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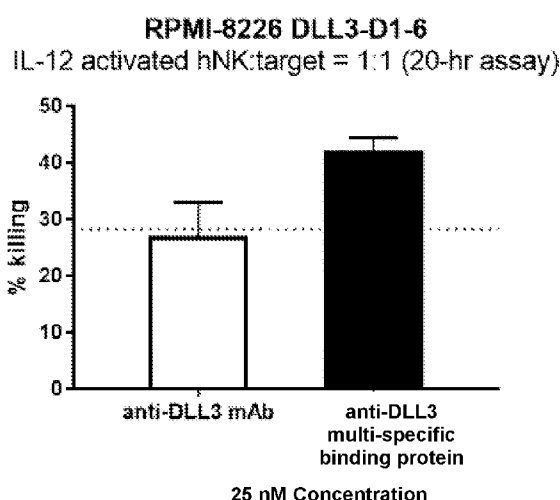
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(54) Title: PROTEINS BINDING NKG2D, CD16 AND AN ANTIGEN ASSOCIATED WITH TUMORS, MDSCS AND/OR TAMs

FIG. 30B



(57) Abstract: Multi-specific binding proteins that bind the NKG2D receptor, CD 16, and a tumor-associated antigen on tumor cells, or an antigen on myeloid-derived suppressor cells or tumor-associated macrophages are described, as well as pharmaceutical compositions and therapeutic methods useful for the treatment of cancer.

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**PROTEINS BINDING NKG2D, CD16 AND AN ANTIGEN ASSOCIATED WITH
TUMORS, MDSCS AND/OR TAMS**

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 62/651,951, filed April 3, 2018, the disclosure of which is hereby incorporated by reference in its entirety for all purposes; U.S. Provisional Patent Application No. 62/667,844, filed May 7, 2018; U.S. Provisional Patent Application No. 62/672,299, filed May 16, 2018; and U.S. Provisional Patent Application No. 62/663,607, filed April 27, 2018, the disclosure of which is hereby incorporated by reference in its entirety for all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 2, 2019, is named DFY-053WO_SL.txt and is 541,768 bytes in size.

FIELD OF THE INVENTION

[0003] The invention relates to multi-specific binding proteins that bind to the NKG2D receptor, CD16, and a tumor-associated antigen on tumor cells or an antigen on myeloid-derived suppressor cells (MDSCs) or tumor-associated macrophages (TAMs), as well as pharmaceutical compositions and therapeutic methods useful for the treatment of cancer.

BACKGROUND

[0004] Cancer continues to be a significant health problem despite the substantial research efforts and scientific advances reported in the literature for treating this disease. Some of the most frequently diagnosed cancers include prostate cancer, breast cancer, and lung cancer. Prostate cancer is the most common form of cancer in men. Breast cancer remains a leading cause of death in women. Current treatment options for these cancers are not effective for all patients and/or can have substantial adverse side effects. Other types of cancers also remain challenging to treat using existing therapeutic options.

[0005] Cancer immunotherapies are desirable because they are highly specific and can facilitate destruction of cancer cells using the patient's own immune system. Fusion proteins such as bi-specific T-cell engagers are cancer immunotherapies described in the literature that bind to tumor cells and T-cells to facilitate destruction of tumor cells. Antibodies that bind to

certain tumor-associated antigens and to certain immune cells have been described in the literature. *See, e.g.*, WO 2016/134371 and WO 2015/095412.

[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, natural cytotoxicity receptors (NCRs), DNAX accessory molecule 1 (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.

[0008] Delta like canonical Notch ligand 3 (DLL3) belongs to the delta protein ligand family, and acts as a ligand in the notch signaling pathway. DLL3 has been associated with a variety of neuroendocrine cancers. It is expressed on the surface of tumor cells in about 85% of patients with small-cell lung cancer and large-cell neuroendocrine cancer, but not in healthy tissues. It is also implicated in glioblastoma, Ewing Sarcoma and other cancers with neuroendocrine phenotype. DLL3 binds to Notch receptors and promotes the proliferation and inhibits the apoptosis of cancer cells.

[0009] Mucin 1 (MUC1) is a transmembrane mucin family protein having highly conserved 20 amino acid repeats (HGVTSAPDTRPAPGSTAPPA (SEQ ID NO:633)) decorated with a dense O-linked glycosylation pattern. MUC1 lines the apical surface of epithelial cells in the lungs, stomach, intestines, eyes and several other organs, and provides a protective barrier for the epithelial cells. MUC1 is normally expressed at a basal level in human epithelial cells, but is over-expressed in cancers, including gastric cancer, colorectal cancer, pancreatic cancer, breast cancer, endometrial cancer, lung cancer, bladder cancer, cervical cancer, head and neck cancer, ovarian cancer, renal cell cancer, and multiple

myeloma. MUC1 is often shed from cells; in this case, a small extracellular domain (MUC1-C) remains on the cell surface following cleavage of the N-terminal ectodomain.

Furthermore, MUC1 in cancer cells is aberrantly glycosylated. For example, MUC1 is highly expressed in an underglycosylated form in multiple tumor types of epithelial origin, including over 90% of breast cancers.

[0010] Plexins are the signal-transducing elements of semaphorins, which are a large family of evolutionarily conserved molecules implicated in axon guidance, organogenesis, angiogenesis, immune responses, and oncogenesis. In particular, the type A plexins (Plexin-A1, Plexin-A2, Plexin-A3, and Plexin-A4), together with ligand-binding neuropilins, are the signaling moiety of the receptor complex for class 3 semaphorins. Moreover, the type A plexins are also the primary receptors for class 6 transmembrane semaphorins (*e.g.*, Semaphorin-6A and Semaphorin-6D) that do not bind neuropilins. Type A plexins modulate the affinity of the receptor complex for specific semaphorins, and the cytoplasmic domain of type A plexins is required for the activation of down-stream signaling events in the cytoplasm. It has been identified that semaphorins and their receptor plexin-A1 are over-expressed in many cancers, including head and neck cancer, gastric cancer, pancreatic cancer, prostate cancer, and glioma.

[0011] Tumor Necrosis Factor (TNF) is a pro-inflammatory cytokine involved in the progression and development of cancer. Tumor necrosis factor receptor superfamily member 10B (TNFRSF10B) is a cell surface receptor of the TNF-receptor superfamily that binds TRAIL and mediates apoptosis. It is over-expressed in many types of cancers, such as liver cancer, pancreatic cancer, stomach cancer, renal cancer, breast cancer, ovarian cancer, endometrial cancer, and melanoma.

[0012] Six-transmembrane epithelial antigen of prostate member 1 (STEAP1) is a metalloredutase involved in a wide range of biologic processes, such as molecular trafficking in the endocytic and exocytic pathways, and control of cell proliferation and apoptosis. It is over-expressed in several types of human cancers, such as prostate cancer, bladder cancer, colon cancer, pancreatic cancer, ovarian cancer, testicular cancer, breast cancer, cervical cancer and Ewing sarcoma.

[0013] CUB domain-containing protein 1 (CDCP1) is a type I integral membrane glycoprotein that directly interacts with proteins involved in both cell-cell and cell-extracellular matrix adhesion, thereby playing a role in cell motility and adhesion. Increased

CDCP1 expression has been found in various types of cancers, including colon cancer, lung cancer, gastric cancer, breast cancer, pancreatic cancer, head and neck cancer, bladder cancer, ovarian cancer, endometrial cancer, and skin cancer.

[0014] Tyrosine-protein kinase-like 7 (PTK7), also known as colon carcinoma kinase 4 (CCK4), is a member of the receptor protein tyrosine kinase family. PTK7 plays a role in vertebrate tissue morphogenesis, by regulating the canonical and non-canonical Wnt pathways, and orientation of cells in a tissue plane. Expression of PTK7 is upregulated in lung cancer, head and neck cancer, stomach cancer, prostate cancer, testicular cancer, endometrial cancer, breast cancer, melanoma, skin cancer, and leukemia.

[0015] AXL receptor tyrosine kinase (AXL), a cell surface receptor tyrosine kinase, transduces signals from the extracellular matrix into the cytoplasm by binding growth factors, and is involved in stimulation of cell proliferation and survival. AXL is over-expressed in many human cancers, including breast cancer, lung cancer, colon cancer, prostate cancer, renal cancer, esophageal cancer, liver cancer, pancreatic cancer, Kaposi's sarcoma, acute myeloid leukemia, glioma, and mesothelioma. AXL oncogenic signaling promotes cancer cell survival, proliferation, migration, and invasion.

[0016] Receptor tyrosine-protein kinase ERBB-3 (ERBB-3), also known as HER3, is a member of the epidermal growth factor receptor (EGFR/ERBB) family of receptor tyrosine kinases. It forms heterodimers with other EGF receptor family members and heterodimerization leads to the activation of pathways involved in cell proliferation or differentiation. Over-expression of ERBB-3 has been reported in numerous cancers, including prostate cancer, bladder cancer, and breast cancer, ovarian cancer, colon cancer, pancreatic cancer, stomach cancer, oral cavity cancer, head and neck cancer, lung cancer, and melanoma.

[0017] Endothelin receptor type B (EDNRB) is a G protein-coupled receptor which activates a phosphatidylinositol-calcium second messenger system. Its ligand, endothelin, consists of a family of three potent vasoactive peptides: endothelin-1, endothelin-2, and endothelin-3. Tumors over-express EDNRB and the endothelins. The interaction between EDNRB and the endothelins induces tumor growth and metastasis by promoting tumor cell survival and proliferation, angiogenesis, and tissue remodeling. Exemplary tumors include melanoma, uveal melanoma, and glioma.

[0018] Tyrosinase related protein -1 (TYRP1) belongs to a family of $\text{Cu}^{++}/\text{Zn}^{++}$ metalloenzymes, which are expressed in melanocytes where they play key roles in promoting melanogenesis. The mature form of TYRP1, also called gp75, is a 75 kDa transmembrane glycoprotein produced within the endoplasmic reticulum (ER) and
5 transported through the Golgi to specialized organelles called melanosomes. There is growing evidence indicating an important role of Tyrp1 in melanoma progression.

[0019] Oxidized low-density lipoprotein receptor 1 (OLR1) is the main receptor for oxidized low-density lipoprotein on endothelial cells, macrophages, smooth muscle cells, and other cell types. OLR1 binds, internalizes and degrades oxidized low-density lipoprotein.

10 Over-expression of OLR1 has been associated with gastric cancer, colorectal cancer, pancreatic cancer, prostate cancer, breast cancer, and endometrial cancer.

[0020] ADAM12 is a member of the “a disintegrin and metalloprotease” (ADAM) protein family. Members of this family are membrane-anchored proteins structurally related to snake venom disintegrins, and have been implicated in a variety of biological processes
15 involving cell-cell and cell-matrix interactions, including fertilization, muscle development, and neurogenesis. ADAM12 has two alternatively-spliced gene products: a shorter secreted form and a longer membrane-bound form. The shorter form is found to stimulate myogenesis. Numerous studies have demonstrated the importance of ADAM12 in cancer, and ADAM12 is markedly upregulated in a variety of human cancers, including prostate cancer,
20 breast cancer, basal cell carcinoma, squamous cell carcinoma, melanoma, pancreatic cancer, bladder cancer, colorectal cancer, lung cancer, liver cancer, esophageal cancer, Non-Hodgkin's lymphoma, ovarian cancer, and uterine cancer.

[0021] The urokinase receptor, also known as urokinase plasminogen activator receptor, CD87 or PLAUR, is a multidomain glycoprotein tethered to the cell membrane with
25 a glycosylphosphatidylinositol (GPI) anchor. PLAUR is a part of the plasminogen activation system, which in the healthy body is involved in tissue reorganization events such as mammary gland involution and wound healing. Elevated levels of PLAUR is detected in various cancer types (for example, breast cancer, colorectal cancer, non-small cell lung cancer, and oral cancer), and is closely associated with poor prognosis of cancers. Binding of
30 uPA to PLAUR triggers the conversion of plasminogen to plasmin and the subsequent activation of metalloproteinases. These events confer tumor cells with the ability to degrade the components of the surrounding extracellular matrix, thus contributing to tumor cell invasion and metastasis. uPA-PLAR interaction also elicits signals that stimulate cell

proliferation/survival and the expression of tumor-promoting genes, thus assisting tumor development. In addition to its interaction with uPA, PLAUR also interacts with vitronectin and this interaction promotes cancer metastasis by activating Rac and stimulating cell migration. Although underlying mechanisms are yet to be fully elucidated, PLAUR has been shown to facilitate epithelial-mesenchymal transition (EMT) and induce cancer stem cell-like properties in breast cancer cells.

[0022] C-C motif chemokine receptor 6 (CCR6) is a member of the beta chemokine receptor family, which is a seven transmembrane protein similar to G protein-coupled receptors. This receptor is preferentially expressed by immature dendritic cells and memory T cells, and the ligand of this receptor is macrophage inflammatory protein 3 alpha (MIP-3 alpha). CCR6 has been shown to be important for B-lineage maturation and antigen-driven B-cell differentiation, and it may regulate the migration and recruitment of dendritic and T cells during inflammatory and immunological responses. In addition, expression of CCR6 was found to be upregulated in many cancer types, for example, colorectal cancer, breast cancer, cervical cancer, liver cancer, lung cancer, and cutaneous T-cell lymphoma, and to contribute to the proliferation and migration of the cancers.

[0023] Ephrin type-A receptor 4 (EPHA4) belongs to the ephrin receptor subfamily of protein-tyrosine kinases. EPHA4 relays a direct cell-cell contact-mediated bidirectional signaling pathway. EPHA4 signaling mainly affects cell shape and motility by regulating cytoskeletal organization and cellular adhesion. EPHA4 signaling also influences cell proliferation and cell-fate. The genes for Ephrin receptors and ephrins have been recognized to be differentially expressed in various human tumors including melanoma, glioma, prostate cancer, breast cancer, small cell lung cancer, endometrial cancer, esophageal cancer, gastric cancer, and colorectal cancer. Abnormal EPHA4 expression can be correlated with altered tumor behavior such as increased invasiveness or increased metastatic potential and, consequently, poor patient outcome.

[0024] Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous population of immature myeloid cells consisting of precursors for granulocytes, macrophages or dendritic cells (DCs) that accumulate during chronic inflammation and tumor progression. It has been shown that established tumors are able to produce multiple factors that impair myelopoiesis favoring the formation of MDSCs, trafficking of MDSCs to the tumor site and activation of MDSCs. Numerous recent studies have demonstrated that after the generation and migration to the tumor site, MDSCs significantly upregulate their immunosuppressive

functions inhibiting the anti-tumor functions of T cells and NK cells. Moreover, MDSCs directly stimulate tumor development by promoting neovascularization, and tumor cell invasion by creating a pre-metastatic environment.

[0025] There are two different types of MDSCs identified in humans: polymorphonuclear MDSCs (PMN-MDSCs), and monocytic MDSCs (M-MDSCs). In the tumor, M-MDSCs are more prominent, and rapidly differentiate to tumor-associated macrophages (TAMs) (*see Kumar et al. (2016) Trends Immunol.*; 37(3): 208–220.). In addition to being differentiated from MDSCs, TAMs can be tissue resident. Alternatively, peripheral blood monocytes can be recruited locally to the tissue and differentiate into TAMs in response to various chemokines and growth factors produced by stromal and tumor cells in the tumor microenvironment. TAMs play an important role in connecting inflammation with cancer. They can promote proliferation, invasion, and metastasis of tumor cells, stimulate tumor angiogenesis, and inhibit antitumor immune response mediated by T cells (Yang *et al. (2017) Journal of Hematology & Oncology*; 10:58.).

[0026] A variety of antigens may be expressed on MDSCs and/or TAMs, which are present in a tumor microenvironment, including CD14, CD163, colony stimulating factor 3 receptor (CSF3R), sialic acid-binding Ig-like lectin 9 (Siglec-9), integrin alpha M (ITGAM), V-domain Ig suppressor of T cell activation (VISTA), B7-H4 (also known as V-Set Domain Containing T Cell Activation Inhibitor 1; VTCN1), C-C chemokine receptor type 1 (CCR1), leucine rich repeat containing 25 (LRRC25), platelet activating factor receptor (PTAFR), signal regulatory protein beta 1 (SIRPB1), Toll-like receptor 2 (TLR2), Toll-like receptor 4 (TLR4), CD300 molecule like family member b (CD300LB), ATPase Na⁺/K⁺ transporting subunit alpha 3 (ATP1A3), and C-C chemokine receptor type 5 (CCR5).

[0027] The present invention provides certain advantages to improve treatments for the above-mentioned cancers.

SUMMARY

[0028] The invention provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and a tumor-associated antigen selected from DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, and EPHA4. The invention also provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and an antigen selected from CD14, CD163, CSF3R, Siglec-9,

ITGAM, VISTA, B7-H4 (VTCN1), CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and CCR5. Such proteins can engage more than one kind of NK-activating receptor, and may block the binding of natural ligands to NKG2D. In certain embodiments, the proteins can agonize NK cells in humans, and in other species such as rodents and cynomolgus monkeys. Various aspects and embodiments of the invention are described in further detail below.

[0029] Accordingly, in certain embodiments the invention provides a protein that incorporates a first antigen-binding site that binds NKG2D; a second antigen-binding site that binds a tumor-associated antigen selected from DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, and EPHA4; and an antibody fragment crystallizable (Fc) domain, a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.

[0030] In certain embodiments, the present invention provides multi-specific binding proteins that bind to a tumor-associated antigen selected from DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, and EPHA4 on a cancer cell, and to the NKG2D receptor and CD16 receptor on natural killer cells, in which the NKG2D-binding site includes a heavy chain variable domain at least 90% identical to an amino acid sequence selected from: SEQ ID NO:1, SEQ ID NO:41, SEQ ID NO:49, SEQ ID NO: 57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:85, SEQ ID NO:650, SEQ ID NO:653, SEQ ID NO:656, SEQ ID NO:659, SEQ ID NO:662, SEQ ID NO:665, and SEQ ID NO:93.

[0031] In certain other embodiments, the invention provides a protein that incorporates a first antigen-binding site that binds NKG2D; a second antigen-binding site that binds an antigen selected from CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4 (VTCN1), CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and CCR5; and an antibody Fc domain, a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.

[0032] In certain other embodiments, the invention provides a protein that incorporates a first antigen-binding site that binds NKG2D; a second antigen-binding site that binds an antigen selected from CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4 (VTCN1), CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and CCR5; an

antibody Fc domain, or a portion thereof, sufficient to bind CD16, or a third antigen-binding site that binds CD16; and a fourth antigen-binding site that binds to a tumor-associated antigen, which includes any antigen that is associated with cancer, such as, but not limited to, a protein, glycoprotein, ganglioside, carbohydrate, or lipid. 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 infiltrates.

[0033] The antigen-binding sites may each incorporate an antibody heavy chain variable domain and an antibody light chain variable domain (*e.g.*, arranged as in an antibody, or fused together to form an scFv), or one or more of the antigen-binding sites may be a single domain antibody, such as a V_HH antibody like a camelid antibody or a V_{NAR} antibody like those found in cartilaginous fish.

[0034] The first antigen-binding site, which binds to NKG2D, in some embodiments, 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:105), CDR2 (SEQ ID NO:106), and CDR3 (SEQ ID NO:107 or SEQ ID NO:635) sequences of SEQ ID NO:1. The heavy chain variable domain related to SEQ ID NO:1 can be coupled a variety of light chain variable domains to form a NKG2D binding site. For example, the first antigen-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, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, and 40. For example, the first antigen-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, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, and 40.

[0035] Alternatively, in certain embodiments the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:41 and a light chain variable domain related to SEQ ID NO:42. For example, the heavy chain variable domain of the first 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:41, and/or incorporate amino acid

sequences identical to the CDR1 (SEQ ID NO:43 or SEQ ID NO:636), CDR2 (SEQ ID NO:44), and CDR3 (SEQ ID NO:45 or SEQ ID NO:637) sequences of SEQ ID NO:41.

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

5 SEQ ID NO:42, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:46), CDR2 (SEQ ID NO:47), and CDR3 (SEQ ID NO:48) sequences of SEQ ID NO:42.

[0036] In certain embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:49 and a light chain variable domain related to SEQ ID NO:50. For example, the heavy chain variable domain of the first antigen-binding

10 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:49, and/or incorporate amino acid sequences identical to the

CDR1 (SEQ ID NO:51 or SEQ ID NO:638), CDR2 (SEQ ID NO:52), and CDR3 (SEQ ID NO:53 or SEQ ID NO:639) sequences of SEQ ID NO:49. Similarly, the light chain variable

domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%,

15 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:50, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:54), CDR2 (SEQ ID NO:55), and CDR3 (SEQ ID NO:56) sequences of SEQ ID NO:50.

[0037] Alternatively, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:57 and a light chain variable domain related to SEQ ID

20 NO:58, 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:57 and at least 90%

(*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:58 respectively.

[0038] In another embodiment, the first antigen-binding site can incorporate a heavy

25 chain variable domain related to SEQ ID NO:59 and a light chain variable domain related to SEQ ID NO:60, 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:59 and at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to

SEQ ID NO:60 respectively. For example, the heavy chain variable domain of the first

30 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:59, and/or incorporate amino acid sequences

identical to the CDR1 (SEQ ID NO:108), CDR2 (SEQ ID NO:109), and CDR3 (SEQ ID NO:110) sequences of SEQ ID NO:59. 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:60, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:111), CDR2 (SEQ ID NO:112), and CDR3 (SEQ ID NO:113) sequences of SEQ ID NO:60.

