NO:74.

[0051] In some examples the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:78 and a light chain variable domain related to SEQ ID

5 NO:82. For example, the heavy chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:78, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:79 or SEQ ID NO:315), CDR2 (SEQ ID NO:80), and CDR3 (SEQ ID NO:81 or SEQ ID NO:316) sequences of SEQ ID NO:78. Similarly, the light chain variable domain of the
10 NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:82, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:83), CDR2 (SEQ ID NO:84), and CDR3 (SEQ ID NO:85) sequences of SEQ ID NO:82.

[0052] In some examples, the NKG2D binding site can incorporate a heavy chain variable
15 domain related to SEQ ID NO:86 and a light chain variable domain related to SEQ ID

NO:90. For example, the heavy chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:86, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:87 or SEQ ID NO:317), CDR2 (SEQ ID NO:88), and CDR3 (SEQ ID NO:89 or SEQ ID
20 NO:318) sequences of SEQ ID NO:86. Similarly, the light chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:90, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:91), CDR2 (SEQ ID NO:92), and CDR3 (SEQ ID NO:93) sequences of SEQ ID NO:90.

[0053] In some examples, the NKG2D binding site can incorporate a heavy chain variable
25 domain related to SEQ ID NO:94 and a light chain variable domain related to SEQ ID

NO:98. For example, the heavy chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:94, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID
30 NO:95 or SEQ ID NO:319), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:97 or SEQ ID NO:320) sequences of SEQ ID NO:94. Similarly, the light chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, 99%, or 100%) identical to SEQ ID NO:98, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID NO:101) sequences of SEQ ID NO:98.

[0054] In some examples, the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:102 and a light chain variable domain related to SEQ ID NO:106. For example, the heavy chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:102, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:103 or SEQ ID NO:313), CDR2 (SEQ ID NO:104), and CDR3 (SEQ ID NO:105 or SEQ ID NO:321) sequences of SEQ ID NO:102. Similarly, the light chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:106, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:107), CDR2 (SEQ ID NO:108), and CDR3 (SEQ ID NO:109) sequences of SEQ ID NO:106.

[0055] In some examples, the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:322 and a light chain variable domain related to SEQ ID NO:98. For example, the heavy chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:322, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95 or SEQ ID NO:319), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:323 or SEQ ID NO:324) sequences of SEQ ID NO:322. Similarly, the light chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:98, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID NO:101) sequences of SEQ ID NO:98.

[0056] In some examples, the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:325 and a light chain variable domain related to SEQ ID NO:98. For example, the heavy chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:325, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95 or SEQ ID NO:319), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:326 or SEQ ID NO:327) sequences of SEQ ID NO:325. Similarly, the light chain variable domain of the

NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:98, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID NO:101) sequences of SEQ ID NO:98.

5 **[0057]** In some examples, the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:328 and a light chain variable domain related to SEQ ID NO:98. For example, the heavy chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:328, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID
10 NO:95 or SEQ ID NO:319), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:329 or SEQ ID NO:330) sequences of SEQ ID NO:328. Similarly, the light chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:98, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID
15 NO:101) sequences of SEQ ID NO:98.

[0058] In some examples, the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:331 and a light chain variable domain related to SEQ ID NO:98. For example, the heavy chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical
20 to SEQ ID NO:331, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95 or SEQ ID NO:319), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:332 or SEQ ID NO:333) sequences of SEQ ID NO:331. Similarly, the light chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:98, and/or incorporate amino acid sequences
25 identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID NO:101) sequences of SEQ ID NO:98.

[0059] In some examples, the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:334 and a light chain variable domain related to SEQ ID NO:98. For example, the heavy chain variable domain of the NKG2D binding site can be at
30 least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:334, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95 or SEQ ID NO:319), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:335 or SEQ

ID NO:336) sequences of SEQ ID NO:334. Similarly, the light chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:98, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID NO:101) sequences of SEQ ID NO:98.

[0060] In some examples, the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:337 and a light chain variable domain related to SEQ ID NO:98. For example, the heavy chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:337, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95 or SEQ ID NO:319), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:338 or SEQ ID NO:339) sequences of SEQ ID NO:337. Similarly, the light chain variable domain of the NKG2D binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:98, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:99), CDR2 (SEQ ID NO:100), and CDR3 (SEQ ID NO:101) sequences of SEQ ID NO:98.