5 **[0039]** In some embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:101 and a light chain variable domain related to SEQ ID NO:102, 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:101 and at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to
 10 SEQ ID NO:102 respectively. In some embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:103 and a light chain variable domain related to SEQ ID NO:104, 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:103 and at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 15 99%, or 100%) identical to SEQ ID NO:104 respectively.

[0040] The first antigen-binding site, which binds to NKG2D, in some embodiments, can incorporate a heavy chain variable domain related to SEQ ID NO:61 and a light chain variable domain related to SEQ ID NO:62. For example, the heavy chain variable domain of the first antigen binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%,
 20 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:61, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:63 or SEQ ID NO:640), CDR2 (SEQ ID NO:64), and CDR3 (SEQ ID NO:65 or SEQ ID NO:641) sequences of SEQ ID NO:61. 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
 25 SEQ ID NO:62, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:66), CDR2 (SEQ ID NO:67), and CDR3 (SEQ ID NO:68) sequences of SEQ ID NO:62. In some embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:69 and a light chain variable domain related to SEQ ID NO:70. For example, the heavy chain variable domain of the first antigen-binding site can be
 30 at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:69, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:71 or SEQ ID NO:642), CDR2 (SEQ ID NO:72), and CDR3 (SEQ ID NO:73 or SEQ ID NO:643) sequences of SEQ ID NO:69. 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:70, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:74), CDR2 (SEQ ID NO:75), and CDR3 (SEQ ID NO:76) sequences of SEQ ID NO:70.

5 **[0041]** In some embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:77 and a light chain variable domain related to SEQ ID NO:78. For example, the heavy chain variable domain of the first 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:77, and/or incorporate amino acid sequences identical to the CDR1
10 (SEQ ID NO:79 or SEQ ID NO:644), CDR2 (SEQ ID NO:80), and CDR3 (SEQ ID NO:81 or SEQ ID NO:645) sequences of SEQ ID NO:77. 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:78, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:82), CDR2 (SEQ ID NO:83), and CDR3 (SEQ
15 ID NO:84) sequences of SEQ ID NO:78.

[0042] In some embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:85 and a light chain variable domain related to SEQ ID NO:86. For example, the heavy chain variable domain of the first antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%)
20 identical to SEQ ID NO:85, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:87 or SEQ ID NO:646), CDR2 (SEQ ID NO:88), and CDR3 (SEQ ID NO:89 or SEQ ID NO:647) sequences of SEQ ID NO:85. 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:86, and/or incorporate amino acid
25 sequences identical to the CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID NO:92) sequences of SEQ ID NO:86.

[0043] In some embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:650 and a light chain variable domain related to SEQ ID NO:86. For example, the heavy chain variable domain of the first antigen-binding site can
30 be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:650, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:87 or SEQ ID NO:646), CDR2 (SEQ ID NO:88), and CDR3 (SEQ ID NO:651 or SEQ ID NO:652) sequences of SEQ ID NO:650. 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:86, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID NO:92) sequences of SEQ ID NO:86.

5 **[0044]** In some embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:653 and a light chain variable domain related to SEQ ID NO:86. For example, the heavy chain variable domain of the first 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:653, and/or incorporate amino acid sequences identical to the CDR1
10 (SEQ ID NO:87 or SEQ ID NO:646), CDR2 (SEQ ID NO:88), and CDR3 (SEQ ID NO:654 or SEQ ID NO:655) sequences of SEQ ID NO:653. 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:86, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3
15 (SEQ ID NO:92) sequences of SEQ ID NO:86.

[0045] In some embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:656 and a light chain variable domain related to SEQ ID NO:86. For example, the heavy chain variable domain of the first antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%)
20 identical to SEQ ID NO:656, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:87 or SEQ ID NO:646), CDR2 (SEQ ID NO:88), and CDR3 (SEQ ID NO:657 or SEQ ID NO:658) sequences of SEQ ID NO:656. 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:86, and/or incorporate amino
25 acid sequences identical to the CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID NO:92) sequences of SEQ ID NO:86.

[0046] In some embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:659 and a light chain variable domain related to SEQ ID NO:86. For example, the heavy chain variable domain of the first antigen-binding site can
30 be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:659, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:87 or SEQ ID NO:646), CDR2 (SEQ ID NO:88), and CDR3 (SEQ ID NO:660 or SEQ ID NO:661) sequences of SEQ ID NO:659. 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:86, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID NO:92) sequences of SEQ ID NO:86.

5 **[0047]** In some embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:662 and a light chain variable domain related to SEQ ID NO:86. For example, the heavy chain variable domain of the first 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:662, and/or incorporate amino acid sequences identical to the CDR1
10 (SEQ ID NO:87 or SEQ ID NO:646), CDR2 (SEQ ID NO:88), and CDR3 (SEQ ID NO:663 or SEQ ID NO:664) sequences of SEQ ID NO:662. 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:86, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3
15 (SEQ ID NO:92) sequences of SEQ ID NO:86.

[0048] In some embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:665 and a light chain variable domain related to SEQ ID NO:86. For example, the heavy chain variable domain of the first antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%)
20 identical to SEQ ID NO:665, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:87 or SEQ ID NO:646), CDR2 (SEQ ID NO:88), and CDR3 (SEQ ID NO:666 or SEQ ID NO:667) sequences of SEQ ID NO:665. 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:86, and/or incorporate amino
25 acid sequences identical to the CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID NO:92) sequences of SEQ ID NO:86.

[0049] In some embodiments, the first antigen-binding site can incorporate a heavy chain variable domain related to SEQ ID NO:93 and a light chain variable domain related to SEQ ID NO:94. For example, the heavy chain variable domain of the first antigen-binding site can
30 be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:93, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:95 or SEQ ID NO:648), CDR2 (SEQ ID NO:96), and CDR3 (SEQ ID NO:97 or SEQ ID NO:649) sequences of SEQ ID NO:93. 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:94, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:98), CDR2 (SEQ ID NO:99), and CDR3 (SEQ ID NO:100) sequences of SEQ ID NO:94.

5 **[0050]** In certain embodiments, the second antigen-binding site can bind DLL3 and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:114 and a light chain variable domain related to SEQ ID NO:115. For example, the heavy 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:114, and/or incorporate
10 amino acid sequences identical to the CDR1 (SEQ ID NO:116), CDR2 (SEQ ID NO:117), and CDR3 (SEQ ID NO:118) sequences of SEQ ID NO:114. 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:115 and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:119), CDR2 (SEQ ID
15 NO:120), and CDR3 (SEQ ID NO:121) sequences of SEQ ID NO:115.

[0051] Alternatively, the second antigen-binding site binding to DLL3 can incorporate a heavy chain variable domain related to SEQ ID NO:122 and a light chain variable domain related to SEQ ID NO:123. For example, the heavy chain variable domain of the second antigen-binding site can be at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,
20 98%, 99%, or 100%) identical to SEQ ID NO:122, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:124), CDR2 (SEQ ID NO:125), and CDR3 (SEQ ID NO:126) sequences of SEQ ID NO:122. 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:123, and/or incorporate amino acid
25 sequences identical to the CDR1 (SEQ ID NO:127), CDR2 (SEQ ID NO:128), and CDR3 (SEQ ID NO:129) sequences of SEQ ID NO:123.

[0052] In some embodiments, the second antigen-binding site binding to DLL3 can incorporate a heavy chain variable domain related to SEQ ID NO:679, 668, 671, 673, 675, 677, or 130 and a light chain variable domain related to SEQ ID NO:669 or 131. For
30 example, the heavy 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:679, 668, 671, 673, 675, 677, or 130, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:132), CDR2 (SEQ ID NO:133), and CDR3 (SEQ ID NO:

670, 672, 674, 676, 678, 680, or 134) sequences of SEQ ID NO:679, 668, 671, 673, 675, 677, or 130. 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:669 or 131, and/or incorporate amino acid sequences identical to the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:669 or 131.

[0053] In some embodiments, the second antigen-binding site binding to DLL3 can incorporate a heavy chain variable domain related to SEQ ID NO:138 and a light chain variable domain related to SEQ ID NO:139. For example, the heavy 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:138, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:140), CDR2 (SEQ ID NO:141), and CDR3 (SEQ ID NO:142) sequences of SEQ ID NO:138. 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:139, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:143), CDR2 (SEQ ID NO:144), and CDR3 (SEQ ID NO:145) sequences of SEQ ID NO:139.

[0054] In some embodiments, the second antigen-binding site binding to DLL3 can incorporate a heavy chain variable domain related to SEQ ID NO:146 and a light chain variable domain related to SEQ ID NO:147. For example, the heavy 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:146, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:148), CDR2 (SEQ ID NO:149), and CDR3 (SEQ ID NO:150) sequences of SEQ ID NO:146. 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:147, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:151), CDR2 (SEQ ID NO:152), and CDR3 (SEQ ID NO:153) sequences of SEQ ID NO:147.

[0055] In some embodiments, the second antigen-binding site binding to DLL3 can incorporate a heavy chain variable domain related to SEQ ID NO:154 and a light chain variable domain related to SEQ ID NO:155. For example, the heavy 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:154, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:156), CDR2 (SEQ ID NO:157), and CDR3

(SEQ ID NO:158) sequences of SEQ ID NO:154. 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:155, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:159), CDR2 (SEQ ID NO:160), and CDR3 (SEQ ID NO:161) sequences of SEQ ID NO:155.

[0056] In some embodiments, the second antigen-binding site binding to DLL3 can incorporate a heavy chain variable domain related to SEQ ID NO:162 and a light chain variable domain related to SEQ ID NO:163. For example, the heavy 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:162, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:164), CDR2 (SEQ ID NO:165), and CDR3 (SEQ ID NO:166) sequences of SEQ ID NO:162. 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:163, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:167), CDR2 (SEQ ID NO:168), and CDR3 (SEQ ID NO:169) sequences of SEQ ID NO:163.

[0057] In some embodiments, the second antigen-binding site binding to DLL3 can incorporate a heavy chain variable domain related to SEQ ID NO:170 and a light chain variable domain related to SEQ ID NO:171. For example, the heavy 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:170, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:172), CDR2 (SEQ ID NO:173), and CDR3 (SEQ ID NO:174) sequences of SEQ ID NO:170. 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:171, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:175), CDR2 (SEQ ID NO:176), and CDR3 (SEQ ID NO:177) sequences of SEQ ID NO:171.

[0058] In some embodiments, the second antigen-binding site binding to DLL3 can incorporate a heavy chain variable domain related to SEQ ID NO:178 and a light chain variable domain related to SEQ ID NO:179. For example, the heavy 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:178, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:180), CDR2 (SEQ ID NO:181), and CDR3

(SEQ ID NO:182) sequences of SEQ ID NO:178. 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:179, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:183), CDR2 (SEQ ID NO:184), and CDR3 (SEQ ID NO:185) sequences of SEQ ID NO:179.

[0059] In some embodiments, the second antigen-binding site binding to DLL3 can incorporate a heavy chain variable domain related to SEQ ID NO:186 and a light chain variable domain related to SEQ ID NO:187. For example, the heavy 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:186, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:188), CDR2 (SEQ ID NO:189), and CDR3 (SEQ ID NO:190) sequences of SEQ ID NO:186. 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:187, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:191), CDR2 (SEQ ID NO:192), and CDR3 (SEQ ID NO:193) sequences of SEQ ID NO:187.

[0060] In some embodiments, the second antigen-binding site binding to DLL3 can incorporate a heavy chain variable domain related to SEQ ID NO:194 and a light chain variable domain related to SEQ ID NO:195. For example, the heavy 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:194, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:196), CDR2 (SEQ ID NO:197), and CDR3 (SEQ ID NO:198) sequences of SEQ ID NO:194. 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:195, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:199), CDR2 (SEQ ID NO:200), and CDR3 (SEQ ID NO:201) sequences of SEQ ID NO:195.

[0061] In certain embodiments, the second antigen-binding site can bind MUC1 (or MUC1-C) and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:203 and a light chain variable domain related to SEQ ID NO:207. For example, the heavy 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 203, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:204), CDR2 (SEQ ID

NO:205), and CDR3 (SEQ ID NO:206) sequences of SEQ ID NO:203. 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:207, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:208), CDR2 (SEQ ID NO:209), and CDR3 (SEQ ID NO:210) sequences of SEQ ID NO:207.

[0062] Alternatively, the second antigen-binding site binding to MUC1 (or MUC1-C) can incorporate a heavy chain variable domain related to SEQ ID NO:211 and a light chain variable domain related to SEQ ID NO:215. For example, the heavy 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:211, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:212), CDR2 (SEQ ID NO:213), and CDR3 (SEQ ID NO:214) sequences of SEQ ID NO:211. 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:215, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:216), CDR2 (SEQ ID NO:217), and CDR3 (SEQ ID NO:218) sequences of SEQ ID NO:215.

[0063] The certain embodiments, the second antigen-binding site can bind to Plexin-A1 and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:220 and a light chain variable domain related to SEQ ID NO:224. For example, the heavy 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:220, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:221), CDR2 (SEQ ID NO:222), and CDR3 (SEQ ID NO:223) sequences of SEQ ID NO:220. 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:224, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:225), CDR2 (SEQ ID NO:226), and CDR3 (SEQ ID NO:227) sequences of SEQ ID NO:224. Alternatively, the second antigen-binding site binding to Plexin-A1 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:228 and a light chain variable domain related to SEQ ID NO:232. For example, the heavy 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:228, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:229), CDR2 (SEQ ID NO:230), and CDR3 (SEQ ID NO:231) sequences of

SEQ ID NO:228. 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:232, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:233), CDR2 (SEQ ID NO:234), and CDR3 (SEQ ID NO:235) sequences of SEQ ID NO:232.

[0064] In certain embodiments, the second antigen-binding site can bind to TNFRSF10B and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:237 and a light chain variable domain related to SEQ ID NO:241. For example, the heavy 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:237, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:238), CDR2 (SEQ ID NO:239), and CDR3 (SEQ ID NO:240) sequences of SEQ ID NO:237. 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:241, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:242), CDR2 (SEQ ID NO:243), and CDR3 (SEQ ID NO:244) sequences of SEQ ID NO:241. Alternatively, the second antigen-binding site binding to TNFRSF10B can optionally incorporate a heavy chain variable domain related to SEQ ID NO:245 and a light chain variable domain related to SEQ ID NO:249. For example, the heavy 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:245, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:246), CDR2 (SEQ ID NO:247), and CDR3 (SEQ ID NO:248) sequences of SEQ ID NO:248. 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:249, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:250), CDR2 (SEQ ID NO:251), and CDR3 (SEQ ID NO:252) sequences of SEQ ID NO:249. Alternatively, the second antigen-binding site binding to TNFRSF10B can optionally incorporate a heavy chain variable domain related to SEQ ID NO:253 and a light chain variable domain related to SEQ ID NO:257. For example, the heavy 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:253, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:254), CDR2 (SEQ ID NO:255), and CDR3 (SEQ ID NO:256) sequences of SEQ ID NO:253. 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:257, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:258), CDR2 (SEQ ID NO:259), and CDR3 (SEQ ID NO:260) sequences of SEQ ID NO:257.

5 **[0065]** In certain embodiments, the second antigen-binding site can bind to STEAP1 and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:262 and a light chain variable domain related to SEQ ID NO:266. For example, the heavy 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:262, and/or
10 incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:263), CDR2 (SEQ ID NO:264), and CDR3 (SEQ ID NO:265) sequences of SEQ ID NO:262. 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:266, and/or
15 incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:267), CDR2 (SEQ ID NO:268), and CDR3 (SEQ ID NO:269) sequences of SEQ ID NO:266. Alternatively, the second antigen-binding site binding to STEAP1 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:270 and a light chain variable domain related to SEQ ID NO:274. For example, the heavy 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%)
20 identical to SEQ ID NO:270, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:271), CDR2 (SEQ ID NO:272), and CDR3 (SEQ ID NO:273) sequences of SEQ ID NO:270. 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:274, and/or incorporate amino acid sequences identical to the
25 CDR1 (SEQ ID NO:275), CDR2 (SEQ ID NO:276), and CDR3 (SEQ ID NO:277) sequences of SEQ ID NO:274.

[0066] In certain embodiments, the second antigen-binding site can bind to CDCP1 and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:279 and a light chain variable domain related to SEQ ID NO:283 or SEQ ID NO:287. For example, the
30 heavy 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:279, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:280), CDR2 (SEQ ID NO:281), and CDR3 (SEQ ID NO:282) sequences of SEQ ID NO:279.

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:283, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:284), CDR2 (SEQ ID NO:285), and CDR3 (SEQ ID NO:286) sequences of SEQ ID

NO:283. Alternatively, 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:287, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:288), CDR2 (SEQ ID NO:289), and CDR3 (SEQ ID NO:290) sequences of SEQ ID NO:287.

[0067] In certain embodiments, the second antigen-binding site can bind to PTK7 and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:292 and a light chain variable domain related to SEQ ID NO:296. For example, the heavy 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:292, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:293), CDR2 (SEQ ID NO:294), and CDR3 (SEQ ID NO:295) sequences of SEQ ID NO:292. 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:296, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:297), CDR2 (SEQ ID NO:298), and CDR3 (SEQ ID NO:299) sequences of SEQ ID NO:296.

[0068] The second antigen-binding site binding to AXL can optionally incorporate a heavy chain variable domain related to SEQ ID NO:301 and a light chain variable domain related to SEQ ID NO:305. For example, the heavy 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:301, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:302), CDR2 (SEQ ID NO:303), and CDR3 (SEQ ID NO:304) sequences of SEQ ID NO:301. 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:305, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:306), CDR2 (SEQ ID NO:307), and CDR3 (SEQ ID NO:308) sequences of SEQ ID NO:305. Alternatively, the second antigen-binding site binding to AXL can optionally incorporate a heavy chain variable domain related to SEQ ID NO:309 and a light chain variable domain related to SEQ ID NO:313. For example, the

heavy 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:309, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:310), CDR2 (SEQ ID NO:311), and CDR3 (SEQ ID NO:312) sequences of SEQ ID NO:309.

5 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:313, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:314), CDR2 (SEQ ID NO:315), and CDR3 (SEQ ID NO:316) sequences of SEQ ID NO:313.

10 **[0069]** In certain embodiments, the second antigen-binding site can bind to ERBB-3 and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:318 and a light chain variable domain related to SEQ ID NO:322. For example, the heavy 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:318, and/or
15 incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:319), CDR2 (SEQ ID NO:320), and CDR3 (SEQ ID NO:321) sequences of SEQ ID NO:318. 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:322, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:323), CDR2 (SEQ ID
20 NO:324), and CDR3 (SEQ ID NO:325) sequences of SEQ ID NO:322. Alternatively, the second antigen-binding site binding to ERBB-3 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:326 and a light chain variable domain related to SEQ ID NO:330. For example, the heavy 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%)
25 identical to SEQ ID NO:326, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:327), CDR2 (SEQ ID NO:328), and CDR3 (SEQ ID NO:329) sequences of SEQ ID NO:326. 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:330, and/or incorporate amino acid sequences identical to the
30 CDR1 (SEQ ID NO:331), CDR2 (SEQ ID NO:332), and CDR3 (SEQ ID NO:333) sequences of SEQ ID NO:330. Alternatively, the second antigen-binding site binding to ERBB-3 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:334 and a light chain variable domain related to SEQ ID NO:338. For example, the heavy 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:334, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:335), CDR2 (SEQ ID NO:336), and CDR3 (SEQ ID NO:337) sequences of SEQ ID NO:334. 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:338, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:339), CDR2 (SEQ ID NO:340), and CDR3 (SEQ ID NO:341) sequences of SEQ ID NO:338.

[0070] In certain embodiments, the second antigen-binding site can bind to EDNRB and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:343 and a light chain variable domain related to SEQ ID NO:347. For example, the heavy 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:343, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:344), CDR2 (SEQ ID NO:345), and CDR3 (SEQ ID NO:346) sequences of SEQ ID NO:343. 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:347, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:348), CDR2 (SEQ ID NO:349), and CDR3 (SEQ ID NO:350) sequences of SEQ ID NO:347. Alternatively, the second antigen-binding site binding to EDNRB can optionally incorporate a heavy chain variable domain related to SEQ ID NO:351 and a light chain variable domain related to SEQ ID NO:355. For example, the heavy 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:351, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:352), CDR2 (SEQ ID NO:353), and CDR3 (SEQ ID NO:354) sequences of SEQ ID NO:351. 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:355, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:356), CDR2 (SEQ ID NO:357), and CDR3 (SEQ ID NO:358) sequences of SEQ ID NO:355.

[0071] In certain embodiments, the second antigen-binding site can bind to TYRP1 and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:360 and a light chain variable domain related to SEQ ID NO:364. For example, the heavy 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:360, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:361), CDR2 (SEQ ID NO:362), and CDR3 (SEQ ID NO:363) sequences of SEQ ID NO:360. 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:364, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:365), CDR2 (SEQ ID NO:366), and CDR3 (SEQ ID NO:367) sequences of SEQ ID NO:364. Alternatively, the second antigen-binding site binding to TYRP1 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:368 and a light chain variable domain related to SEQ ID NO:372. For example, the heavy 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:368, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:369), CDR2 (SEQ ID NO:370), and CDR3 (SEQ ID NO:371) sequences of SEQ ID NO:368. 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:372, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:373), CDR2 (SEQ ID NO:374), and CDR3 (SEQ ID NO:375) sequences of SEQ ID NO:372. Alternatively, the second antigen-binding site binding to TYRP1 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:376 and a light chain variable domain related to SEQ ID NO:380. For example, the heavy 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:376, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:377), CDR2 (SEQ ID NO:378), and CDR3 (SEQ ID NO:379) sequences of SEQ ID NO:376. 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:380, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:381), CDR2 (SEQ ID NO:382), and CDR3 (SEQ ID NO:383) sequences of SEQ ID NO:380.

[0072] In certain embodiments, the second antigen-binding site can bind to OLR1 and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:385 and a light chain variable domain related to SEQ ID NO:389. For example, the heavy 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:385, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:386), CDR2 (SEQ ID NO:387), and CDR3 (SEQ ID NO:388) sequences of SEQ ID NO:385. 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:389, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:390), CDR2 (SEQ ID NO:391), and CDR3 (SEQ ID NO:392) sequences of SEQ ID NO:389. Alternatively, the second antigen-binding site binding to OLR1 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:393 and a light chain variable domain related to SEQ ID NO:397. For example, the heavy 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:393, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:394), CDR2 (SEQ ID NO:395), and CDR3 (SEQ ID NO:396) sequences of SEQ ID NO:393. 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:397, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:398), CDR2 (SEQ ID NO:399), and CDR3 (SEQ ID NO:400) sequences of SEQ ID NO:397.

[0073] In certain embodiments, the second antigen-binding site can bind to PLAUR and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:405 and a light chain variable domain related to SEQ ID NO:409. For example, the heavy 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:405, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:406), CDR2 (SEQ ID NO:407), and CDR3 (SEQ ID NO:408) sequences of SEQ ID NO:405. 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:409, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:410), CDR2 (SEQ ID NO:411), and CDR3 (SEQ ID NO:412) sequences of SEQ ID NO:409.

[0074] Alternatively, the second antigen-binding site binding to PLAUR can optionally incorporate a heavy chain variable domain related to SEQ ID NO:413 and a light chain variable domain related to SEQ ID NO:417. For example, the heavy 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:413, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:414), CDR2 (SEQ ID NO:415), and CDR3 (SEQ ID NO:416) sequences of SEQ ID NO:413. 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:417, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:418), CDR2 (SEQ ID NO:419), and CDR3 (SEQ ID NO:420) sequences of SEQ ID NO:417.

[0075] In certain embodiments, the second antigen-binding site can bind to CCR6 and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:422 and a light chain variable domain related to SEQ ID NO:426. For example, the heavy 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:422, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:423), CDR2 (SEQ ID NO:424), and CDR3 (SEQ ID NO:425) sequences of SEQ ID NO:422. 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:426, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:427), CDR2 (SEQ ID NO:428), and CDR3 (SEQ ID NO:429) sequences of SEQ ID NO:426.

[0076] Alternatively, the second antigen-binding site binding to CCR6 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:430 and a light chain variable domain related to SEQ ID NO:434. For example, the heavy 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:430, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:431), CDR2 (SEQ ID NO:432), and CDR3 (SEQ ID NO:433) sequences of SEQ ID NO:430. 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:434, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:435), CDR2 (SEQ ID NO:436), and CDR3 (SEQ ID NO:437) sequences of SEQ ID NO:434.

[0077] In certain embodiments, the second antigen-binding site can bind to EPHA4 and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:439 and a light chain variable domain related to SEQ ID NO:443. For example, the heavy 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:439, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:440), CDR2 (SEQ ID NO:441), and CDR3 (SEQ ID NO:442) sequences of SEQ ID NO:439. 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:443, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:444), CDR2 (SEQ ID NO:445), and CDR3 (SEQ ID NO:446) sequences of SEQ ID NO:443.

[0078] Alternatively, the second antigen-binding site binding to EPHA4 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:447 and a light chain variable domain related to SEQ ID NO:451. For example, the heavy 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:447, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:448), CDR2 (SEQ ID NO:449), and CDR3 (SEQ ID NO:450) sequences of SEQ ID NO:447. 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:451, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:452), CDR2 (SEQ ID NO:453), and CDR3 (SEQ ID NO:454) sequences of SEQ ID NO:451.

[0079] In certain embodiments, the second antigen-binding site can bind to CD14 and can optionally incorporate a heavy chain variable domain related to SEQ ID NO:456 and a light chain variable domain related to SEQ ID NO:460. For example, the heavy 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:456, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:457), CDR2 (SEQ ID NO:458), and CDR3 (SEQ ID NO:459) sequences of SEQ ID NO:456. 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:460, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:461), CDR2 (SEQ ID NO:462), and CDR3 (SEQ ID NO:463) sequences of SEQ ID NO:460.

[0080] Alternatively, the second antigen-binding site binding to CD14 can incorporate a heavy chain variable domain incorporating amino acid sequences identical to the CDR1 (SEQ ID NO:464), CDR2 (SEQ ID NO:465), and CDR3 (SEQ ID NO:466) sequences; and a light

chain variable domain incorporating amino acid sequences identical to the CDR1 (SEQ ID NO:467), CDR2 (SEQ ID NO:468), and CDR3 (SEQ ID NO:469) sequences.

[0081] The second antigen-binding site binding to CD163 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:471 and a light chain variable domain related to SEQ ID NO:475. For example, the heavy 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:471, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:472), CDR2 (SEQ ID NO:473), and CDR3 (SEQ ID NO:474) sequences of SEQ ID NO:471. 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:475, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:476), CDR2 (SEQ ID NO:477), and CDR3 (SEQ ID NO:478) sequences of SEQ ID NO:475.

[0082] Alternatively, the second antigen-binding site binding to CD163 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:479 and a light chain variable domain related to SEQ ID NO:483. For example, the heavy 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:479, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:480), CDR2 (SEQ ID NO:481), and CDR3 (SEQ ID NO:482) sequences of SEQ ID NO:479. 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:483, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:484), CDR2 (SEQ ID NO:485), and CDR3 (SEQ ID NO:486) sequences of SEQ ID NO:483.

[0083] The second antigen-binding site binding to CSF3R can optionally incorporate a heavy chain variable domain related to SEQ ID NO:488 and a light chain variable domain related to SEQ ID NO:492. For example, the heavy 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:488, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:489), CDR2 (SEQ ID NO:490), and CDR3 (SEQ ID NO:491) sequences of SEQ ID NO:488. 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:492, and/or incorporate amino acid

sequences identical to the CDR1 (SEQ ID NO:493), CDR2 (SEQ ID NO:494), and CDR3 (SEQ ID NO:495) sequences of SEQ ID NO:492.

[0084] The second antigen-binding site binding to Siglec-9 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:497 and a light chain variable domain related to SEQ ID NO:501. For example, the heavy 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:497, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:498), CDR2 (SEQ ID NO:499), and CDR3 (SEQ ID NO:500) sequences of SEQ ID NO:497. 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:501, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:502), CDR2 (SEQ ID NO:503), and CDR3 (SEQ ID NO:504) sequences of SEQ ID NO:501.

[0085] Alternatively, the second antigen-binding site binding to Siglec-9 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:505 and a light chain variable domain related to SEQ ID NO:509. For example, the heavy 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:505, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:506), CDR2 (SEQ ID NO:507), and CDR3 (SEQ ID NO:508) sequences of SEQ ID NO:505. 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:509, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:510), CDR2 (SEQ ID NO:511), and CDR3 (SEQ ID NO:512) sequences of SEQ ID NO:509.

[0086] The second antigen-binding site binding to ITGAM can optionally incorporate a heavy chain variable domain related to SEQ ID NO:514 and a light chain variable domain related to SEQ ID NO:518. For example, the heavy 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:514, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:515), CDR2 (SEQ ID NO:516), and CDR3 (SEQ ID NO:517) sequences of SEQ ID NO:514. 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:518, and/or incorporate amino acid

sequences identical to the CDR1 (SEQ ID NO:519), CDR2 (SEQ ID NO:520), and CDR3 (SEQ ID NO:521) sequences of SEQ ID NO:518.

[0087] The second antigen-binding site binding to CCR1 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:523 and a light chain variable domain related to SEQ ID NO:527. For example, the heavy 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:523, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:524), CDR2 (SEQ ID NO:525), and CDR3 (SEQ ID NO:526) sequences of SEQ ID NO:523. 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:527, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:528), CDR2 (SEQ ID NO:529), and CDR3 (SEQ ID NO:530) sequences of SEQ ID NO:527.

[0088] Alternatively, the second antigen-binding site binding to CCR1 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:531 and a light chain variable domain related to SEQ ID NO:535. For example, the heavy 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:531, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:532), CDR2 (SEQ ID NO:533), and CDR3 (SEQ ID NO:534) sequences of SEQ ID NO:531. 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:535, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:536), CDR2 (SEQ ID NO:537), and CDR3 (SEQ ID NO:538) sequences of SEQ ID NO:535.

[0089] The second antigen-binding site binding to TLR2 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:544 and a light chain variable domain related to SEQ ID NO:548. For example, the heavy 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:544, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:545), CDR2 (SEQ ID NO:546), and CDR3 (SEQ ID NO:547) sequences of SEQ ID NO:544. 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:548, and/or incorporate amino acid

sequences identical to the CDR1 (SEQ ID NO:549), CDR2 (SEQ ID NO:550), and CDR3 (SEQ ID NO:551) sequences of SEQ ID NO:548.

[0090] Alternatively, the second antigen-binding site binding to TLR2 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:552 and a light chain variable domain related to SEQ ID NO:556. For example, the heavy 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:552, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:553), CDR2 (SEQ ID NO:554), and CDR3 (SEQ ID NO:555) sequences of SEQ ID NO:552. 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:556, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:557), CDR2 (SEQ ID NO:558), and CDR3 (SEQ ID NO:559) sequences of SEQ ID NO:556.

[0091] The second antigen-binding site binding to TLR4 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:561 and a light chain variable domain related to SEQ ID NO:565. For example, the heavy 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:561, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:562), CDR2 (SEQ ID NO:563), and CDR3 (SEQ ID NO:564) sequences of SEQ ID NO:561. 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:565, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:566), CDR2 (SEQ ID NO:567), and CDR3 (SEQ ID NO:568) sequences of SEQ ID NO:565.

[0092] Alternatively, the second antigen-binding site binding to TLR4 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:569 and a light chain variable domain related to SEQ ID NO:573. For example, the heavy 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:569, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:570), CDR2 (SEQ ID NO:571), and CDR3 (SEQ ID NO:572) sequences of SEQ ID NO:569. 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:573, and/or incorporate

amino acid sequences identical to the CDR1 (SEQ ID NO:574), CDR2 (SEQ ID NO:575), and CDR3 (SEQ ID NO:576) sequences of SEQ ID NO:573.

[0093] The second antigen-binding site binding to CCR5 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:581 and a light chain variable domain related to SEQ ID NO:585. For example, the heavy 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:581, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:582), CDR2 (SEQ ID NO:583), and CDR3 (SEQ ID NO:584) sequences of SEQ ID NO:581. 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:585, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:586), CDR2 (SEQ ID NO:587), and CDR3 (SEQ ID NO:588) sequences of SEQ ID NO:585.

[0094] Alternatively, the second antigen-binding site binding to CCR5 can optionally incorporate a heavy chain variable domain related to SEQ ID NO:589 and a light chain variable domain related to SEQ ID NO:593. For example, the heavy 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:589, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:590), CDR2 (SEQ ID NO:591), and CDR3 (SEQ ID NO:592) sequences of SEQ ID NO:589. 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:593, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:594), CDR2 (SEQ ID NO:595), and CDR3 (SEQ ID NO:596) sequences of SEQ ID NO:593.

[0095] The second antigen-binding site binding to B7-H4 (VTCN1) can optionally incorporate a heavy chain variable domain related to SEQ ID NO:598 and a light chain variable domain related to SEQ ID NO:602. For example, the heavy 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:598, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:599), CDR2 (SEQ ID NO:600), and CDR3 (SEQ ID NO:601) sequences of SEQ ID NO:598. 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:602, and/or incorporate

amino acid sequences identical to the CDR1 (SEQ ID NO:603), CDR2 (SEQ ID NO:604), and CDR3 (SEQ ID NO:605) sequences of SEQ ID NO:602.

[0096] Alternatively, the second antigen-binding site binding to B7-H4 (VTCN1) can optionally incorporate a heavy chain variable domain related to SEQ ID NO:606 and a light chain variable domain related to SEQ ID NO:610. For example, the heavy 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:606, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:607), CDR2 (SEQ ID NO:608), and CDR3 (SEQ ID NO:609) sequences of SEQ ID NO:606. 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:610, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:611), CDR2 (SEQ ID NO:612), and CDR3 (SEQ ID NO:613) sequences of SEQ ID NO:610.

[0097] The second antigen-binding site binding to VISTA can optionally incorporate a heavy chain variable domain related to SEQ ID NO:615 and a light chain variable domain related to SEQ ID NO:619. For example, the heavy 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:615, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:616), CDR2 (SEQ ID NO:617), and CDR3 (SEQ ID NO:618) sequences of SEQ ID NO:615. 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:619, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:620), CDR2 (SEQ ID NO:621), and CDR3 (SEQ ID NO:622) sequences of SEQ ID NO:619.

[0098] Alternatively, the second antigen-binding site binding to VISTA can optionally incorporate a heavy chain variable domain related to SEQ ID NO:623 and a light chain variable domain related to SEQ ID NO:627. For example, the heavy 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:623, and/or incorporate amino acid sequences identical to the CDR1 (SEQ ID NO:624), CDR2 (SEQ ID NO:625), and CDR3 (SEQ ID NO:626) sequences of SEQ ID NO:623. 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:627, and/or incorporate

amino acid sequences identical to the CDR1 (SEQ ID NO:628), CDR2 (SEQ ID NO:629), and CDR3 (SEQ ID NO:630) sequences of SEQ ID NO:627.

[0099] In some embodiments, the second antigen binding site incorporates a light chain variable domain having an amino acid sequence identical to the amino acid sequence of the light chain variable domain present in the first antigen binding site.

[0100] In some embodiments, the protein incorporates a portion of an antibody Fc domain sufficient to bind CD16, wherein the antibody Fc domain comprises hinge and CH2 domains, and/or amino acid sequences at least 90% identical to amino acid sequence 234-332 of a human IgG antibody.

[0101] In certain embodiments, the protein further incorporates a fourth antigen-binding site that binds to a tumor-associated antigen, which includes any antigen that is associated with cancer. For example, the fourth antigen-binding site may bind to human epidermal growth factor receptor 2 (HER2), CD20, CD33, B-cell maturation antigen (BCMA), prostate-specific membrane (PSMA), DLL3, ganglioside GD2 (GD2), CD123, anoctamin-1 (Ano1), mesothelin, carbonic anhydrase IX (CAIX), tumor-associated calcium signal transducer 2 (TROP2), carcinoembryonic antigen (CEA), claudin-18.2, receptor tyrosine kinase-like orphan receptor 1 (ROR1), trophoblast glycoprotein (5T4), glycoprotein non-metastatic melanoma protein B (GPNMB), folate receptor-alpha (FR-alpha), pregnancy-associated plasma protein A (PAPP-A), CD37, epithelial cell adhesion molecule (EpCAM), CD2, CD19, CD30, CD38, CD40, CD52, CD70, CD79b, fms-like tyrosine kinase 3 (FLT3), glypican 3 (GPC3), B7 homolog 6 (B7H6), C-C chemokine receptor type 4 (CCR4), C-X-C motif chemokine receptor 4 (CXCR4), receptor tyrosine kinase-like orphan receptor 2 (ROR2), CD133, HLA class I histocompatibility antigen, alpha chain E (HLA-E), epidermal growth factor receptor (EGFR/ERBB1), insulin-like growth factor 1-receptor (IGF1R), human epidermal growth factor receptor 3 (HER3)/ERBB-3, human epidermal growth factor receptor 4 (HER4)/ERBB-4, MUC1, tyrosine protein kinase MET (cMET), signaling lymphocytic activation molecule F7 (SLAMF7), prostate stem cell antigen (PSCA), MHC class I polypeptide-related sequence A (MICA), MHC class I polypeptide-related sequence B (MICB), TNF-related apoptosis inducing ligand receptor 1 (TRAILR1), TNF-related apoptosis inducing ligand receptor 2 (TRAILR2), melanoma associated antigen 3 (MAGE-A3), B-lymphocyte activation antigen B7.1 (B7.1), B-lymphocyte activation antigen B7.2 (B7.2), cytotoxic T-lymphocyte associated protein 4 (CTLA4), programmed cell death

protein 1 (PD1), programmed cell death 1 ligand 1 (PD-L1), or CD25 antigen expressed on cancer cells.

[0102] Formulations containing one of these proteins; cells containing one or more nucleic acids expressing these proteins, and methods of enhancing tumor cell death using these proteins are also provided.

[0103] Another aspect of the invention 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 protein described herein. Exemplary cancers for

treatment using the DLL3-targeting multi-specific binding proteins include, for example,

small cell lung cancer, large cell neuroendocrine carcinoma, glioblastoma, Ewing sarcoma, and cancers with neuroendocrine phenotype. Cancers to be treated using MUC1 (or MUC1-

C)-targeting multi-specific binding proteins include, for example, gastric cancer, colorectal cancer, pancreatic cancer, breast cancer, endometrial cancer, lung cancer, bladder cancer, cervical cancer, head and neck cancer, ovarian cancer, renal cell cancer, and multiple

myeloma. Cancers to be treated using Plexin-A1-targeting multi-specific binding proteins include, for example, head and neck cancer, gastric cancer, pancreatic cancer, prostate cancer, and glioma. Cancers to be treated using TNFRSF10B-targeting multi-specific binding proteins include, for example, liver cancer, pancreatic cancer, stomach cancer, renal cancer, breast cancer, ovarian cancer, endometrial cancer, and melanoma. Cancers to be treated using

STEAP1-targeting multi-specific binding proteins include, for example, prostate cancer, bladder cancer, colon cancer, pancreatic cancer, ovarian cancer, testicular cancer, breast cancer, cervical cancer, and Ewing sarcoma. Cancers to be treated using CDCP1-targeting

multi-specific binding proteins include, for example, colon cancer, lung cancer, gastric cancer, breast cancer, pancreatic cancer, head and neck cancer, bladder cancer, ovarian

cancer, endometrial cancer, and skin cancer. Cancers to be treated using PTK7-targeting multi-specific binding proteins include, for example, lung cancer, head and neck cancer, stomach cancer, prostate cancer, testicular cancer, endometrial cancer, breast cancer,

melanoma, skin cancer, and leukemia. Cancers to be treated using Axl-targeting multi-specific binding proteins include, for example, breast cancer, lung cancer, colon cancer,

prostate cancer, renal cancer, esophageal cancer, liver cancer, pancreatic cancer, Kaposi's sarcoma, acute myeloid leukemia, glioma, and mesothelioma. Cancers to be treated using

ERBB-3-targeting multi-specific binding proteins include, for example, prostate cancer, bladder cancer, breast cancer, ovarian cancer, colon cancer, pancreatic cancer, stomach

cancer, oral cavity cancer, head and neck cancer, lung cancer, and melanoma. Cancers to be treated using EDNRB-targeting multi-specific binding proteins include, for example, melanoma, uveal melanoma, and glioma. Cancers to be treated using TYRP1-targeting multi-specific binding proteins include, for example, melanoma. Cancers to be treated using OLR1-targeting multi-specific binding proteins include, for example, gastric cancer, colorectal cancer, pancreatic cancer, prostate cancer, breast cancer, and endometrial cancer. Cancers to be treated using ADAM12-targeting multi-specific binding proteins include any cancers that express ADAM12, for example, prostate cancer, breast cancer, basal cell carcinoma, squamous cell carcinoma, melanoma, pancreatic cancer, bladder cancer, colorectal cancer, lung cancer, liver cancer, esophageal cancer, non-Hodgkin's lymphoma, ovarian cancer, and uterine cancer. Cancers to be treated using PLAUR-targeting multi-specific binding proteins include any cancers that express PLAUR, for example, breast cancer, colorectal cancer, non-small cell lung cancer, multiple myeloma, and oral cancer. Cancers to be treated using CCR6-targeting multi-specific binding proteins include any cancers that express CCR6, for example, colorectal cancer, breast cancer, cervical cancer, liver cancer, lung cancer, prostate cancer, and cutaneous T-cell lymphoma. Cancers to be treated using EPHA4-targeting multi-specific binding proteins include any cancers that express EPHA4, for example, melanoma, glioma, prostate cancer, breast cancer, small cell lung cancer, endometrial cancer, esophageal cancer, gastric cancer, and colorectal cancer.

[0104] In certain embodiments, the invention provides a method of treating cancer in a patient by targeting MDSCs and/or TAMs present in the tumor environment, and the method comprises administering to a patient in need thereof a therapeutically effective amount of the multi-specific binding proteins described herein. In some embodiments, the multi-specific binding proteins target the MDSCs and/or TAMs in a tumor environment. In some embodiments, the multi-specific binding proteins target cancer cells as well as the MDSCs and/or TAMs in the same tumor environment. Exemplary cancers to be treated may be hematological malignancies such as acute myeloid leukemia, myelodysplastic and/or myeloproliferative neoplasms, acute lymphoblastic leukemia, B-cell lymphoma, chronic neutrophilic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia. Alternatively, the cancers to be treated may be solid tumors such as bladder cancer, colon cancer, prostate cancer, breast cancer, glioblastoma, hepatocellular carcinoma, head and neck cancer, lung cancer, ovarian cancer, pancreatic cancer, kidney cancer and melanoma.

BRIEF DESCRIPTION OF THE DRAWINGS

[0105] **FIG. 1** is a representation of a heterodimeric multi-specific binding protein. Each arm can represent either an NKG2D-binding domain, or a binding domain to a tumor-associated antigen, MDSC-associated antigen, or TAM-associated antigen. The multi-specific binding protein further comprises an Fc domain or a portion thereof that binds to CD16. In some embodiments, the NKG2D-binding and antigen-binding domains can share a common light chain.