[0061] In some examples, the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:110 and a light chain variable domain related to SEQ ID NO:111, such as by having amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:110 and at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:111 respectively. In some examples, the NKG2D binding site can incorporate a heavy chain variable domain related to SEQ ID NO:112 and a light chain variable domain related to SEQ ID NO:113, such as by having amino acid sequences at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:112 and at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:113 respectively.

[0062] Formulations containing any one of the proteins described herein; cells containing one or more nucleic acids expressing the proteins, and methods of enhancing tumor cell death using the proteins are also provided.

[0063] Another example of the disclosure provides a method of treating cancer in a patient. The method comprises administering to a patient in need thereof a therapeutically effective amount of the multi-specific binding proteins described herein. Cancers to be treated may include acute myeloid leukemia, acute myelomonocytic leukemia, B cell

5 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
10 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 infiltrated by T regulatory cells, cancers with extracellular
matrix deposition, cancers with high levels of reactive stroma, and cancers with
neoangiogenesis.

SEQ ID NO:111

QSALTQPASVSGSPGQSITISCSGSSSNIGNNAVNWYQQLPGKAPKLLIYYDDLPSG
VSDRFSGSKSGTSAFLAISGLQSEDEADYYCAAWDDSLNGPVFGGGTKLTVL

- 5 **[0127]** Alternatively, a heavy chain variable domain represented by SEQ ID NO:112 can be paired with a light chain variable domain represented by SEQ ID NO:113 to form an antigen-binding site that can bind to NKG2D, as illustrated in US 7,879,985.

SEQ ID NO:112

QVHLQESGPGLVKPSETLSLTCTVSDDISISSYYWSWIRQPPGKGLEWIGHISYSGSAN
10 YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCANWDDAFNIWGQGTMTVTS
S

SEQ ID NO:113

EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGI
PDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK

15 ***Tumor-associated antigen binding site***

- [0128]** Tumor-associated antigen used herein means any antigen including but not limited to a protein, glycoprotein, ganglioside, carbohydrate, lipid that is associated with cancer. Such antigen can be expressed on malignant cells or in the tumor microenvironment such as on tumor-associated blood vessels, extracellular matrix, mesenchymal stroma, or immune
20 infiltrates. For example, the tumor-associated antigen can include ANO1, BCMA, EpCAM, CAIX, CEA, CCR4, CD2, CD123, CD133, CD19, CD20, CD22, CD25, CD30, CD33, CD37, CD38, CD40, CD52, CD70, CLAUDIN-18.2, DLL3, EGFR/ERBB1, GD2, IGF1R, HER2, HER3/ERBB3, HER4/ERBB4, MUC1, cMET, SLAMF7, PSMA, mesothelin, MICA, MICB, TRAILR1, TRAILR2, TROP2, MAGE-A3, B7.1, B7.2, CTLA4, PD1, 5T4, GPNMB, FR-
25 alpha, PAPP-A, FLT3, GPC3, CXCR4, ROR1, ROR2, HLA-E, PD-L1, VLA4, CD44, CD13, CD15, CD47, CLL1, CD81, CD23, CD79a, CD79b, CD80, CRLF2, SLAMF7, CD138, CA125, NaPi2b, Nectin4, ADAM8, ADAM9, SLC44A4, CA19-9, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LILRA1, LILRA2, LILRA3, LILRA4, LILRA5, and LILRA6, CCR8, CD7, CTLA4, CX3CR1, ENTPD1, HAVCR2, IL-1R2, PDCD1LG2, TIGIT,
30 TNFRSF4, TNFRSF8, TNFRSF9, GEM, NT5E, TNFRSF18, MUC1, P-cadherin, Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, Axl, erbB-3, EDNRB, Tyrp1, CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4 (VTCN1), CCR1, LRRC25, PTAFR, SIRPB1,

Claims:

1. A polypeptide comprising a single-chain variable fragment (scFv) linked to an antibody constant domain via a hinge comprising Ala-Ser, wherein the scFv comprises a heavy chain variable domain and a light chain variable domain.
- 5 2. The polypeptide according to claim 1, wherein the heavy chain variable domain forms a disulfide bridge with the light chain variable domain, optionally between C44 from the heavy chain variable domain and C100 from the light chain variable domain.
3. A polypeptide according to claim 1 or 2, wherein the heavy chain variable domain is linked to the light chain variable domain via a flexible linker, optionally wherein the flexible
10 linker comprises (G₄S)₄.
4. A polypeptide according to any one of claims 1-3, wherein the heavy chain variable domain is positioned at the N-terminus or the C-terminus of the light chain variable domain.
5. A polypeptide according to any one of claims 1-4, wherein the hinge further comprises amino acid sequence Thr-Lys-Gly.
- 15 6. A polypeptide according to any one of claims 1-5, wherein the antibody constant domain comprises:
 - (a) an antibody Fc domain or a portion thereof sufficient to bind CD16;
 - (b) a CH2 and a CH3 domain of a human IgG1 antibody;
 - (c) an amino acid sequence at least 90% identical to amino acids 234-332 of a human
20 IgG1 antibody; and/or
 - (d) an amino acid sequence at least 90% identical to the Fc domain of human IgG1 and differing at one or more positions selected from the group consisting of Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439.

7. A polypeptide according to claim 6, wherein the antibody constant domain comprises amino acid sequence at least 90% identical to the Fc domain of human IgG1 and differing by one or more substitutions 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.
8. A polypeptide according to any one of claims 1-7, wherein the scFv binds NKG2D.
9. A polypeptide according to claim 8, wherein:
- (a) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:94 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:98;
 - (b) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:322 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:98;
 - (c) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:325 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:98;
 - (d) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:328 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:98;
 - (e) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:331 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:98;

- (f) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:334 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:98;
- 5 (g) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:337 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:98;
- (h) wherein the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:44 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:48;
- 10 (i) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:52 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:56.
- (j) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:60 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:61;
- 15 (k) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:62 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:66;
- (l) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:70 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:74;
- 20 (m) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:78 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:82;
- 25 (n) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:86 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:90;

- (o) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:102 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:106;
- (p) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:110 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:111; or
- (q) the heavy chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:112 and the light chain variable domain comprises an amino acid sequence at least 90% identical to SEQ ID NO:113.
10. A polypeptide according to any one of claims 1-7, wherein the scFv binds to a tumor-associated antigen, optionally wherein the tumor-associated antigen is selected from the group consisting of ANO1, CAIX, CCR4, CD2, CD123, CD133, CD20, CD22, CD25, CD30, CD37, CD38, CD40, CD52, CD70, CLAUDIN-18.2, DLL3, GD2, IGF1R, HER2, HER3/ERBB3, HER4/ERBB4, MUC1, SLAMF7, PSMA, mesothelin, MICA, MICB, TRAILR1, TRAILR2, TROP2, MAGE-A3, B7.1, B7.2, CTLA4, PD1, 5T4, GPNMB, FR-alpha, PAPP-A, GPC3, CXCR4, ROR1, ROR2, PD-L1, VLA4, CD44, CD13, CD15, CD47, CD81, CD23, CD79a, CD79b, CD80, CRLF2, SLAMF7, CD138, CA125, NaPi2b, ADAM8, ADAM9, SLC44A4, CA19-9, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LILRA1, LILRA2, LILRA3, LILRA4, LILRA5, and LILRA6, CCR8, CD7, CTLA4, CX3CR1, ENTPD1, HAVCR2, IL-1R2, PDCD1LG2, TIGIT, TNFRSF4, TNFRSF8, TNFRSF9, GEM, NT5E, TNFRSF18, P-cadherin, Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, Axl, erbB-3, EDNRB, Tyrp1, CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4 (VTCN1), CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, CCR5, Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, EDNRB, OLR1, and TYRP1.
11. A protein comprising:
- (a) the polypeptide according to any one of claims 1-10, wherein the scFv of the polypeptide comprises a first antigen-binding site;
- (b) a second antigen-binding site; and

(c) a second antibody constant domain.

12. The protein of claim 11, wherein the first antigen-binding site binds NKG2D, and the second antigen-binding site binds an tumor-associated antigen.

13. The protein of claim 11, wherein the first antigen-binding site binds an tumor-associated antigen, and the second antigen-binding site binds NKG2D.