[0106] **FIG. 2** is a representation of a heterodimeric multi-specific binding protein. Either the NKG2D binding domain, or the binding domain to a tumor-associated antigen, MDSC-associated antigen, or TAM-associated antigen can take an scFv format (right arm).

[0107] **FIG. 3** is a line graph showing the binding affinity of NKG2D-binding domains (listed as clones) to human recombinant NKG2D in an ELISA assay.

[0108] **FIG. 4** is a line graph showing the binding affinity of NKG2D-binding domains (listed as clones) to cynomolgus recombinant NKG2D in an ELISA assay.

[0109] **FIG. 5** is a line graph showing the binding affinity of NKG2D-binding domains (listed as clones) to mouse recombinant NKG2D in an ELISA assay.

[0110] **FIG. 6** is a bar graph showing the binding of NKG2D-binding domains (listed as clones) to EL4 cells expressing human NKG2D, measured by flow cytometry as mean fluorescence intensity (MFI) fold-over-background (FOB).

[0111] **FIG. 7** is a bar graph showing the binding of NKG2D-binding domains (listed as clones) to EL4 cells expressing mouse NKG2D, measured by flow cytometry as mean fluorescence intensity (MFI) fold-over-background (FOB).

[0112] **FIG. 8** is a line graph showing the binding affinity of NKG2D-binding domains (listed as clones) for recombinant human NKG2D-Fc in a competitive binding assay with NKG2D's natural ligand, ULBP-6.

[0113] **FIG. 9** is a line graph showing the binding affinity of NKG2D-binding domains (listed as clones) for recombinant human NKG2D-Fc in a competitive binding assay with NKG2D's natural ligand, MICA.

[0114] **FIG. 10** is a line graph showing the binding affinity of NKG2D-binding domains (listed as clones) for recombinant mouse NKG2D-Fc in a competitive binding assay with NKG2D's natural ligand, Rae-1 delta.

[0115] FIG. 11 is a bar graph showing activation of cells expressing human NKG2D-CD3 zeta fusion proteins by NKG2D-binding domains (listed as clones) as measured by flow cytometry and quantified as the percentage of TNF α -positive cells.

[0116] FIG. 12 is a bar graph showing activation of cells expressing mouse NKG2D-CD3 zeta fusions proteins by NKG2D-binding domains (listed as clones) as measured by flow cytometry and quantified as the percentage of TNF α -positive cells.

[0117] FIG. 13 is a bar graph showing activation of human NK cells by NKG2D-binding domains (listed as clones) as measured by flow cytometry and quantified as the percentage of IFN γ ⁺/CD107a⁺ cells.

[0118] FIG. 14 is a bar graph showing activation of human NK cells by NKG2D-binding domains (listed as clones) as measured by flow cytometry and quantified as the percentage of IFN γ ⁺/CD107a⁺ cells.

[0119] FIG. 15 is a bar graph showing activation of mouse NK cells by NKG2D-binding domains (listed as clones) as measured by flow cytometry and quantified as the percentage of IFN γ ⁺/CD107a⁺ cells.

[0120] FIG. 16 is a bar graph showing activation of mouse NK cells by NKG2D-binding domains (listed as clones) as measured by flow cytometry and quantified as the percentage of IFN γ ⁺/CD107a⁺ cells.

[0121] FIG. 17 is a bar graph showing the cytotoxic effect of NKG2D-binding domains (listed as clones) on THP-1 tumor cells as measured using a Perkin Elmer DELFIA[®] Cytotoxicity kit assay.

[0122] FIG. 18 is a bar graph showing the melting temperature of NKG2D-binding domains (listed as clones) measured by differential scanning fluorimetry.

[0123] FIGS. 19A-19C are bar graphs showing synergistic activation of NK cells by CD16 and NKG2D binding as measured by flow cytometry and quantified as the percentage of positive cells for NK activation markers. FIG. 19A shows the percentage of CD107a⁺ cells 4 hours post-treatment with plate-bound anti-CD16 monoclonal antibody alone, anti-NKG2D antibody alone, or anti-CD16 antibody in combination with anti-NKG2D antibody. FIG. 19B shows the percentage of IFN γ ⁺ cells 4 hours post-treatment with plate-bound anti-CD16 monoclonal antibody alone, anti-NKG2D antibody alone, or anti-CD16 antibody in combination with anti-NKG2D antibody. FIG. 19C shows the percentage of CD107a⁺/IFN γ ⁺

cells 4 hours post-treatment with plate-bound anti-CD16 monoclonal antibody alone, anti-NKG2D antibody alone, or anti-CD16 antibody in combination with anti-NKG2D antibody. . Graphs indicate the mean ($n = 2$) $\pm$ SD. Data are representative of five independent experiments using five different healthy donors.

5 [0124] FIG. 20 are line graphs showing binding kinetics of murine anti-DLL3 antibodies to the extracellular domain (ECD) of DLL3 obtained by surface plasmon resonance (SPR) analysis at 37 °C. Antibodies have affinities from <0.011 up to 8.44 nM. Stemcentrx antibody was used as a control.

[0125] FIG. 21A are line graphs showing binding kinetics of a murine anti-DLL3
10 antibody, that includes the 5E7 clone, to the different domains of DLL3 obtained by SPR analysis at 37 °C. FIG. 21B is an illustration of different domains of DLL3, including an N-terminal domain (N-term), a DSL domain, EGF domains, and a C-terminal domains (C). “PM” indicates the plasma membrane.

[0126] FIG. 22A is a time-response curve showing the epitope binning of an anti-DLL3
15 antibody corresponding to the 9E6 clone with the Stemcentrx anti-DLL3 antibody measured by SPR analysis at 25 °C. FIG. 22B is a time-response curve showing the epitope binning of an anti-DLL3 antibody corresponding to the 2F7 clone with the Stemcentrx anti-DLL3 antibody measured by SPR analysis at 25 °C. FIG. 22C is a time-response curve showing the epitope binning of an anti-DLL3 antibody corresponding to the 5E7 clone with the
20 Stemcentrx anti-DLL3 antibody measured by SPR analysis at 25 °C. Anti-DLL3 antibodies were captured in a uniform orientation on the anti-mouse Fc Biacore chip, followed by injection of DLL3 ECD, followed by injection of the Stemcentrx antibody.

[0127] FIG. 23 is a bar graph showing melting temperatures of different anti-DLL3
25 antibodies as measured by differential scanning fluorimetry. All antibodies demonstrate melting temperatures above 70 °C.

[0128] FIG. 24 is a line graph showing dose-dependent binding of anti-DLL3 antibodies to human DLL3. Antibody for DLL3 (R&D Systems, Minneapolis, MN; Cat# MAB4215) was used as a positive control.

[0129] FIGs. 25A-25B are line graphs showing limited cross-reactive binding of anti-
30 DLL3 antibodies to recombinant DLL1 and DLL4. FIG. 25A shows binding of anti-DLL3 antibodies to human DLL1. Antibody for DLL1 (BioLegend, San Diego, CA; Cat# MHD1-314) was used as a positive control. FIG. 25B shows binding of anti-DLL3 antibodies to

human DLL4. Antibody for DLL4 (BioLegend, San Diego, CA; Cat# MHD4-46) was used as a positive control.

[0130] FIG. 26A is a flow cytometry histogram profile showing the binding of anti-DLL3 antibodies (2 µg/mL) to DLL3 expressed on NCI-H82 cells. FIG. 26B is a line graph showing a dose-response profile of anti-DLL3 antibody binding to DLL3 on NCI-H82 cells.

[0131] FIG. 27A is a line graph showing antibody internalization by SHP-77 cells expressing DLL3 after 1-3 hours of incubation with anti-DLL3 antibodies. FIG. 27B is a line graph showing antibody internalization by DMS-79 cells expressing DLL3 after 1-3 hours of incubation with anti-DLL3 antibodies.

[0132] FIG. 28A is a line graph showing the dose-response curves of an anti-DLL3 multi-specific binding protein and an anti-DLL3 monoclonal antibody binding to the full-length extracellular domain of DLL3 (DLL3-D1-6). FIG. 28B is a line graph showing the dose-response curves of anti-DLL3 multi-specific binding protein and anti-DLL3 monoclonal antibody binding to a truncated form of the DLL3 extracellular domain (DLL3-1-2).

[0133] FIG. 29A is a line graph showing activation of purified human NK cells co-cultured with RPMI-8226 cells expressing DLL3-D1-6, as measured by flow cytometry and quantified as the percentage of IFN γ ⁺/CD107a⁺ cells. FIG. 29B is a line graph showing activation of purified human NK cells co-cultured with RPMI-8226 cells expressing DLL3-D1-6, as measured by flow cytometry and quantified as the mean fluorescence intensity (MFI) of CD69⁺-labelled cells.

[0134] FIG. 30A is a line graph showing lysis of RPMI-8226 cells expressing DLL3-D1-2 by IL-2 activated peripheral blood mononuclear cells (PBMCs) in the presence of anti-DLL3 multi-specific binding protein or anti-DLL3 monoclonal antibody. FIG. 30B is a bar graph showing % killing of RPMI-8226 cells expressing DLL3-D1-6 by IL-2 activated human NK cells in the presence of anti-DLL3 multi-specific binding protein or anti-DLL3 monoclonal antibody.

[0135] FIG. 31 is a representative illustration of a multi-specific binding protein in a Triomab form.

[0136] FIG. 32 is a representative illustration of a multi-specific binding protein in a KiH Common Light Chain (LC) form.

[0137] FIG. 33 is a representative illustration of a mutli-specific binding protein in a dual-variable domain immunoglobulin (DVD-Ig™) form.

[0138] FIG. 34 is representative illustration of a mutli-specific binding protein in an Orthogonal Fab interface (Ortho-Fab) form.

5 [0139] FIG. 35 is a representative illustration of a mutli-specific binding protein in a 2-in-1 Ig form.

[0140] FIG. 36 is a representative illustration of a mutli-specific binding protein in an electrostatic-steering (ES) form.

[0141] FIG. 37 is a representative illustration of a mutli-specific binding protein in a
10 controlled Fab-Arm Exchange (cFAE) form.

[0142] FIG. 38 is representative illustration of a mutli-specific binding protein in a strand-exchange engineered domain (SEED) body form.

[0143] FIG. 39 is a representative illustration of a mutli-specific binding protein in a LuZ-Y form.

15 [0144] FIG. 40 is a representative illustration of a mutli-specific binding protein in a Cov-X-Body form.

[0145] FIGS. 41A-41B are representative illustrations of a mutli-specific binding protein in a $\kappa\lambda$ -Body form. FIG. 41A is an exemplary representative illustration of one form of a $\kappa\lambda$ -Body; FIG. 41B is an exemplary representative illustration of another $\kappa\lambda$ -Body.

20 [0146] FIG. 42 is a representative illustration of a mutli-specific binding protein in a one-arm single chain (OAsc)-Fab form.

[0147] FIG. 43 is a representative illustration of a mutli-specific binding protein in a DuetMab form.

[0148] FIG. 44 is a representative illustration of a mutli-specific binding protein in a
25 CrossmAb form.

[0149] FIG. 45 is a representative illustration of a mutli-specific binding protein in a Fit-Ig form.

[0150] FIG. 46 is a series of sensograms generated from a Biacore analysis of DLL3-His binding to murine and humanized variants of the 5E7 antibody.

[0151] FIG. 47 is a graph showing the binding of humanized variants of the 5E7 antibody to RPMI-8226 cells transduced to express DLL3, compared to binding of a chimeric protein of murine 5E7 variable regions and human IgG1/Igk constant regions to the same cells.

5

DETAILED DESCRIPTION

[0152] The invention provides multi-specific binding proteins that bind a tumor-associated antigen selected from DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, and EPHA4 on a cancer cell, and the NKG2D receptor and CD16 receptor on natural
10 killer cells to activate the natural killer cells. In certain embodiments, the multi-specific binding proteins further include an additional antigen-binding site that binds a tumor-associated antigen.

[0153] The invention also provides multi-specific binding proteins that bind the NKG2D receptor and CD16 receptor on natural killer cells, and an antigen selected from CD14,
15 CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and CCR5. In some embodiments, the multi-specific binding proteins further include an additional antigen-binding site that binds a tumor-associated antigen.

[0154] The invention additionally provides pharmaceutical compositions comprising such
20 multi-specific binding proteins, and therapeutic methods using such multi-specific binding proteins and pharmaceutical compositions, for purposes such as treating cancer. Various aspects of the invention are set forth below in sections; however, aspects of the invention described in one particular section are not to be limited to any particular section.

[0155] To facilitate an understanding of the present invention, a number of terms and
25 phrases are defined below.

[0156] The terms “a” and “an” as used herein mean “one or more” and include the plural unless the context is inappropriate.

[0157] As used herein, the term “antigen-binding site” refers to the part of the immunoglobulin molecule that participates in antigen binding. In human antibodies,
30 the antigen binding site is formed by amino acid residues of the N-terminal variable (“V”) regions of the heavy (“H”) and light (“L”) chains. Three highly divergent stretches within the V regions of the heavy and light chains are referred to as “hypervariable regions” which are

interposed between more conserved flanking stretches known as “framework regions,” or “FR.” Thus the term “FR” refers to amino acid sequences which are naturally found between and adjacent to hypervariable regions in immunoglobulins. In a human antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as “complementarity-determining regions,” or “CDRs.” In certain animals, such as camels and cartilaginous fish, the antigen-binding site is formed by a single antibody chain providing a “single domain antibody.” Antigen-binding sites can exist in an intact antibody, in an antigen-binding fragment of an antibody that retains the antigen-binding surface, or in a recombinant polypeptide such as an scFv, using a peptide linker to connect the heavy chain variable domain to the light chain variable domain in a single polypeptide.

[0158] The term “tumor associated antigen” as used herein means any antigen including but not limited to a protein, glycoprotein, ganglioside, carbohydrate, or 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 infiltrates.

[0159] As used herein, the terms “subject” and “patient” refer to an organism to be treated by the methods and compositions described herein. Such organisms preferably include, but are not limited to, mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably include humans.

[0160] As used herein, the term “effective amount” refers to the amount of a compound (*e.g.*, a compound of the present invention) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route. As used herein, the term “treating” includes any effect, *e.g.*, lessening, reducing, modulating, ameliorating or eliminating, that results in the improvement of the condition, disease, disorder, and the like, or ameliorating a symptom thereof.

[0161] As used herein, the term “pharmaceutical composition” refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*.

[0162] As used herein, the term “pharmaceutically acceptable carrier” refers to any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, *see, e.g., Remington's Pharmaceutical Sciences*, 15th Ed., Mack Publishing Co., Easton, PA [1975].

[0163] As used herein, the term “pharmaceutically acceptable salt” refers to any pharmaceutically acceptable salt (*e.g.*, acid or base) of a compound of the present invention which, upon administration to a subject, is capable of providing a compound of this invention or an active metabolite or residue thereof. As is known to those of skill in the art, “salts” of the compounds of the present invention may be derived from inorganic or organic acids and bases. Exemplary acids include, but are not limited to, hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic, benzenesulfonic acid, and the like. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

[0164] Exemplary bases include, but are not limited to, alkali metal (*e.g.*, sodium) hydroxides, alkaline earth metal (*e.g.*, magnesium) hydroxides, ammonia, and compounds of formula NW_4^+ , wherein W is C_{1-4} alkyl, and the like.

[0165] Exemplary salts include, but are not limited to: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, flucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate, persulfate, phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, undecanoate, and the like. Other examples of salts include anions of the compounds

of the present invention compounded with a suitable cation such as Na^+ , NH_4^+ , and NW_4^+ (wherein W is a C_{1-4} alkyl group), and the like.

[0166] For therapeutic use, salts of the compounds of the present invention are contemplated as being pharmaceutically acceptable. However, salts of acids and bases that are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound.

[0167] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

[0168] As a general matter, compositions specifying a percentage are by weight unless otherwise specified. Further, if a variable is not accompanied by a definition, then the previous definition of the variable controls.

I. PROTEINS

[0169] The invention provides multi-specific binding proteins that bind a tumor-associated antigen selected from DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, and EPHA4 on cancer cells and the NKG2D receptor and CD16 receptor on natural killer cells to activate the natural killer cell. The multi-specific binding proteins are useful in the pharmaceutical compositions and therapeutic methods described herein. Binding of the multi-specific binding protein to the NKG2D receptor and CD16 receptor on a natural killer cell enhances the activity of the natural killer cell toward destruction of a cancer cell. Binding of the multi-specific binding protein to DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, and/or EPHA4 on a cancer cell brings the cancer cell into proximity to the natural killer cell, which facilitates direct and indirect destruction of the cancer cell by the natural killer cell.

[0170] The invention also provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and an antigen selected from CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR,

SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and CCR5. In some embodiments, the multi-specific binding proteins further include an additional antigen-binding site that binds a tumor-associated antigen. The multi-specific binding proteins are useful in the pharmaceutical compositions and therapeutic methods described herein. Binding of the multi-specific binding proteins to the NKG2D receptor and CD16 receptor on a natural killer cell enhances the activity of the natural killer cell toward destruction of cells expressing CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and/or CCR5 antigen. Binding of the multi-specific binding proteins to CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and/or CCR5-expressing cells brings the cells into proximity with the natural killer cell, which facilitates direct and indirect destruction of the cells by the natural killer cell. In some embodiments, the cells are MDSCs. In some other embodiments, the cells are TAMs. Destruction of the MDSCs and/or TAMs by the natural killer cell may de-repress/enhance the immune response against tumor cells, which co-exist with the MDSCs and/or TAMs in a tumor microenvironment. In some embodiments, the multi-specific binding proteins that include an additional antigen-binding site for a tumor-associated antigen, enhances the activity of the natural killer cell toward destruction of cells expressing the tumor-associated antigen as well as the cells expressing CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and/or CCR5. Further description of some exemplary multi-specific binding proteins is provided below.

[0171] The first component of the multi-specific binding proteins binds to NKG2D receptor-expressing cells, which can include but are not limited to NK cells, $\gamma\delta$ T cells and CD8⁺ $\alpha\beta$ T cells. Upon NKG2D binding, the multi-specific binding proteins may block natural ligands, such as ULBP6 and MICA, from binding to NKG2D and activating NKG2D receptors.

[0172] In certain embodiments, the second component of the multi-specific binding proteins binds to DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4-expressing cells. DLL3-expressing cells may be found for example in, but not limited to, small cell lung cancer, large cell neuroendocrine carcinoma, glioblastoma, Ewing sarcoma, and cancers with neuroendocrine phenotype. MUC1 (or MUC1-C)-expressing cells may be found for example in, but not limited to, gastric cancer, colorectal cancer, pancreatic cancer,

breast cancer, endometrial cancer, lung cancer, bladder cancer, cervical cancer, head and neck cancer, ovarian cancer, renal cell cancer, and multiple myeloma. Plexin-A1-expressing cells may be found for example in, but not limited to, head and neck cancer, gastric cancer, pancreatic cancer, prostate cancer, and glioma. TNFRSF10B-expressing cells may be found
5 for example in, but not limited to, liver cancer, pancreatic cancer, stomach cancer, renal cancer, breast cancer, ovarian cancer, endometrial cancer, and melanoma. STEAP1-expressing cells may be found for example in, but not limited to, prostate cancer, bladder cancer, colon cancer, pancreas cancer, ovarian cancer, testicular cancer, breast cancer, cervical cancer and Ewing sarcoma. CDCP1-expressing cells may be found for example in,
10 but not limited to, colon cancer, lung cancer, gastric cancer, breast cancer, pancreatic cancer, head and neck cancer, bladder cancer, ovarian cancer, endometrial cancer, and skin cancer. PTK7-expressing cells may be found for example in, but not limited to, lung cancer, head and neck cancer, stomach cancer, prostate cancer, testicular cancer, endometrial cancer, breast cancer, melanoma, skin cancer, and leukemia. AXL-expressing cells may be found for
15 example in, but not limited to, breast cancer, lung cancer, colon cancer, prostate cancer, renal cancer, esophageal cancer, liver cancer, pancreatic cancer, Kaposi's sarcoma, acute myeloid leukemia, glioma, and mesothelioma. ERBB-3-expressing cells may be found for example in, but not limited to, prostate cancer, bladder cancer, breast cancer, ovarian cancer, colon cancer, pancreatic cancer, stomach cancer, oral cavity cancer, head and neck cancer,
20 lung cancer and melanoma. EDNRB-expressing cells may be found for example in, but not limited to, melanoma, uveal melanoma, and glioma. TYRP1-expressing cells may be found for example in, but not limited to, melanoma. OLR1-expressing cells may be found for example in, but not limited to, gastric cancer, colorectal cancer, pancreatic cancer, prostate cancer, breast cancer, and endometrial cancer. ADAM12-expressing cells may be found, for
25 example in, but not limited to, prostate cancer, breast cancer, basal cell carcinoma, squamous cell carcinoma, melanoma, pancreatic cancer, bladder cancer, colorectal cancer, lung cancer, liver cancer, esophageal cancer, Non-Hodgkin's lymphoma, ovarian cancer, and uterine cancer. PLAUR-expressing cells may be found, for example in, but not limited to, breast cancer, colorectal cancer, non-small cell lung cancer, and oral cancer. CCR6-expressing cells
30 may be found, for example in, but not limited to, colorectal cancer, breast cancer, cervical cancer, liver cancer, lung cancer, and cutaneous T-cell lymphoma. EPHA4-expressing cells may be found, for example in, but not limited to, melanoma, glioma, prostate cancer, breast cancer, small cell lung cancer, endometrial cancer, esophageal cancer, gastric cancer, and colorectal cancer.

[0173] In certain other embodiments, the second component of the multi-specific binding proteins binds to an antigen selected from CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and CCR5. In some embodiments, one or more of CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and CCR5 antigens are expressed by MDSCs and/or TAMs in the micro-microenvironment of a variety of hematological and solid tumors, such as acute myeloid leukemia, myelodysplastic and/or myeloproliferative neoplasms, acute lymphoblastic leukemia, B-cell lymphoma, chronic neutrophilic leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, as well as bladder cancer, colon cancer, prostate cancer, breast cancer, glioblastoma, hepatocellular carcinoma, head and neck cancer, lung cancer, ovarian cancer, pancreatic cancer, kidney cancer and melanoma.

[0174] In some embodiments, multi-specific binding proteins described herein further incorporate an additional antigen-binding site that binds to a tumor-associated antigen, which includes any antigen that is associated with cancer, such as but not limited to a protein, glycoprotein, ganglioside, carbohydrate, or lipid. Such antigens can be expressed on malignant cells or in the tumor microenvironment such as on tumor-associated blood vessels, extracellular matrix, mesenchymal stroma, or immune infiltrates. For example, the additional antigen-binding site can bind to HER2, CD20, CD33, BCMA, PSMA, DLL3, GD2, CD123, Anol, Mesothelin, CAIX, TROP2, CEA, Claudin-18.2, ROR1, 5T4, GPNMB, FR-alpha, PAPP-A, CD37, EpCAM, CD2, CD19, CD30, CD38, CD40, CD52, CD70, CD79b, FLT3, GPC3, B7H6, CCR4, CXCR4, ROR2, CD133, HLA-E, EGFR/ERBB-1, IGF1R, HER3/ERBB-3, HER4/ERBB-4, MUC1, cMET, SLAMF7, PSCA, MICA, MICB, TRAILR1, TRAILR2, MAGE-A3, B7.1, B7.2, CTLA4, PD1, PD-L1, or CD25 antigen expressed on cancer cells. Accordingly, in some embodiments, binding of the multi-specific binding proteins to a tumor-associated antigen expressed on cancer cells brings the cells into proximity with the natural killer cell, which facilitates direct and indirect destruction of the cancer cells by the natural killer cell in addition to the destruction of MDSCs and/or TAMs by the natural killer cell.

[0175] The third component for the multi-specific binding proteins binds to cells expressing CD16, an Fc receptor on the surface of leukocytes including natural killer cells, macrophages, neutrophils, eosinophils, mast cells, and follicular dendritic cells.

[0176] The multi-specific binding proteins described herein can take various formats. For example, one format is a heterodimeric, multi-specific antibody including a first immunoglobulin heavy chain, a first immunoglobulin light chain, a second immunoglobulin heavy chain and a second immunoglobulin light chain (FIG. 1). The first immunoglobulin heavy chain includes a first Fc (hinge-CH2-CH3) domain, a first heavy chain variable domain and optionally a first CH1 heavy chain domain. The first immunoglobulin light chain includes a first light chain variable domain and a first light chain constant domain. The first immunoglobulin light chain, together with the first immunoglobulin heavy chain, forms an antigen-binding site that binds NKG2D. The second immunoglobulin heavy chain comprises a second Fc (hinge-CH2-CH3) domain, a second heavy chain variable domain and optionally a second CH1 heavy chain domain. The second immunoglobulin light chain includes a second light chain variable domain and a second light chain constant domain. In certain embodiments, the second immunoglobulin light chain, together with the second immunoglobulin heavy chain, forms an antigen-binding site that binds a tumor-associated antigen selected from DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, and EPHA4. In certain other embodiments, the second immunoglobulin light chain, together with the second immunoglobulin heavy chain, forms an antigen-binding site that binds CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, or CCR5. The first Fc domain and second Fc domain together are able to bind to CD16 (FIG. 1). In some embodiments, the first immunoglobulin light chain is identical to the second immunoglobulin light chain.

[0177] Another exemplary format involves a heterodimeric, multi-specific antibody including a first immunoglobulin heavy chain, a second immunoglobulin heavy chain and an immunoglobulin light chain (FIG. 2). The first immunoglobulin heavy chain includes a first Fc (hinge-CH2-CH3) domain fused via either a linker or an antibody hinge to a single-chain variable fragment (scFv) composed of a heavy variable domain and light chain variable domain which pair and bind NKG2D; bind a tumor-associated antigen selected from DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, and EPHA4; or bind a MDSC-associated or TAM-associated antigen selected from CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and CCR5. The second immunoglobulin heavy chain includes a second Fc (hinge-

CH2-CH3) domain, a second heavy chain variable domain and optionally a CH1 heavy chain domain. The immunoglobulin light chain includes a light chain variable domain and a light chain constant domain. The second immunoglobulin heavy chain pairs with the immunoglobulin light chain and binds to NKG2D; binds a tumor-associated antigen selected from DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, and EPHA4; or binds a MDSC-associated or TAM-associated antigen selected from CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and CCR5. The first Fc domain and the second Fc domain together are able to bind to CD16 (FIG. 2).

[0178] One or more additional binding motifs may be fused to the C-terminus of the constant region CH3 domain, optionally via a linker sequence. In certain embodiments, the antigen-binding site could be a single-chain or disulfide-stabilized variable region (scFv) or could form a tetravalent or trivalent molecule.

[0179] In some embodiments, the multi-specific binding protein is in a Triomab form, which is a trifunctional, bispecific antibody that maintains an IgG-like shape (*e.g.*, the multi-specific binding protein represented in FIG. 31). This chimeric bispecific antibody comprises of two half antibodies, each with one light and one heavy chain, that originate from two parental antibodies. The Triomab form may be a heterodimer, comprising of $\frac{1}{2}$ of a rat antibody and $\frac{1}{2}$ of a mouse antibody.

[0180] In some embodiments, the multi-specific binding protein is in a KiH Common Light Chain (LC) form, which incorporates the knobs-into-holes (KiH) technology (*e.g.*, the multi-specific binding protein represented in FIG. 32). The KiH Common LC form is a heterodimer comprising a Fab which binds to a first target, a Fab which binds to a second target, and an Fc domain stabilized by heterodimerization mutations. The two Fabs each comprise a heavy chain and light chain, wherein the heavy chain of each Fab differs from the other, and the light chain that pairs with each respective heavy chain is common to both Fabs.

[0181] The KiH technology involves engineering CH3 domains to create either a “knob” or a “hole” in each heavy chain to promote heterodimerization. Introduction of a “knob” in one CH3 domain (CH3A) comprises substitution of a small residue with a bulky one (*e.g.*, T366W_{CH3A} in EU numbering). To accommodate the “knob,” a complementary “hole” surface is introduced on the other CH3 domain (CH3B) by replacing the closest neighboring

residues to the knob with smaller ones (*e.g.*, T366S/L368A/Y407V_{CH3B}). The “hole” mutation was optimized by structure-guided phage library screening (Atwell S., *et al.* (1997) *J. Mol. Biol.*; 270(1):26–35.). X-ray crystal structures of KiH Fc variants (Elliott J.M., *et al.* (2014) *J. Mol. Biol.*; 426(9):1947–57.; Mimoto F., *et al.* (2014) *Mol.*

Immunol.; 58(1):132–8.) demonstrated that heterodimerization is thermodynamically favored by hydrophobic interactions driven by steric complementarity at the inter-CH3 domain core interface, whereas the knob–knob and the hole–hole interfaces do not favor homodimerization owing to steric hindrance and disruption of the favorable interactions, respectively.

[0182] In some embodiments, the multi-specific binding protein is in a dual-variable domain immunoglobulin (DVD-IgTM) form, which is a tetravalent IgG-like structure comprising the target-binding domains of two monoclonal antibodies and flexible naturally occurring linkers (*e.g.*, FIG. 33). The DVD-IgTM form is homodimeric comprising a variable domain targeting antigen 2 fused to the N-terminus of a Fab variable domain targeting antigen 1. The representative multi-specific binding protein shown in FIG. 33 comprises an unmodified Fc.

[0183] In some embodiments, the multi-specific binding protein is in an Orthogonal Fab interface (Ortho-Fab) form (*e.g.*, the multi-specific binding protein represented in FIG. 34). In the Ortho-Fab IgG approach (Lewis S.M., *et al.* (2014) *Nat. Biotechnol.*; 32(2):191–8.), structure-based regional design introduces complementary mutations at the LC and HC_{VH-CH1} interface in only one Fab, without any changes being made to the other Fab.

[0184] In some embodiments, the multi-specific binding protein is in a 2-in-1 Ig form (*e.g.*, the multi-specific binding protein represented in FIG. 35).

[0185] In some embodiments, the multi-specific binding protein is an electrostatic-steering (ES) form, which is a heterodimer comprising two different Fabs binding to targets 1 and target 2, and an Fc domain (*e.g.*, the multi-specific binding protein represented in FIG. 36). Heterodimerization is ensured by electrostatic steering mutations in the Fc domain.

[0186] In some embodiments, the multi-specific binding protein is in a controlled Fab-Arm Exchange (cFAE) form (*e.g.*, the multi-specific binding protein represented in FIG. 37).

The cFAE form is a bispecific heterodimer comprising two different Fabs binding to targets 1 and 2, wherein a LC-HC pair (half-molecule) has been swapped with a LC-HC pair from another molecule. Heterodimerization is ensured by mutations in the Fc.

[0187] In some embodiments, the multi-specific binding protein is in a strand-exchange engineered domain (SEED) body form (*e.g.*, the multi-specific binding protein represented in FIG. 38). The SEED platform was designed to generate asymmetric and bispecific antibody-like molecules in order to expand the therapeutic applications of natural antibodies. This protein engineering platform is based on exchanging structurally related sequences of immunoglobulin classes within the conserved CH3 domains (*e.g.*, alternating segments of IgA and IgG CH3 domain sequences). The SEED design allows efficient generation of heterodimers, while disfavoring homodimerization of SEED CH3 domains. (Muda M. *et al.* (2011) *Protein Eng. Des. Sel.*; 24(5):447-54.).

[0188] In some embodiments, the multi-specific binding protein is in a LuZ-Y form (*e.g.*, the multi-specific binding protein represented in FIG. 39). The LuZ-Y form is a heterodimer comprising two different scFabs binding to targets 1 and 2, fused to an Fc domain. Heterodimerization is ensured through the introduction of leucine zipper motifs fused to the C-terminus of the Fc domain (Wranik B.J. *et al.* (2012) *J. Biol. Chem.*; 287:43331-9.).

[0189] In some embodiments, the multi-specific binding protein is in a Cov-X-Body form (*e.g.*, the multi-specific binding protein represented in FIG. 40). Bispecific Cov-X-Bodies comprise a scaffold antibody having a pharmacophore peptide heterodimer covalently linked to each Fab arm, wherein one molecule of the peptide heterodimer binds to a first target and the other molecule of the peptide heterodimer binds to a second target, and wherein the two molecules are joined by an azetidinone linker. Whereas the pharmacophores are responsible for functional activities, the antibody scaffold imparts long half-life and Ig-like distribution. The pharmacophores can be chemically optimized or replaced with other pharmacophores to generate optimized or unique bispecific antibodies. (Doppalapudi V.R. *et al.* (2010) *PNAS*; 107(52):22611-22616.).

[0190] In some embodiments, the multi-specific binding protein is in a $\kappa\lambda$ -Body form, which is a heterodimer comprising two different Fabs fused to Fc domains stabilized by heterodimerization mutations (*e.g.*, the multi-specific binding protein represented in FIG. 41). A first Fab binding target 1 comprises a kappa LC, and a second Fab binding target 2 comprises a lambda LC. FIG. 41A is an exemplary representation of one form of a $\kappa\lambda$ -Body; FIG. 41B is an exemplary representation of another $\kappa\lambda$ -Body.

[0191] In some embodiments, the multi-specific binding protein is in a one-arm single chain (OAsc)-Fab form (*e.g.*, the multi-specific binding protein represented in FIG. 42). The

OAsc-Fab form is a heterodimer that includes a Fab binding to target 1 and an scFab binding to target 2 fused to an Fc domain. Heterodimerization is ensured by mutations in the Fc domain.

[0192] In some embodiments, the multi-specific binding protein is in a DuetMab form (e.g., the multi-specific binding protein represented in FIG. 43). The DuetMab form is a heterodimer comprising two different Fabs binding to targets 1 and 2, and an Fc domain stabilized by heterodimerization mutations. The two different Fabs comprise different S-S bridges that ensure correct LC and HC pairing.

[0193] In some embodiments, the multi-specific binding protein is in a CrossmAb form (e.g., the multi-specific binding protein represented in FIG. 44). The CrossmAb form is a heterodimer comprising two different Fabs binding to targets 1 and 2, and an Fc domain stabilized by heterodimerization mutations. CL and CH1 domains and VH and VL domains are switched, e.g., CH1 is fused in-line with VL, while CL is fused in-line with VH.

[0194] In some embodiments, the multi-specific binding protein is in a Fit-Ig form (e.g., the multi-specific binding protein represented in FIG. 45). The Fit-Ig form is a homodimer comprising a Fab binding to target 2 fused to the N-terminus of the HC of a Fab that binds to target 1. The representative multi-specific binding protein of FIG. 45 comprises an unmodified Fc domain.

[0195] Table 1 lists peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to NKG2D. Unless indicated otherwise, the CDR sequences provided in Table 1 are determined under Kabat. The NKG2D binding domains can vary in their binding affinity to NKG2D, nevertheless, they all activate human NKG2D and NK cells.

Table 1		
Clones	Heavy chain variable region amino acid sequence	Light chain variable region amino acid sequence
ADI-27705	QVQLQQWGAGLLKPSETLSLTCAY YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS	DIQMTQSPSTLSASVGDRVIT TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF SGSGSGTEFTLTISLQPDFF

	(SEQ ID NO:1) CDR1 (SEQ ID NO:105) – GSFSGGYYWS (non-Kabat) or GYYWS [SEQ ID NO:634] CDR2 (SEQ ID NO:106) – EIDHSGSTNYPNPSLKS CDR3 (SEQ ID NO:107) – ARARGPWSFDP (non-Kabat) or ARGPWSFDP [SEQ ID NO:635]	ATYYCQQYNSYPITFGGGTK VEIK (SEQ ID NO:2)
ADI- 27724	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:3)	EIVLTQSPGTLSPGERATL SCRASQSVSSSYLAWYQQKP GQAPRLLIYGASSRATGIPDR FSGSGSGTDFTLTISRLEPEDF AVYYCQQYGSSPITFGGGTK VEIK (SEQ ID NO:4)
ADI- 27740 (A40)	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:5)	DIQMTQSPSTLSASVGDRVITI TCRASQSIGSWLAWYQQKP GKAPKLLIYKASSLESQVPSR FSGSGSGTEFTLTITSLQPDDF ATYYCQQYHSFYTFGGGTK VEIK (SEQ ID NO:6)
ADI- 27741	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:7)	DIQMTQSPSTLSASVGDRVITI TCRASQSIGSWLAWYQQKP GKAPKLLIYKASSLESQVPSR FSGSGSGTEFTLTITSLQPDDF ATYYCQQSNSYYTFGGGTK VEIK (SEQ ID NO:8)

ADI- 27743	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:9)	DIQMTQSPSTLSASVGDRVITI TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF SGSGSGTEFTLTISLQPDFF ATYYCQQYNSYPTFGGGTK VEIK (SEQ ID NO:10)
ADI- 28153	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWGFDPWGQGTLVTVSS (SEQ ID NO:11)	ELQMTQSPSSLSASVGDRVITI TCRTSQSISSYLNWYQQKPG QPPKLLIYWASTRESGVPRD FSGSGSGTDFTLTISLQPEDS ATYYCQQSYDIPYTFGQGTK LEIK (SEQ ID NO:12)
ADI- 28226 (C26)	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:13)	DIQMTQSPSTLSASVGDRVITI TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF SGSGSGTEFTLTISLQPDFF ATYYCQQYGSFPITFGGGTK VEIK (SEQ ID NO:14)
ADI- 28154	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:15)	DIQMTQSPSTLSASVGDRVITI TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF SGSGSGTDFTLTISLQPDFF ATYYCQQSKEVPWTFGQGT KVEIK (SEQ ID NO:16)
ADI- 29399	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS	DIQMTQSPSTLSASVGDRVITI TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF

	KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:17)	SGSGSGTEFTLTISLQPDDF ATYYCQQYNSFPTFGGGTK VEIK (SEQ ID NO:18)
ADI- 29401	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:19)	DIQMTQSPSTLSASVGDRVITI TCRASQSIGSWLAWYQQKP GKAPKLLIYKASSLESGVPSR FSGSGSGTEFTLTISLQPDDF ATYYCQQYDIYPTFGGGTKV EIK (SEQ ID NO:20)
ADI- 29403	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:21)	DIQMTQSPSTLSASVGDRVITI TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF SGSGSGTEFTLTISLQPDDF ATYYCQQYDSYPTFGGGTK VEIK (SEQ ID NO:22)
ADI- 29405	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:23)	DIQMTQSPSTLSASVGDRVITI TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF SGSGSGTEFTLTISLQPDDF ATYYCQQYGSFPTFGGGTK VEIK (SEQ ID NO:24)
ADI- 29407	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS	DIQMTQSPSTLSASVGDRVITI TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF SGSGSGTEFTLTISLQPDDF

	(SEQ ID NO:25)	ATYYCQQYQSFPFSGGGTK VEIK (SEQ ID NO:26)
ADI- 29419	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:27)	DIQMTQSPSTLSASVGDRVITI TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF SGSGSGTEFTLTISLQPDFF ATYYCQQYSSSFSTFGGGTKV EIK (SEQ ID NO:28)
ADI- 29421	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:29)	DIQMTQSPSTLSASVGDRVITI TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF SGSGSGTEFTLTISLQPDFF ATYYCQQYESYSTFGGGTK VEIK (SEQ ID NO:30)
ADI- 29424	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:31)	DIQMTQSPSTLSASVGDRVITI TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF SGSGSGTEFTLTISLQPDFF ATYYCQQYDSFITFGGGTKV EIK (SEQ ID NO:32)
ADI- 29425	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:33)	DIQMTQSPSTLSASVGDRVITI TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF SGSGSGTEFTLTISLQPDFF ATYYCQQYQSYPTFGGGTK VEIK

		(SEQ ID NO:34)
ADI- 29426	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:35)	DIQMTQSPSTLSASVGDRVITI TCRASQSIGSWLAWYQQKP GKAPKLLIYKASSLESGVPSR FSGSGSGTEFTLTISLQPDFF ATYYCQQYHSFPTFGGGTK VEIK (SEQ ID NO:36)
ADI- 29429	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:37)	DIQMTQSPSTLSASVGDRVITI TCRASQSIGSWLAWYQQKP GKAPKLLIYKASSLESGVPSR FSGSGSGTEFTLTISLQPDFF ATYYCQQYELYSYTFGGGT KVEIK (SEQ ID NO:38)
ADI- 29447 (F47)	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYPNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGTLVTVSS (SEQ ID NO:39)	DIQMTQSPSTLSASVGDRVITI TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF SGSGSGTEFTLTISLQPDFF ATYYCQQYDTFITFGGGTKV EIK (SEQ ID NO:40)
ADI- 27727	QVQLVQSGAEVKKPGSSVKVSCA SGGTFSSYAISWVRQAPGQGLEWM GGIPIFGTANYAQKFQGRVTITADE STSTAYMELSSLRSEDVAVYYCAR GDSSIRHAYYYYGMDVWGQGT TVSS (SEQ ID NO:41) CDR1 (SEQ ID NO:43) –	DIVMTQSPDSLAVSLGERATI NCKSSQSVLYSSNNKNYLA WYQQKPGQPPLLIYWAST RESGVPDRFSGSGSGTDFTLT ISLQAEDVAVYYCQQYYST PITFGGGTKVEIK (SEQ ID NO:42) CDR1 (SEQ ID NO:46) –

	GTFSSY AIS (non-Kabat) or SY AIS [SEQ ID NO:636] CDR2 (SEQ ID NO:44) – GIIPFGTANYAQKFQG CDR3 (SEQ ID NO:45) – ARGDSSIRHAYYYYGMDV (non-Kabat) or GDSSIRHAYYYYGMDV [SEQ ID NO:637]	KSSQSVLYSSNNKNYLA CDR2 (SEQ ID NO:47) – WASTRES CDR3 (SEQ ID NO:48) – QQYYSTPIT
ADI-29443 (F43)	QLQLQESGPGLVKPSETLSLTCTVS GGSISSSSYYWGWIRQPPGKGLEWI GSIYYSGSTYYNPSLKSRTISVDTS KNQFSLKLSSVTAADTAVYYCARG SDRFHPYFDYWGGTLTVSS (SEQ ID NO:49) CDR1 (SEQ ID NO:51) – GSISSSSYYWG (non-Kabat) or SSSYYWG [SEQ ID NO:638] CDR2 (SEQ ID NO:52) – SIYYSGSTYYNPSLKS CDR3 (SEQ ID NO:53) – ARGSDRFHPYFDY (non-Kabat) or GSDRFHPYFDY [SEQ ID NO:639]	EIVLTQSPATLSLSPGERATL SCRASQSVSRYLAWYQQKP GQAPRLLIYDASN RATGIPA RFSGSGSGTDFTLTIS SLEPED FAVYYCQQFDTWPPTFGGG TKVEIK (SEQ ID NO:50) CDR1 (SEQ ID NO:54) – RASQSVSRYLA CDR2 (SEQ ID NO:55) – DASN RAT CDR3 (SEQ ID NO:56) – QQFDTWPPT
ADI-29404 (F04)	QVQLQQWGAGLLKPSETLSLTCAV YGGSFSGYYWSWIRQPPGKGLEWI GEIDHSGSTNYNPSLKSRTISVDTS KNQFSLKLSSVTAADTAVYYCARA RGPWSFDPWGQGT LTVSS (SEQ ID NO:57)	DIQMTQSPSTLSASVGDRVTI TCRASQSISSWLAWYQQKPG KAPKLLIYKASSLESGVPSRF SGSGSGTEFTLTIS SLQPDDF ATYYCEQYDSYPTFGGGTK VEIK (SEQ ID NO:58)