14. A protein according to any one of claims 11-13, wherein the second antigen binding site comprises a Fab.

15. A protein according to any one of claims 11-14, wherein the second antibody constant domain comprises:

- 10 (a) hinge and CH2 domain of a human IgG1 antibody;
- (b) an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody; and/or
- (c) an amino acid sequence at least 90% identical to the Fc domain of human IgG1 and differing at one or more positions selected from the group consisting of Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439.

16. A protein comprising:

- (a) a first antigen-binding site that binds a tumor-associated antigen;
- (b) a second antigen-binding site that binds the same tumor-associated antigen as the first antigen-binding site;
- (c) a third antigen-binding site that binds NKG2D; and
- (d) an antibody constant region or a portion thereof sufficient to bind CD16, or a fourth antigen-binding site that binds CD16.

17. A protein according to claim 16, wherein the first antigen-binding site and the second antigen-binding site each comprises a Fab.

18. A protein according to claim 16 or 17, wherein the third antigen-binding site comprises an scFv.

19. A protein according to any one of claims 16-18, wherein the tumor-associated antigen is selected from the group consisting of ANO1, CAIX, CCR4, CD2, CD123, CD133, CD20, CD22, CD25, CD30, CD37, CD38, CD40, CD52, CD70, CLAUDIN-18.2, DLL3, GD2, IGF1R, HER2, HER3/ERBB3, HER4/ERBB4, MUC1, SLAMF7, PSMA, mesothelin, MICA, MICB, TRAILR1, TRAILR2, TROP2, MAGE-A3, B7.1, B7.2, CTLA4, PD1, 5T4, GPNMB, FR-alpha, PAPP-A, GPC3, CXCR4, ROR1, ROR2, PD-L1, VLA4, CD44, CD13, CD15, CD47, CD81, CD23, CD79a, CD79b, CD80, CRLF2, SLAMF7, CD138, CA125, NaPi2b, ADAM8, ADAM9, SLC44A4, CA19-9, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, LILRA1, LILRA2, LILRA3, LILRA4, LILRA5, and LILRA6, CCR8, CD7, CTLA4, CX3CR1, ENTPD1, HAVCR2, IL-1R2, PDCD1LG2, TIGIT, TNFRSF4, TNFRSF8, TNFRSF9, GEM, NT5E, TNFRSF18, P-cadherin, Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, Axl, erbB-3, EDNRB, Tyrp1, CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4 (VTCN1), CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, CCR5, Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, EDNRB, OLR1, and TYRP1.

20. A protein according to any one of claims 16-19, wherein the third antigen-binding site comprises:

- (a) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:94 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:98;
- (b) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:322 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:98;
- (c) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:325 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:98;

- (d) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:328 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:98;
- 5 (e) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:331 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:98;
- (f) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:334 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:98;
- 10 (g) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:337 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:98;
- (h) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:44 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:48;
- 15 (i) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:52 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:56;
- (j) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:60 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:61;
- 20 (k) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:62 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:66;
- (l) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:70 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:74;
- 25

- 5 (m) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:78 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:82;
- (n) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:86 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:90;
- (o) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:102 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:106;
- 10 (p) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:110 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:111; or
- (q) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:112 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:113.
- 15
21. A protein according to any one of claims 16-20, wherein the antibody constant region or the portion thereof sufficient to bind CD16 comprises:
- (a) hinge and CH2 domain of a human IgG1 antibody;
- (b) an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody; and/or
- 20 (c) an amino acid sequence at least 90% identical to the Fc domain of human IgG1 and differing at one or more positions selected from the group consisting of Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439.
- 25 22. A protein comprising:
- (a) an antigen-binding site that binds NKG2D;

- (b) an antigen-binding T cell receptor (TCR) fragment; and
- (c) an antibody constant region or a portion thereof sufficient to bind CD16, or an additional antigen-binding site that binds CD16.

23. A formulation comprising a protein according to any one of claims 11-22 and a
5 pharmaceutically acceptable carrier.
24. A cell comprising one or more nucleic acids encoding a protein according to any one of claims 11-22.
25. The protein according to any one of claims 11-22 or the formulation of claim 23 for use in therapy.
- 10 26. Use of the protein according to any one of claims 11-22 or the formulation of claim 23 in the manufacture of a medicament for treating cancer.
27. The use of claim 26, 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,
15 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
20 cancer, cancers infiltrated by myeloid-derived suppressor cells, cancers with extracellular matrix deposition, cancers with high levels of reactive stroma, and cancers with neoangiogenesis.