ADI-28200	QVQLVQSGAEVKKPGSSVKVSCA SGGTFSSYAISWVRQAPGQGLEWM GGIPIFGTANYAQKFQGRVTITADE STSTAYMELSSLRSEDVAVYYCAR RGRKASGSFYFYYGMDVWGQGT VTVSS (SEQ ID NO:59) CDR1 (SEQ ID NO:108) – GTFSSYAIS CDR2 (SEQ ID NO:109) – GIPIFGTANYAQKFQG CDR3 (SEQ ID NO:110) – ARRGRKASGSFYFYYGMDV	DIVMTQSPDSLAVSLGERATI NCESSQSLLNSGNQKNYLT WYQQKPGQPPLIYWAST RESGVPDRFSGSGSGTDFTLT ISSLQAEDVAVYYCQNDYSY PYTFGQGTKLEIK (SEQ ID NO:60) CDR1 (SEQ ID NO:111) – ESSQSLLNSGNQKNYLT CDR2 (SEQ ID NO:112) – WASTRES CDR3 (SEQ ID NO:113) – QNDYSYPYT
ADI-29379 (E79)	QVQLVQSGAEVKKPGASVKVSCK ASGYTFTSYMHVVRQAPGQGLE WMGIINPSGGSTSYAQKFQGRVTM TRDTSTSTVYMELSSLRSEDVAVYY CARGAPNYGDTTHDYYYMDVWG KGTTVTVSS (SEQ ID NO:61) CDR1 (SEQ ID NO:63) – YTFTSYMH (non-Kabat) or SYMH [SEQ ID NO:640] CDR2 (SEQ ID NO:64) - IINPSGGSTSYAQKFQG CDR3 (SEQ ID NO:65) – ARGAPNYGDTTHDYYYMDV (non- Kabat) or GAPNYGDTTHDYYYMDV [SEQ ID NO:641]	EIVMTQSPATLSVSPGERATL SCRASQSVSSNLAWYQQKP GQAPRLLIYGASTRATGIPAR FSGSGSGTEFTLTISSLQSEDF AVYYCQQYDDWPFTFGGT KVEIK (SEQ ID NO:62) CDR1 (SEQ ID NO:66) - RASQSVSSNLA CDR2 (SEQ ID NO:67) - GASTRAT CDR3 (SEQ ID NO:68) - QQYDDWPFT

ADI- 29463 (F63)	QVQLVQSGAEVKKPGASVKVSCK ASGYTFTGYMHWRQAPGQGLE WMGWINPNSGGTNYAQKFQGRVT MTRDTSISTAYMELSRLRSDDTAV YYCARDTGEYYDTDDHGMDVWG QGTTVTVSS (SEQ ID NO:69) CDR1 (SEQ ID NO:71) - YTFTGYMH (non-Kabat) or GYMH [SEQ ID NO:642] CDR2 (SEQ ID NO:72) - WINPNSGGTNYAQKFQG CDR3 (SEQ ID NO:73) - ARDTGEYYDTDDHGMDV (non- Kabat) or DTGEYYDTDDHGMDV [SEQ ID NO:643]	EIVLTQSPGTLSPGERATL SCRASQSVSSNLAWYQQKP GQAPRLLIYGASTRATGIPAR FSGSGSGTEFTLTISSLQSEDF AVYYCQQDDYWPPTFGGGT KVEIK (SEQ ID NO:70) CDR1 (SEQ ID NO:74) - RASQSVSSNLA CDR2 (SEQ ID NO:75) - GASTRAT CDR3 (SEQ ID NO:76) - QQDDYWPPT
ADI- 27744 (A44)	EVQLLESGLLVQPGGSLRLSCAAS GFTFSSYAMSWVRQAPGKGLEWV SAISGSGGSTYYADSVKGRFTISR NSKNTLYLQMNSLRAEDTAVYYC AKDGGYYDSGAGDYWGQGLTVT VSS (SEQ ID NO:77) CDR1 (SEQ ID NO:79) - FTFSSYAMS (non-Kabat) or SYAMS [SEQ ID NO:644] CDR2 (SEQ ID NO:80) - AISGSGGSTYYADSVKG	DIQMTQSPSSVSASVGDRVITI TCRASQGIDSWLAWYQQKP GKAPKLLIYAASSLQSGVPS RFSGSGSGTDFTLTISSLQPE DFATYYCQQGVSYPRTFGG GTKVEIK (SEQ ID NO:78) CDR1 (SEQ ID NO:82) - RASQGIDSWLA CDR2 (SEQ ID NO:83) - AASSLQS CDR3 (SEQ ID NO:84) - QQGVSYPRT

	CDR3 (SEQ ID NO:81) – AKDGGYYDSGAGDY (non-Kabat) or DGGYYDSGAGDY [SEQ ID NO:645]	
ADI- 27749 (A49)	EVQLVESGGGLVKPGGSLRLSCAA SGFTFSSYSMNWVRQAPGKGLEW VSSISSSSSYIYYADSVKGRFTISR NAKNSLYLQMNSLRAEDTAVYYC ARGAPMGAAAGWFDPWGQGT LVTVSS (SEQ ID NO:85) CDR1 (SEQ ID NO:87) – FTFSSYSMN or SYSMN [SEQ ID NO:646] CDR2 (SEQ ID NO:88) - SISSSSSYIYYADSVKG CDR3 (SEQ ID NO:89) – ARGAPMGAAAGWFDP (non-Kabat) or GAPMGAAAGWFDP [SEQ ID NO:647]	DIQMTQSPSSVSASVGDRVTI TCRASQGISSWLAWYQQKP GKAPKLLIYAASSLQSGVPS RFSGSGSGTDFTLTISLQPE DFATYYCQQGVSPRTFGGG TKVEIK (SEQ ID NO:86) CDR1 (SEQ ID NO:90) - RASQGISSWLA CDR2 (SEQ ID NO:91) - AASSLQS CDR3 (SEQ ID NO:92) - QQGVSPRT
ADI- 29378 (E78)	QVQLVQSGAEVKKPGASVKV SCKASGYTFTSYMHVVRQAPGQGLE WMGIINPSGGTSYAQKFQGRVTM TRDTSTSTVYMELSSLRSEDTAVYY CAREGAGFAYGMDYYMDVWGK GTTVTVSS (SEQ ID NO:93) CDR1 (SEQ ID NO:95) – YTFTSYMH (non-Kabat) or SYMH [SEQ ID NO:648]	EIVLTQSPATLSLSPGERATL SCRASQSVSSYLAWYQQKP GQAPRLLIYDASNRTGIPA RFSGSGSGTDFTLTISLQPED FAVYYCQQSDNWPFTFGGG TKVEIK (SEQ ID NO:94) CDR1 (SEQ ID NO:98) - RASQSVSSYLA

	CDR2 (SEQ ID NO:96) - IINPSGGSTSYAQKFQG CDR3 (SEQ ID NO:97) – AREGAGFAYGMDYYYMDV (non-Kabat) or EGAGFAYGMDYYYMDV [SEQ ID NO:649]	CDR2 (SEQ ID NO:99) - DASNRAT CDR3 (SEQ ID NO:100) - QQSDNWPFT
A49MI	EVQLVESGGGLVKPGGSLRLSCAA SGFTFSSYSMNWVRQAPGKGLEW VSSISSSSSYIYYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYC ARGAP<u>I</u>GAAAGWFDPWGQGTLVT VSS [SEQ ID NO:650] CDR1: FTFSSYSMN [SEQ ID NO:87] non kabat or SYSMN [SEQ ID NO:646] CDR2: SISSSSSYIYYADSVKG [SEQ ID NO:88] CDR3: (non-Kabat) ARGAP<u>I</u>GAAAGWFDP [SEQ ID NO:651] or GAP<u>I</u>GAAAGWFDP [SEQ ID NO:652]	DIQMTQSPSSVSASVGDRVITI TCRASQGISSWLAWYQQKP GKAPKLLIYAASSLQSGVPS RFSGSGSGTDFTLTISSLQPE DFATYYCQQGVSPRTFGGG TKVEIK (SEQ ID NO:86) CDR1 (SEQ ID NO:90) - RASQGISSWLA CDR2 (SEQ ID NO:91) - AASSLQS CDR3 (SEQ ID NO:92) - QQGVSPRT
A49MQ	EVQLVESGGGLVKPGGSLRLSCAA SGFTFSSYSMNWVRQAPGKGLEW VSSISSSSSYIYYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYC ARGAP<u>Q</u>GAAAGWFDPWGQGTLVT VSS [SEQ ID NO:653] CDR1: FTFSSYSMN [SEQ ID NO:87] non kabat or SYSMN [SEQ ID NO:646] CDR2: SISSSSSYIYYADSVKG	DIQMTQSPSSVSASVGDRVITI TCRASQGISSWLAWYQQKP GKAPKLLIYAASSLQSGVPS RFSGSGSGTDFTLTISSLQPE DFATYYCQQGVSPRTFGGG TKVEIK (SEQ ID NO:86) CDR1 (SEQ ID NO:90) - RASQGISSWLA

	[SEQ ID NO:88] CDR3 (non-Kabat) [SEQ ID NO:654] - ARGAP QG AAAGWFDP or CDR3 [SEQ ID NO:655] - GAP QG AAAGWFDP	CDR2 (SEQ ID NO:91) - AASSLQS CDR3 (SEQ ID NO:92) - QQGVSPRT
A49ML	EVQLVESGGGLVKPGGSLRLSCAA SGFTFSSYSMNWVRQAPGKGLEW VSSISSSSSYIYYADSVKGRFTISR NAKNSLYLQMNSLRAEDTAVYYC ARGAPLGAAAGWFDPWGQGTLVT VSS [SEQ ID NO:656] CDR1: FTFSSYSMN [SEQ ID NO:87] non kabat or SYSMN [SEQ ID NO:646] CDR2: SISSSSSYIYYADSVKG [SEQ ID NO:88] CDR3 (non-Kabat) [SEQ ID NO:657] - ARGAPLGAAAGWFDP or CDR3 [SEQ ID NO:658] - GAPLGAAAGWFDP	DIQMTQSPSSVSASVGDRVIT TCRASQGISSWLAWYQQK PKAPKLLIYAASSLQSGVPS RFSGSGSGTDFTLTISLQPE DFATYYCQQGVSPRTFGGG TKVEIK (SEQ ID NO:86) CDR1 (SEQ ID NO:90) - RASQGISSWLA CDR2 (SEQ ID NO:91) - AASSLQS CDR3 (SEQ ID NO:92) - QQGVSPRT
A49MF	EVQLVESGGGLVKPGGSLRLSCAA SGFTFSSYSMNWVRQAPGKGLEW VSSISSSSSYIYYADSVKGRFTISR NAKNSLYLQMNSLRAEDTAVYYC ARGAPFGAAAGWFDPWGQGTLVT VSS [SEQ ID NO:659] CDR1: FTFSSYSMN [SEQ ID NO:87] non kabat or SYSMN [SEQ ID NO:646]	DIQMTQSPSSVSASVGDRVIT TCRASQGISSWLAWYQQK PKAPKLLIYAASSLQSGVPS RFSGSGSGTDFTLTISLQPE DFATYYCQQGVSPRTFGGG TKVEIK (SEQ ID NO:86) CDR1 (SEQ ID NO:90) - RASQGISSWLA

	CDR2: SISSSSSYIYYADSVKG [SEQ ID NO:88] CDR3 (non-Kabat) [SEQ ID NO:660] - ARGAPFGAAAGWFDP or CDR3 [SEQ ID NO:661] - GAPFGAAAGWFDP	CDR2 (SEQ ID NO:91) - AASSLQS CDR3 (SEQ ID NO:92) - QQGVSPRT
A49MV	EVQLVESGGGLVKPGGSLRLSCAA SGFTFSSYSMNWVRQAPGKGLEW VSSISSSSSYIYYADSVKGRFTISR NAKNSLYLQMNSLRAEDTAVYYC ARGAPVGAAAGWFDPWGQGTLVT VSS [SEQ ID NO:662] CDR1: FTFSSYSMN [SEQ ID NO:87] non kabat or SYSMN [SEQ ID NO:646] CDR2: SISSSSSYIYYADSVKG [SEQ ID NO:88] CDR3 (non-Kabat) [SEQ ID NO:663] - ARGAPVGAAAGWFDP or CDR3 [SEQ ID NO:664] - GAPVGAAAGWFDP	DIQMTQSPSSVSASVGDRVITI TCRASQGISSWLAWYQQKP GKAPKLLIYAASSLQSGVPS RFSGSGSGTDFTLTISLQPE DFATYYCQQGVSPRTFGGG TKVEIK (SEQ ID NO:86) CDR1 (SEQ ID NO:90) - RASQGISSWLA CDR2 (SEQ ID NO:91) - AASSLQS CDR3 (SEQ ID NO:92) - QQGVSPRT
A49- consensus	EVQLVESGGGLVKPGGSLRLSCAA SGFTFSSYSMNWVRQAPGKGLEW VSSISSSSSYIYYADSVKGRFTISR NAKNSLYLQMNSLRAEDTAVYYC ARGAPXGAAAGWFDPWGQGTLVT VSS, wherein X is M, L, I, V, Q, or F [SEQ ID NO:665] CDR1: FTFSSYSMN [SEQ ID NO:87] non kabat or SYSMN [SEQ ID NO:646]	DIQMTQSPSSVSASVGDRVITI TCRASQGISSWLAWYQQKP GKAPKLLIYAASSLQSGVPS RFSGSGSGTDFTLTISLQPE DFATYYCQQGVSPRTFGGG TKVEIK (SEQ ID NO:86) CDR1 (SEQ ID NO:90) - RASQGISSWLA

	CDR2: SISSSSSYIYYADSVKG [SEQ ID NO:88] CDR3 (non-Kabat) [SEQ ID NO:666] - ARGAPXGAAAGWFDP or CDR3 [SEQ ID NO:667] – GAPXGAAAGWFDP, wherein X is M, L, I, V, Q, or F	CDR2 (SEQ ID NO:91) - AASSLQS CDR3 (SEQ ID NO:92) - QQGVSPRT
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[0196] Alternatively, a heavy chain variable domain defined by SEQ ID NO:101 can be paired with a light chain variable domain defined by SEQ ID NO:102 to form an antigen-binding site that can bind to NKG2D, as illustrated in US 9,273,136.

SEQ ID NO:101

5 QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAFI
RYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDRGL
GDGTYFDYWGGQTTTVTVSS

SEQ ID NO:102

10 QSALTQPASVSGSPGQSITISCSGSSSNIGNNAVNWYQQLPGKAPKLLIYYDDL
LPSGVSDRFSGSKSGTSAFLAISGLQSEDEADYYCAAWDDSLNGPVFGGGTK
LTVL

[0197] Alternatively, a heavy chain variable domain defined by SEQ ID NO:103 can be paired with a light chain variable domain defined by SEQ ID NO:104 to form an antigen-binding site that can bind to NKG2D, as illustrated in US 7,879,985.

15 SEQ ID NO:103

QVHLQESGPGLVKPSETLSLTCTVSDDSISSYYWSWIRQPPGKGLEWIGHISYS
GSANYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCANWDDAFNIWG
QGTMTVTVSS

SEQ ID NO:104

20 EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASS
RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK

[0198] In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-associated antigen DLL3. Table 2 lists peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to DLL3.

Table 2		
Clones	Heavy chain variable domain peptide sequence	Light chain variable domain peptide sequence
2F7	QVQLQQSGAELMKPGASVKLS CKATGYTFTGYWIDWIKQRPG HGLEWVGEILPGSDNINYNKFF RGKATFTADTSSNTAYIQLSSL TTEDSAIYFCARCGTGPWFTY WGQGTLVTVSA [SEQ ID NO:114] CDR1: GYWID [SEQ ID NO:116] CDR2: EILPGSDNINYNKFFRG [SEQ ID NO:117] CDR3: CGTGPWFTY [SEQ ID NO:118]	NIMMTQSPSSLAVSAGEKVTMS CKSSQSVLYSSNQKNYLAWYQ QKPGQSPRLLIYWASTRASGVP DRFTGSGSGTDFTLTITNIQPED LAVYYCHQFLSSTWTFGGGTK LEIK [SEQ ID NO:115] CDR1: KSSQSVLYSSNQKNYLA [SEQ ID NO:119] CDR2: WASTRAS [SEQ ID NO:120] CDR3: HQFLSSTWT [SEQ ID NO:121]
9E6 or 10F5	QLQLVQSGPELMRPGETVKISC KASGYTFTTYGMNWVKQAPG KGLKWVGWINTYSGVPTYAD DFKGRFAFSLESSASTAFLQINN LKDEDTATYFCARFGNYGFDC WGQGTTLTVSS [SEQ ID NO:122] CDR1: TYGMN [SEQ ID NO:124]	DIVMTQSPSSLSVSAGEKVTMS CKSSQSLNLSGNQKNYLAWYQ QKPGQPPKLLIYGASTRESGVP DRFTGSGSGTDFTLTISVQAED LAVYYCQNDHIYPYTFGGGTK LEIK [SEQ ID NO:123] CDR1: KSSQSLNLSGNQKNYLA [SEQ ID NO:127]

	CDR2: WINTYSGVPTYADDFKG [SEQ ID NO:125] CDR3: FGNYGFDC [SEQ ID NO:126]	CDR2: GASTRES [SEQ ID NO:128] CDR3: QNDHIYPYT [SEQ ID NO:129]
5E7	EVQLQQSGAELVRPGASVKLS CTASGFNIKDDYIHWVKQWPE QGLEWIGWIDSENGDTEYASK FQGKATMTADTSSNTAYLQLS GLTSEDTA VYYCTTSSYYSYDL FVYWGQGTLVTVSA [SEQ ID NO:130] CDR1: DDYIH [SEQ ID NO:132] CDR2: WIDSENGDTEYASKFQG [SEQ ID NO:133] CDR3: SSYYSYDLFVY [SEQ ID NO:134]	DVLMTQTPLTLSVPIGQPASISC KSSQSLLHSNGKTYLNWLLQRP GQSPKLLIYLVSKLESGVPDRFS GSGSGTDFTLKISRVEAEDLGV YYCLQTTHLYTFGGGKLEIK [SEQ ID NO:131] CDR1: KSSQSLLHSNGKTYLN [SEQ ID NO:135] CDR2: LVSKLES [SEQ ID NO:136] CDR3: LQTTHLYT [SEQ ID NO:137]
2H6	QIQLVQSGPELKKPGETVKISC KASGYTFTTYGVNWKQAPG KGLKWMGWINTYSGVPTYAD DFKGRFAFSLETIATTAYLQINN LKNEDTATYFCARFGNYGFDY WGQGTTTLTVSS [SEQ ID NO:138] CDR1: TYGVN [SEQ ID NO:140] CDR2: WINTYSGVPTYADDFKG [SEQ ID NO:141] CDR3: FGNYGFDY [SEQ ID NO:142]	DIVMTQSPSSLSVSAGEKVTMS CKSSQSLVNSGNQKNYLAWYQ QKPGQPPKLLISGASTRESGVPD RFTGSGSGTDFTLTISVQAEDL AVYYCQNDHNYPYTFGGGKLEIK EIK [SEQ ID NO:139] CDR1: KSSQSLVNSGNQKNYLA [SEQ ID NO:143] CDR2: GASTRES [SEQ ID NO:144] CDR3: QNDHNYPYT [SEQ ID NO:145]
h5E7	QVQLVQSGAEVKKPGASVKVS CKASGFNIKDDYIHWVRQAPG	DVQMTQSPSSLSASVGDRVTIT CKSSQSLLHSNGKTYLNWLQQ

	QGLEWMGWIDSENGDTEYAS KFQGRVTITADTSANTAYMELS SLRSED TAVYYCATSSYYSYDL FVYWGQGTLVTVSS [SEQ ID NO:668] CDR1: DDYIH [SEQ ID NO:132] CDR2: WIDSENGDTEYASKFQG [SEQ ID NO:133] CDR3: SSYYSYDLFVY [SEQ ID NO:670]	KPGQAPKLLLYLVSKLESGVPS RFSGSGSGTDYTLTISSLQPEDF ATYYCLQTTHLYTFGQGGTKLEI K [SEQ ID NO:669] CDR1: KSSQSLHLSNGKTYLN [SEQ ID NO:135] CDR2: LVSKLES [SEQ ID NO:136] CDR3: LQTTHLYT [SEQ ID NO:137]
h5E7-YD- C6	QVQLVQSGAEVKKPGASVKVS CKASGFNIKDDYIHWVRQAPG QGLEWMGWIDSENGDTEYAS KFQGRVTITADTSANTAYMELS SLRSED TAVYYCATSEYYSYDL FVYWGQGTLVTVSS [SEQ ID NO:671] CDR1: DDYIH [SEQ ID NO:132] CDR2: WIDSENGDTEYASKFQG [SEQ ID NO:133] CDR3: SEYYSYDLFVY [SEQ ID NO:672]	DVQMTQSPSSLSASVGDRVTIT CKSSQSLHLSNGKTYLNWLQQ KPGQAPKLLLYLVSKLESGVPS RFSGSGSGTDYTLTISSLQPEDF ATYYCLQTTHLYTFGQGGTKLEI K [SEQ ID NO:669] CDR1: KSSQSLHLSNGKTYLN [SEQ ID NO:135] CDR2: LVSKLES [SEQ ID NO:136] CDR3: LQTTHLYT [SEQ ID NO:137]
h5E7-YD- F3	QVQLVQSGAEVKKPGASVKVS CKASGFNIKDDYIHWVRQAPG QGLEWMGWIDSENGDTEYAS KFQGRVTITADTSANTAYMELS SLRSED TAVYYCATSSYWSYD LLVYWGQGTLVTVSS [SEQ ID NO:673] CDR1: DDYIH [SEQ ID NO:132]	DVQMTQSPSSLSASVGDRVTIT CKSSQSLHLSNGKTYLNWLQQ KPGQAPKLLLYLVSKLESGVPS RFSGSGSGTDYTLTISSLQPEDF ATYYCLQTTHLYTFGQGGTKLEI K [SEQ ID NO:669] CDR1: KSSQSLHLSNGKTYLN [SEQ ID NO:135]

	CDR2: WIDSENGDTEYASKFQG [SEQ ID NO:133] CDR3: SSYWSYDLLVY [SEQ ID NO:674]	CDR2: LVSKLES [SEQ ID NO:136] CDR3: LQTTHLYT [SEQ ID NO:137]
h5E7-YD-A6	QVQLVQSGAEVKKPGASVKVS CKASGFNIKDDYIHWVRQAPG QGLEWMGWIDSENGDTEYAS KFQGRVTITADTSANTAYMELS SLRSEDVAVYYCATSSYWSYD LFVYWGQGTLVTVSS [SEQ ID NO:675] CDR1: DDYIH [SEQ ID NO:132] CDR2: WIDSENGDTEYASKFQG [SEQ ID NO:133] CDR3: SSYWSYDLFVY [SEQ ID NO:676]	DVQMTQSPSSLSASVGDRVTIT CKSSQSLLSNGKTYLNWLQQ KPGQAPKLLLYLVSKLESGVPS RFGSGSGTDYTLTISSLQPEDF ATYYCLQTTHLYTFGQGKLEIK [SEQ ID NO:669] CDR1: KSSQSLLSNGKTYLN [SEQ ID NO:135] CDR2: LVSKLES [SEQ ID NO:136] CDR3: LQTTHLYT [SEQ ID NO:137]
h5E7-YD-B5	QVQLVQSGAEVKKPGASVKVS CKASGFNIKDDYIHWVRQAPG QGLEWMGWIDSENGDTEYAS KFQGRVTITADTSANTAYMELS SLRSEDVAVYYCATSTYWSYD LFVYWGQGTLVTVSS [SEQ ID NO:677] CDR1: DDYIH [SEQ ID NO:132] CDR2: WIDSENGDTEYASKFQG [SEQ ID NO:133] CDR3: STYWSYDLFVY [SEQ ID NO:678]	DVQMTQSPSSLSASVGDRVTIT CKSSQSLLSNGKTYLNWLQQ KPGQAPKLLLYLVSKLESGVPS RFGSGSGTDYTLTISSLQPEDF ATYYCLQTTHLYTFGQGKLEIK [SEQ ID NO:669] CDR1: KSSQSLLSNGKTYLN [SEQ ID NO:135] CDR2: LVSKLES [SEQ ID NO:136] CDR3: LQTTHLYT [SEQ ID NO:137]

h5E7 variants consensus	QVQLVQSGAEVKKPGASVKVS CKASGFNIKDDYIHWVRQAPG QGLEWMGWIDSENGDTEYAS KFQGRVTITADTSANTAYMELS SLRSEDNAVYYCATSX ₁ YX ₂ SY DLX ₃ VYWGQGTLVTVSS, wherein X ₁ is S, T, or E; X ₂ is Y or W; and X ₃ is F or L [SEQ ID NO:679] CDR1: DDYIH [SEQ ID NO:132] CDR2: WIDSENGDTEYASKFQG [SEQ ID NO:133] CDR3: SX ₁ YX ₂ SYDLX ₃ VY, wherein X ₁ is S, T, or E; X ₂ is Y or W; and X ₃ is F or L [SEQ ID NO:680]	DVQMTQSPSSLSASVGDRVIT CKSSQSLLSHNGKTYLNWLQQ KPGQAPKLLLYLVSKLESGVPS RFSGSGSGTDYTLTISSLQPEDF ATYYCLQTTHLYTFTGQGTKLEI K [SEQ ID NO:669] CDR1: KSSQSLLSHNGKTYLN [SEQ ID NO:135] CDR2: LVSKLES [SEQ ID NO:136] CDR3: LQTTHLYT [SEQ ID NO:137]
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[0199] In certain embodiments, the DLL3 binding site binds DLL3 with a K_D of 0.001 nM – 10 nM, *e.g.*, 0.001 nM – 9 nM, 0.001 nM – 8 nM, 0.001 nM – 7 nM, 0.001 nM – 6 nM, 0.001 nM – 5 nM, 0.001 nM – 4 nM, 0.001 nM – 3 nM, 0.001 nM – 2 nM, 0.001 nM – 1 nM, 0.001 nM – 0.9 nM, 0.001 nM – 0.8 nM, 0.001 nM – 0.7 nM, 0.001 nM – 0.6 nM, 0.001 nM – 0.5 nM, 0.001 nM – 0.4 nM, 0.001 nM – 0.3 nM, 0.001 nM – 0.2 nM, 0.001 nM – 0.1 nM, 0.05 nM – 10 nM, 0.1 nM – 10 nM, 0.2 nM – 10 nM, 0.3 nM – 10 nM, 0.4 nM – 10 nM, 0.5 nM – 10 nM, 1 nM – 10 nM, 2 nM – 10 nM, 3 nM – 10 nM, 4 nM – 10 nM, 5 nM – 10 nM, 6 nM – 10 nM, 7 nM – 10 nM, 8 nM – 10 nM, or 9 nM – 10 nM, as measured using standard binding assays, for example, surface plasmon resonance or bio-layer interferometry. In certain embodiments, the antibody binds to DLL3 with a K_D of <0.011 nM, about 0.203 nM, about 0.669 nM, about 0.184 nM, about 1.12 nM, about 1.92 nM, about 5.11 nM, about 6.1 nM, or about 8.44 nM, as measured using surface plasmon resonance.

[0200] Table 3 lists publicly available peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to DLL3.

Table 3		
Clones	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Rovalpituzumab (US Patent No. 9,089,615)	QVQLVQSGAEVKKPGASVK VSKASGYTFTNYGMNWVR QAPGQGLEWMGWINTYTGE PTYADDFKGRVTMTTDTSTS TAYMELRSLRSDDTAVYYC ARIGDSSPSDYWGQGTLVTV SS (SEQ ID NO:146) CDR1(SEQ ID NO:148) - GYTFTNY CDR2 (SEQ ID NO:149) - NTYTGE CDR3 (SEQ ID NO:150) - IGDSSPSDY	EIVMTQSPATLSVSPGERATLSCK ASQSVSNDVVWYQQKPGQAPRL LIYYASNRYTGIPARFSGSGSGTE FTLTISSLQSEDFAVYYCQQDYTS PWTFGQGTKLEIKR (SEQ ID NO:147) CDR1(SEQ ID NO:151) - QSVSNDVV CDR2 (SEQ ID NO:152) - YASNRYT CDR3 (SEQ ID NO:153) - QQDYTSPWT
Chugai DL301 (US Patent No. 9,127,071)	QVQLQQSGADLVRPGTSVK VSKASGYAFTNYLIEWIKQ RPGQGLEWIGVMNPGSGGT HYSEKFRGKATLTADKSSST AYMQLISLTSDDSAVYFCAR SDYDYVTYAMDYWGQGTS VTVSS (SEQ ID NO:154) CDR1(SEQ ID NO:156) - NYLIE	DIKMTQSPSSMYASLGERVTITC KASQDINSYLIWFQQKPGKSPKT LIYRTNRLVDGVPSRFSGSGSGGQ DYSLTISSLEYGDMGIYYCLQYD EFPFTFGSGTKLEIK (SEQ ID NO:155) CDR1(SEQ ID NO:159) - KASQDINSYLI CDR2 (SEQ ID NO:160) - RTNRLVD

	CDR2 (SEQ ID NO:157) - VMNPGSGGTHYSEKFRG CDR3 (SEQ ID NO:158) - SDYDYVTYAMDY	CDR3 (SEQ ID NO:161) - LQYDEFPFT
Chugai DL306 (US Patent No. 9,127,071)	EVQLQQSGPELVKPGASVK MSCRASGYTFTDYYMKWV KQSHGKSLEWIGDINPNNGD TFYNQKFKGKATLTIDKSSS TAYMQLNSLTSDDSAVYYC ARDGNYAYFDYWGQGTTLT VSS (SEQ ID NO:162) CDR1(SEQ ID NO:164) - DYYMK CDR2 (SEQ ID NO:165) - DINPNNGDTFYNQKFKG CDR3 (SEQ ID NO:166) - DGNYAYFDY	DIVLTQSPASLAVSLGQRATISCR ASKSVSTSGYSYMHYQQKPGQ PPKLLIFLASNLESGVPARFSGSG SGTDFTLNIHPVEEEDAATYYCQ HSRHLPWTFGGGTKLEIK (SEQ ID NO:163) CDR1(SEQ ID NO:167) - RASKSVSTSGYSYMH CDR2 (SEQ ID NO:168) - LASNLES CDR3 (SEQ ID NO:169) - QHSRHLPWT
Chugai DL309 (US Patent No. 9,127,071)	QVQLQQSGGDLMKPGASVK ISCKAAGYTFSNYYIEWVKQ RPGHGLEWIGEILPGSGSTTY NEKFKGKASFTADTSSNTAY MQLSSLTSEDSAVYYCARW GAREPGFPYWGQGTLLTVS A (SEQ ID NO:170) CDR1(SEQ ID NO:172) - NYYIE CDR2 (SEQ ID NO:173) - EILPGSGSTTYNEKFKG	DIVMTQSQKFMSTSVGDRVSVTC KASQNVGTNVAWYQQKPGQSP KALIYSASYRYSQVPRFTGSGS GTDFTLTISNVQSEDLAEYFCQQ YNNYPLTFGAGTKLELK (SEQ ID NO:171) CDR1(SEQ ID NO:175) - KASQNVGTNVA CDR2 (SEQ ID NO:176) - SASYRYS CDR3 (SEQ ID NO:177) - QQYNNYPLT

	CDR3 (SEQ ID NO:174) - WGAREPGFPY	
Chugai DL312 (US Patent No. 9,127,071)	EVKLVESGGGLVQPGGSLSL SCAASGFTFTDYIMNWVRQ PPGKALEWLALIRNKANGYT TEYNASVKGRFTISRDN SQNI LYLQMNALRAEDSATYYCA RDS DGYEYFYFDYWGQGTT LTVSS (SEQ ID NO:178) CDR1(SEQ ID NO:180) - DYIMN CDR2 (SEQ ID NO:181) - LIRNKANGYTTEYNASVKG CDR3 (SEQ ID NO:182) - DSDGYEYFYFDY	DIQMTQSPSSLSASLGERVSLTCR ASQEISDYLSWLQKKPDGTIKRLI FAASTLDSGVPKRFSGSRSGSD FSLSISSLESEDFADYYCLQYASY PYTFGSGTKLEIK (SEQ ID NO:179) CDR1(SEQ ID NO:183) – RASQEISDYLS CDR2 (SEQ ID NO:184) – AASTLDS CDR3 (SEQ ID NO:185) - LQYASYPYT
Amgen DLL3-4 (US Patent Publication No. 201700371 30)	QVQLQESGPGLVKPSETLSL TCTVSGGSISSYYWSWIRQPP GKGLEWIGYVYYSGTTNYN PSLKSRVTISVDTSKNQFSLK LSSVTAADTAVYYCASI AVT GFYFDYWGQGT LTVSS (SEQ ID NO:186) CDR1(SEQ ID NO:188) - SYYWS CDR2 (SEQ ID NO:189) - YVYYSGTTNYNPSLK S CDR3 (SEQ ID NO:190) - IAVTGFYFDY	EIVLTQSPGTLSPGERVTLSCR ASQRVNNNNYLAWYQQRPGQAP RLLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQQY DRSPLTFGGGTKLEIK (SEQ ID NO:187) CDR1(SEQ ID NO:191) - RASQRVNNNNYLA CDR2 (SEQ ID NO:192) - GASSRAT CDR3 (SEQ ID NO:193) - QQYDRSPLT

Amgen DLL3-9 (US Patent Publication No. 201700371 30)	QVQLQESGPGLVKPSSETLSL TCTVSGASISSFYWSWIRQPP GKGLEWIGYIYYSGTTNYP SLKSRVTISVDTSKNQFSLKL SSVTAADTAVYYCARIAVA GFFFDYWGQGTLVTVSS (SEQ ID NO:194) CDR1(SEQ ID NO:196) - SFYWS CDR2 (SEQ ID NO:197) - YIYYSGTTNYPNPSLKS CDR3 (SEQ ID NO:198) - IAVAGFFFDY	EIVLTQSPGTLSSLSPGESATLSR ASQSVNKNYLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCQQYD RSPLTFGGGTRLEIK (SEQ ID NO:195) CDR1(SEQ ID NO:199) - RASQSVNKNYLA CDR2 (SEQ ID NO:200) - GASSRAT CDR3 (SEQ ID NO:201) - QQYDRSPLT
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[0201] Alternatively, novel antigen-binding sites that can bind to DLL3 can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:202.

SEQ ID NO:202

5 MVSPRMSGLLSQTVILALIFLPQTRPAGVFELQIHSFGPGPGPGAPRSPCSARLPCRLFF
RVCLKPGLSEEAESPICALGAALSARGPVYTEQPGAPAPDLPLPDGLLQVPFRDAWP
GTFSFIIETWREELGDQIGGPAWSLLARVAGRRLAAGGPWARDIQRAGAWELRFSY
RARCEPPAVGTACTRLCRPRSAPSRCGPGLRPCAPLEDECEAPLVCRAGCSPEHGFCE
QPGECRCLEGWTGPLCTVPVSTSSCLSPRGPSATTGCLVPGPGPCDGNPCANGGSCS
10 ETPRSFECTCPRGFYGLRCEVSGVTCADGPCFNGLCVGGADPDSAYICHCPPGFQG
SNCEKRVDRCSLQPCRNGGLCLDLGHALRCRCRAGFAGPRCEHDLDDCAGRACAN
GGTCVEGGGAHRCSCALGFGGRDCRERADPCAARPCAHHGGRCYAHFSGLVACAP
GYMGARCEFPVHPDGASALPAAPPGLRPGDPQRYLLPPALGLLVAAGVAGAALLLV
HVRRRGHSQDAGSRLLAGTPEPSVHALPDALNNLRTQEGSGDGPSSSVDWNRPEDV
15 DPQGIYVISAPSIYAREVATPLFPPLHTGRAGQRQHLLFPYPSSILSVK

[0202] In certain aspects, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor associated-antigen MUC1 (or MUC1-C). Table 4 lists some exemplary sequences of heavy

chain variable domains and light chain variable domains that, in combination, can bind to MUC1 (or MUC1-C).

Table 4		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Immunomedics Clivatuzumab tetraxetan (US Patent No. 9,452,228)	QVQLQQSGAEVKKPGAS VKVSCEASGYTFPSYVLH WVKQAPGQGLEWIGYIN PYNDGTQYNEKFKGKAT LTRDTSINTAYMELSRLR SDDTAVYYCARGFGGSY GFAYWGQGTLVTVSSA (SEQ ID NO:203) CDR1(SEQ ID NO:204) - GYTFPSY CDR2 (SEQ ID NO:205) - NPYNDG CDR3 (SEQ ID NO:206) - GFGGSYGFAY	DIQLTQSPSSLSASVGDRV MTCSASSSVSSSYLYWYQQ KPGKAPKLWIYSTSNLASG VPARFSGSGSGTDFTLTIS LPEDSASYFCHQWNRYPYT FGGGTRLEIKR (SEQ ID NO:207) CDR1(SEQ ID NO:208) - SSVSSSYLY CDR2 (SEQ ID NO:209) - STSNLAS CDR3 (SEQ ID NO:210) - HQWNRYPYT
Glycotope Gatipotuzumab (US Patent No. 9,217,038)	EVQLVESGGGLVQPGGS MRLSCVASGFPSNYWM NWVRQAPGKGLEWVGEI RLKSNNYTTHYAESVKG RFTISRDDSKNSLYLQMN SLKTEDTAVYYCTRHYY FDYWGQGTLVTVSSA (SEQ ID NO:211) CDR1(SEQ ID NO:212) - NYWMN	DIVMTQSPLSNPVTPGEPAS I SCRSSKSLLSNGITYFFWY LQKPGQSPQLLIYQMSNLAS GVPDRFSGSGSGTDFTLRIS RVEAEDVGVYYCAQNLRLP PTFGQGTKVEIKR (SEQ ID NO:215) CDR1(SEQ ID NO:216) - RSSKSLLSNGITYFF CDR2 (SEQ ID NO:217) - QMSNLAS

	CDR2 (SEQ ID NO:213) - EIRLKSNNYTTHYAES CDR3 (SEQ ID NO:214) - HYYFDY	CDR3 (SEQ ID NO:218) – AQNLELPPT
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[0203] Alternatively, novel antigen-binding sites that can bind to MUC1 (or MUC1-C) can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:219.

SEQ ID NO:219

5 MTPGTQSPFFLLLLLLTVLTVVTGSGHASSTPGGEKETSATQRSSVPSSTEKNAVSMTS
SVLSSHSPGSGSSTTQGQDVT LAPATEPASGSAATWGQDVTSVPVTRPALGSTTPPA
HDVTSAPDNKPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAP
PAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGST
APPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPG
10 STAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPA
PGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTR
PAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPD
TRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPD
PDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTS
15 SAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHG
VTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPA
HGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAP
PAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGST
APPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPG
20 STAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPA
PGSTAPPAHGVTSAPDNRPALGSTAPPVHNVTASGSASGSASTLVHNGTSARATTTT
ASKSTPFSIPSHSDTPTTLASHSTKTDASSTHHSSVPPLTSSNHSTSPQLSTGVSTFFFLS
FHISNLQFNSSLEDPSTDYYQELQRDISEMFLQIYKQGGFLGLSNIKFRPGSVVVQLTL
AFREGTINVHDTVETQFNQYKTEAASRYNLTISDVSVSDVPFPFSAQSGAGVPGWGIA
25 LLVLVCVLVALAIVYLIALAVCQCRRKNYGQLDIFPARDTYHPMSEYPTYHTHGRY
VPPSSTD RSPYEKVSAGNGGSSLSYTNPAVAATSANL

[0204] In certain aspects, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the antigen Plexin-A1. Table 5 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to Plexin-A1.

Table 5		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Chugai (US Patent Publication No. 20180193451)	QVQLQQSGPGLVKPSQTL SLTCAISGDSLSSTSA AWNWRQSPSGGLEW LGRTYYRSKWYNDY AVSVKSRITINPDTSK NQFSLQLNSVTPEDTS VYYCARDRGY YNGVDVWGQGTM VTVSS (SEQ ID NO:220) CDR1(SEQ ID NO:221) - STSAAWN CDR2 (SEQ ID NO:222) - RTYYRSKWYNDYAVSVKS CDR3 (SEQ ID NO:223) - DRGYNGVDV	SYELTQPPSVSVSPGQTASITCS GDKLGDKYASWYQQRPGQSPL LVIYQDNKRPSGIPQRFSGSNSG NTATLTISGTQAMDEADYFCQA WDSGTFVFGTGTKVTIL (SEQ ID NO:224) CDR1(SEQ ID NO:225) - SGDKLGDKYAS CDR2 (SEQ ID NO:226) - QDNKRPS CDR3 (SEQ ID NO:227) - QAWDSGTFV
Chugai (US Patent Publication No. 20180193451)	QVQLVQSGAEVKKPGASVK VSCKASGYTFTSYMH WVRQAPGQGLEWMGI NPSGGSTSYAQKFQGR VTMTRDTSTSTVYME LSSLRSEDNAVYYCAR APGHYYYGMDVWGQG TTVTVSS (SEQ ID NO:228) CDR1(SEQ ID NO:229) - SYMH	SYELTQPPSVSVSPGQTASITCS GDKLEDKYASWYQLKPGHSPV LVIYQDSKRPSGIPERFSGSNSG NTATLTISGTQAMDEADYYCRA WDSNTGDVVFGGGTKLTVL (SEQ ID NO:232) CDR1(SEQ ID NO:233) - SGDKLEDKYAS CDR2 (SEQ ID NO:234) - QDSKRPS

	CDR2 (SEQ ID NO:230) - IINPSGGSTSYAQKFQ CDR3 (SEQ ID NO:231) - APGHYYYGMDV	CDR3 (SEQ ID NO:235) - RAWDSNTGDVV
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[0205] Alternatively, novel antigen-binding sites that can bind to Plexin-A1 can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:236.

SEQ ID NO:236

MPLPPRSLQVLLLLLLLLLLLLPGMWAEAGLPRAGGGSQPPFRTFSASDWGLTHLVVH
 5 EQTGEVYVGAVNRIYKLSGNLTLLRAHVTGPVEDNEKCYPPPSVQSCPHGLGSTDN
 VNKLLLLDYAANRLACGSASQGICQFLRLDDLFKLGEPPHHRKEHYLSSVQEAGSM
 AGVLIAGPPGQGQAKLFVGTPIDGKSEYFPTLSSRRLMANEEDADMFGFVYQDEFVS
 SQLKIPSDTLSKFPAFDIYYVYSFRSEQFVYYLTLQLDTQLTSPDAAGEHFFTSKIVRL
 CVDDPKFYSSYVEFFIGCEQAGVEYRLVQDAYLSRPGRALAHQLGLAEDEDVLFTVF
 10 AQQQKNRVKPPKESALCLFTLRAIKEKIKERIQSCYRGEGKLSLPWLLNKGELGCINSP
 LQIDDDFCGQDFNQPLGGTVTIEGTPLFVDKDDGLTAVAAAYDYRGRTVVVFAGTRSG
 RIRKILVDLSNPGGRPALAYESVVAQEGSPILRDLVLSPNHQYLYAMTEKQVTRVPV
 ESCVQYTSCCLGSRDPHCGWCVLHSICSRDADERAEPQRFADLLQCVQLTV
 QPRNVSVTMSQVPLVLQAWNVPDLSAGVNCSFEDFTESESVLEDGRIHCRSPSAREV
 15 APITRGQGDQRVVKLYLKSKETGKKFASVDFVFYNCSVHQSCLSVCNNGSFPCHWCK
 YRHVCTHNVADCAFLEGRVNVSEDCPQILPSTQIYVPVGVVVKPITLAARNLPQPQSG
 QRGYECLFHIPGSPARVTALRFNSSLQCQNSSYSYEGNDVSDLPVNLVSVWNGNFW
 IDNPQNIQAHLKYCPALRESCGLCLKADPRFECGWCVAERRCSLRHHCAADTPASW
 MHARHGSSRCTDPKILKLSPETGPRQGGTRLTITGENLGRLFEDVRLGVRVGKVLCS
 20 PVESEYISAEQIVCEIGDASSVRAHDALVEVCVRDCSPHYRALSPKRFTFVTPTFYRVS
 PSRGPLSGGTWIGIEGSHLNAGSDVAVSVGGRPCFSWRNSREIRCLTPPGQSPGSAPI
 IININRAQLTNPEVKYNYTEDPTILRIDPEWSINSGGTLLTVTGTLNATVREPRIRAKY
 GGIERENGCLVYNDTTMVCRAPSVANPVRSPPELGERPDELGFVMDNVRSLLVLNST
 SFLYYDPDVLEPLSPTGLLELKPSPLILKGRNLLPPAPGNSRLNYTVLIGSTPCTLTVS
 25 ETQLLCEAPNLTGQHKVTVRAGGFESPGTLQVYSDSLTLPALVIGIGGGGGGLLLLVI
 VAVLIAAYKRKSRDADRTLKRLQLQMDNLESRVALECKEAFELQTDIHELNDLDG
 AGIPFLDYRTYAMRVLPFGIEDHPVLKEMEYQANVEKSLTLFGQLLTKKHFLFTFIRT
 LEAQRSFSMRDRGNVASLIMTALQGEMEYATGVLKQLLSDLIEKNLESKNHPKLLLR

RTESVAEKMLTNWFTFLLYKFLKECAGEPLFMLYCAIKQQMEKGPIDAITGEARYSL
 SEDKLIRQQIDYKTLTLNVCNPNENAPEVPVKGLDCDVTQTQAKEKLLDAAYKGV
 YSQRPKAADMDLEWRQGRMARIILQDEDVTTKIDNDWKRLNTLAHYQVTDGSSVA
 LVPKQTSAYNISNSSTFTKSLSRYESMLRTASSPDSLRSRTPMITPDLESGTKLWHLVK
 5 NHDHLDQREGDRGSKMVSEIYLTRLLATKGTLQKFVDDLFETIFSTAHRGSA
 LPLAIKYMFDLDEQADKHQIHDADVRHTWKSNCPLRFWVNVIKNPQFVFDIHKNSITDAC
 LSVVAQTFMDSCSTSEHKLGKDSPSNKLLYAKDIPNYKSWVERYADI
 AKMPAISDQDMSAYLAEQSRLHLSQFNMSALHEIYSYITKYKDEILAALEKDEQARRQRLRSKLE
 QVVDTMALSS

- 10 **[0206]** In certain aspects, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-associated antigen TNFRSF10B. Table 6 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to TNFRSF10B.

Table 6		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Tigatuzumab	EVQLVESGGGLVQPGGSLR LSCAASGFTFSSYVMSWVR QAPGKGLEWVATISSGGSY TYYPDSVKGRFTISRDN AKNTLYLQMNSLRAEDTAVY YCARRGDSMITTDYWGQG TLVTVSSA (SEQ ID NO:237) CDR1(SEQ ID NO:238) - GFTFSSY CDR2 (SEQ ID NO:239) - SSGGSY CDR3 (SEQ ID NO:240) - RGDSMITTDY	DIQMTQSPSSLSASVGDRVTIT CKASQDVGTA VAWYQQKPGKAPKLLIYWASTRHTGVPSRF SGSGSGTDFTLTIS SLQPEDFATYYCQYSSYRTFGQG TKVEIKR (SEQ ID NO:241) CDR1(SEQ ID NO:242) - QDVGTA VA CDR2 (SEQ ID NO:243) - WASTRHT CDR3 (SEQ ID NO:244) - QYSSYRT

Conatumumab	QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYFWSWIRQLPGKGLEWIGHIHNSGTTYNP SLKSRVTISVDTSKKQFSLRLSSVTAADTAVYYCARDRGGDY YYGMDVWGQGTTVTVSSA (SEQ ID NO:245) CDR1(SEQ ID NO:246) - GGSISSGDY CDR2 (SEQ ID NO:247) - HNSGT CDR3 (SEQ ID NO:248) - DRGGDY YYGMDV	EIVLTQSPGTLSTLSPGERATLS CRASQGISRSYLA WYQQKPGQAPSL LIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQFGSSPWTFG QGTKVEIKR (SEQ ID NO:249) CDR1(SEQ ID NO:250) - QGISRSYLA CDR2 (SEQ ID NO:251) - GASSRAT CDR3 (SEQ ID NO:252) - QQFGSSPWT
Drozitumab	EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSGINWQGGSTGYADSVKGRVTISRDNANKNSLYLQMNSLRAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTVSSA (SEQ ID NO:253) CDR1(SEQ ID NO:254) - GFTFDDY CDR2 (SEQ ID NO:255) - NWQGGG CDR3 (SEQ ID NO:256) - ILGAGRGWYFDY	SELTQDPAVSVALGQTVRITCSGDSLRSYYASWYQQKPGQAPVLVIYGANNRPSGIPDRFSGSSGNTASLTITGAQAED EADYYCNSADSSGNHVVFGGGTKLTVLG (SEQ ID NO:257) CDR1(SEQ ID NO:258) - SLRSYYAS CDR2 (SEQ ID NO:259) - GANNRPS CDR3 (SEQ ID NO:260) - NSADSSGNHVV

[0207] Alternatively, novel antigen-binding sites that can bind to TNFRSF10B can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:261.

SEQ ID NO:261

MEQRGQNAPAASGARKRHGPGPREARGARPGPRVPKTLVLVVA AVL LLSAESALI
 5 TQQDLAPQQRAAPQQRSSPSEGLCPPGHHISEDGRDCISCKYGDYSTHWNDLLFC
 LRCTRCDSGEVELSPCTTTRNTVCQCEEGTFREEDSPEMCRKCRTGCPRGMVKVGD
 CTPWSDIECVHKESGTKHSGEVP AVEETVTSSPGTPASPCSLSGIIIGVTVA AVL LIVA
 VVFKSLLWKKVLPYLKGICSGGGGDPERVDRSSQRPGAEDNVLNEIVSILQPTQVP
 EQEMEVQEPAEPTGVNMLSPGESEHLLPEAE AERSQRRRLLPANEGDPTETLRQCF
 10 DDFADLVPFDSWEPLMRKLGLMDNEIKVAKAEAAGHRDTLYTMLIKWVNKTGRDA
 SVHTLLDALETGERLAKQKIEDHLLSSGKFMYLEGNADSAMS

[0208] In certain aspects, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-associated antigen STEAP1. Table 7 lists some exemplary peptide sequences of heavy chain
 15 variable domains and light chain variable domains that, in combination, can bind to STEAP1.

Table 7		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Vandortuzumab vedotin	EVQLVESGGGLVQPGGSL RLSCAASGYSITSDYAWN WVRQAPGKGLEWVGYISN SGSTSYNPSLKSRTFTISRDN SKNTLYLQMNSLRAEDTA VYYCARERNYDYDDYYY AMDYWGQGTLLVTVSS (SEQ ID NO:262) CDR1(SEQ ID NO:263) - GYSITSDY CDR2 (SEQ ID NO:264) - SNSGS	DIQMTQSPSSLSASVGDRV ITCKSSQSLLYRSNQKNYLA WYQQKPGKAPKLLIYWAST RESGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQYYN YPRTFGQGTKVEIKR (SEQ ID NO:266) CDR1(SEQ ID NO:267) - QSLLYRSNQKNYLA CDR2 (SEQ ID NO:268) - WASTRES

	CDR3 (SEQ ID NO:265) - ERNYDYDDYYYAMDY	CDR3 (SEQ ID NO:269) - QQYYNYPRT
Roche (US Patent Publication No. 20170096495)	EVQLVESGGGLVQPGGSL RLSCAVSGYSITSDYAWN WVRQAPGKGLEWVGYSN SGSTSYNPSLKSRTISRDN SKNTLYLQMNSLRAEDTA VYYCARERNYDYDDYYY AMDYWGQGTLLTVSS (SEQ ID NO:270) CDR1(SEQ ID NO:271) - DYAWN CDR2 (SEQ ID NO:272) - YISNSGSTSYNPSLKS CDR3 (SEQ ID NO:273) - ERNYDYDDYYYAMDY	DIQMTQSPSSLSASVGDRV ITCKSSQSLLYRSNQKNYLA WYQQKPGKAPKLLIYWAST RESGVPSRFSGSGSGTDFTL TISSLQPEDFATYYCQQYYN YPRTFGQGKVEIK (SEQ ID NO:274) CDR1(SEQ ID NO:275) - KSSQSLLYRSNQKNYLA CDR2 (SEQ ID NO:276) - WASTRES CDR3 (SEQ ID NO:277) - QQYYNYPRT

[0209] Alternatively, novel antigen-binding sites that can bind to STEAP1 can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:278.

SEQ ID NO:278

MESRKDITNQEELWKMKPRRNLEEDDYLHKDTGETSMLKRPVLLHLHQTAAHADEF
 5 DCPSELQHTQELFPQWHLPIKIAAIIASLTFLYTLLREVIHPLATSHQQYFYKIPILVINK
 VLPMVSITLLALVYLPGVIAAIVQLHNGTKYKKFPHWLDKWMLTRKQFGLLSFFFA
 VLHAIYSLSYPMRRSYRYKLLNWAYQQVQQNKEDAWIEHDVWRMEIYVSLGIVGL
 AILALLAVTSIPSVSDSLTWREFHYIQSKLGIVSLLLGTIHALIFAWNKWIDIKQFVWY
 TPPTFMIAVFLPIVVLIFKSILFLPCLRKKILKIRHGWEDVTKINKTEICSQL

10 **[0210]** In certain aspects, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-associated antigen CDCP1. Table 8 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to CDCP1.

Table 8		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Roche RG7287 (US Patent No. 9,346,886)	EVQLVESGGGLVQPGGSL RLSCAASGFTFNSYGMWS VRQAPGKGLEWVATISSG GSYKYYVDSVKGRFTISR NAKNSLYLQMNSLRAEDT AVYYCARHPDYDGVWFA YWGQGTLLTVSS (SEQ ID NO:279) CDR1(SEQ ID NO:280) - SYGMS CDR2 (SEQ ID NO:281) - TISSGGSYKYYVDSVKG CDR3 (SEQ ID NO:282) - HPDYDGVWFAY	EIVLTQSPATLSLSPGERATMS CSVSSSVFYLHWYQQKPGQAP RLWIYDTSKSLASGIPARFSGSG SGTDFTLTISSLEPEDFAVYYC QQWNSNPPTFGGGTKVEIK (SEQ ID NO:283) CDR1(SEQ ID NO:284) - SVSSSVFYLH CDR2 (SEQ ID NO:285) - DTSKSLAS CDR3 (SEQ ID NO:286) - QQWNSNPPT
Roche RG7287 (US Patent No. 9,346,886)	EVQLVESGGGLVQPGGSL RLSCAASGFTFNSYGMWS VRQAPGKGLEWVATISSG GSYKYYVDSVKGRFTISR NAKNSLYLQMNSLRAEDT AVYYCARHPDYDGVWFA YWGQGTLLTVSS (SEQ ID NO:279) CDR1(SEQ ID NO:280) - SYGMS CDR2 (SEQ ID NO:281) - TISSGGSYKYYVDSVKG	DIQMTQSPSSLSASVGDRVTIT CSVSSSVFYVHWYQQKPGKA PKLLIYDTSKSLASGVPSRFSGS GSGTDFTFTISLQPEDATYY CQQWNSNPPTFGGGTKVEIK (SEQ ID NO:287) CDR1(SEQ ID NO:288) - SVSSSVFYVH CDR2 (SEQ ID NO:289) - DTSKSLAS CDR3 (SEQ ID NO:290) - QQWNSNPPT

	CDR3 (SEQ ID NO:282) - HPDYDGVWFAY	
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[0211] Alternatively, novel antigen-binding sites that can bind to CDCP1 can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:291.

SEQ ID NO:291

MAGLNCGVSIALLGVLLLGAARLPRGAFAFEIALPRESNITVLIKLGTPDLLAKPCYIV
 5 ISKRHITMLSIKSGERIVFTFSCQSPENHFVIEIQKNIDCMMSGPCPFGEVQLQPSTSLPT
 LNRTFIWDVKAHKSIGLELQFSIPRLRQIGPGESCPDGVTHSISGRIDATVVRIGTFCSN
 GTVSRIKMQEGVKMALHLPWFHPRNVSGFSIANRSSIKRLCIIESVFEGEGSATLMSA
 NYPEGFPEDELMTWQFVVPAPHLRASVSFLNFNLSNCERKEERVEYYIPGSTTNPEVF
 KLEDKQPGNMAGNFNLSLQGCDDQAQSPGILRLQFQVLVQHPQNESNKIYVVDLSN
 10 ERAMSLTIEPRPVKQSRKFVPGCFVCLESRTCSSNLTLTSGSKHKISFLCDDLTRLWM
 NVEKTISCTDHRYSQRKSYSLQVPSDILHLPVELHDFSWKLLVPKDRLSLVLVPAQK
 LQQHTHEKPCNTSFSYLVASAIPSQDLYFGSFCPGGSIKQIQVKQNISVTLRTFAPSQ
 QEASRQGLTVSFIPYFKEEGVFTVTPDTKSKVYLRTPNWDRGLPSLTSVSWNISVPRD
 QVACLTFKERSGVVCQTGRAFMIIQEQRTRAEEIFSLDEDVLPKPSFHHHSFWVNIS
 15 NCSPTSGKQLDLLFSVTLTPTRTVDLTVILIAAVGGGVLLLSALGLIICCVKKKKKKTN
 KGPAVGIYNDNINTEMPRQPKKFQKGRKDNDSHVYAVIEDTMVYGHLLQDSSGSFL
 QPEVDTYRPFQGTMGVCPSPPTICSRAPTAKLATEEPPPRSPPESESEPYTFSHPNNG
 DVSSKDTDIPLLNTQEPMEPAE

[0212] In certain aspects, the present disclosure provides multi-specific binding proteins
 20 that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-
 associated antigen PTK7. Table 9 lists some exemplary peptide sequences of heavy chain
 variable domains and light chain variable domains that, in combination, can bind to PTK7.

Table 9		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Pfizer Cofetuzumab	QVQLVQSGPEVKKPGASV KVSCKASGYTFTDYAVHW VRQAPGKRLEWIGVISTYN	EIVLTQSPATLSLSPGERATLS CRASEVDSYGKSFMHYQ QKPGQAPRLLIYRASNLGIP

(US Patent Publication No. 20150315293)	DYTYNNQDFKGRVTMTRD TSASTAYMELSRLRSEDTA VYYCARGNSYFYALDYW GQGTSVTVSSA (SEQ ID NO:292) CDR1(SEQ ID NO:293) - DYAVH CDR2 (SEQ ID NO:294) - VISTYNDYTYNNQDFK CDR3 (SEQ ID NO:295) - GNSYFYALDY	ARFSGSGSGTDFTLTISSLEPE DFAVYYCQQSNEDPWTFGGG TKLEIKR (SEQ ID NO:296) CDR1(SEQ ID NO:297) - RASESVDSYGKSFMH CDR2 (SEQ ID NO:298) - RASNLES CDR3 (SEQ ID NO:299) - QQSNEDPWT
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[0213] Antigen-binding sites that can bind to PTK7 can also be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:300.

SEQ ID NO:300

MGAARGSPARPRRLPLLSVLLLPLLGGTQTAIVFIKQPSSQDALQGRRALLRCEVEAP
 5 GPVHVYWLLDGAPVQDTERRFAQGSSLSFAAVDRLQDSGTFQCVARDDVTGEEARS
 ANASFNIKWIEAGPVVLKHPASEAEIQPQTQVTLRCHIDGHPRPTYQWFRDGTPLSD
 GQSNHTVSSKERNLTLRPAGPEHSGLYSCCAHSAFGQACSSQNFTLSIADESFARVVL
 APQDVVVARYEEAMFHCQFSAQPPPSLQWLFEDETPITNRSRPPHLRRATVTFANGSL
 LLTQVRPRNAGIYRCIGQGQRGPPIILEATLHLAEIEDMPLFEPRVFTAGSEERVTCPLP
 10 KGLPEPSVWWEHAGVRLPTHGRVYQKGHELVLANIAESDAGVYTCHAANLAGQRR
 QDVNITVATVPSWLKKPQDSQLEEGKPGYLDCLTQATPKPTVVWYRNQMLISED
 FEVFKNGTLRINSVEVYDGTWYRCMSSTPAGSIEAARVQVLEKLKFTPPPQPQQCM
 EFDKEATVPCSATGREKPTIKWERADGSSLPEWVTDNAGTLHFARVTRDDAGNYTC
 IASNGPQGQIRAHVQLTVAVFITFKVEPERTTVYQGHTALLQCEAQGDPKPLIQWKG
 15 KDRILDPTKLGPRMHIFQNGSLVIHDVAPEDSGRYTCIAGNSCNIKHTEAPLYVVDKP
 VPEESEGGPGSPPPYKMIQTIGLSVGAAYIIAVLGLMFYCKKRCKAKRLQKQPEGEE
 PEMECLNGGPLQNGQPSAEIQEEVALTSLGSGPAATNKRHSTSDKMHFPRSSLQPITT
 LGKSEFGEVFLAKAQGLEEGVAETLVLVKSLQSKDEQQQLDFRRELEMFGKLNHAN
 VVRLGLCREAEPHYMVLEYVDLGDLDKQFLRISKSKDEKLKSQPLSTKQKVALCTQ
 20 VALGMEHLSNNRFVHKDLAARNCLVSAQRQVKVSALGLSKDVYNSEYYHFRQAW

VPLRWMSPEAILEGDFSTKSDVWAFGVLMWEVFTHGEMPHGGQADDEV LADLQA
GKARLPQPEGCP SKLYRLMQRCWALSPKDRPSFSEIASALGDSTVDSKP

[0214] In certain aspects, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-associated antigen AXL. Table 10 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to AXL.

Table 10		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Genentech (US Patent No. 8,853,369)	EVQLVESGGGLVQPGGSL RLSCAASGFSLSGSIHW VRQAPGKGLEWVGWINP YRGYAYYADSVKGRFTIS ADTSKNTAYLQMNSLRAE DTAVYYCAREYSGWGGG SVGYAMDYWGQGTLLV (SEQ ID NO:301) CDR1(SEQ ID NO:302) - GFSLSGS CDR2 (SEQ ID NO:303) - NPYRGY CDR3 (SEQ ID NO:304) - EYSGWGGSSVG YAMDY	DIQMTQSPSSLSASVGDRVTIT CRASQDVSTAVAWYQQKPGK APKLLIYSASFLYSGVPSRFSG SGSGTDFTLTISSLQPEDFATY YCQQSYTTPPTFGQGTKVEIK R (SEQ ID NO:305) CDR1(SEQ ID NO:306) - QDVSTAVA CDR2 (SEQ ID NO:307) - SASFLYS CDR3 (SEQ ID NO:308) - QQSYTTPPT
Inserm (US Patent No. 9,249,228)	QVQLQQSGAELMKPGAS VKMSCKAAGYTFSSYWIE WVRQRP GHGLEWIGEIFP GSDSTNYNEKFND RATFT ADTSSNTAYMQLSSLTSE DSAVYYCARPLYYGSSA WFAYWGQGTLLVTVSA	DIQMTQSPASLSASVGETVTIT CRASENIYSYLTWYQQKQRKS PQLLVYNAKT LAEGVPSRFSG SGSGTQFSLKINSLQPEDFGTY YCQHHYATPWTFGGGTKVEI K (SEQ ID NO:313)

	(SEQ ID NO:309)	CDR1(SEQ ID NO:314) -
	CDR1(SEQ ID NO:310) -	ENIYSYLT
	GYTFSSY	CDR2 (SEQ ID NO:315) -
	CDR2 (SEQ ID NO:311) -	NAKTLAE
	FPGSDS	CDR3 (SEQ ID NO:316) -
	CDR3 (SEQ ID NO:312) -	QHXYATPWT
	PLYYGSSAWFAY	

[0215] The antigen-binding sites that can bind to AXL can be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:317.

SEQ ID NO:317

MAWRCPRMGRVPLAWCLALCGWACMAPRGTAEESEPFVGNPGNITGARGLTGTLR
5 CQLQVQGEPPEVHWRDGGQILELADSTQTQVPLGEDEQDDWIVVSQLRITSLQLSDT
GQYQCLVFLGHQTFVSQPGYVGLEGLPYFLEPEDRTVAANTPFNLSCQAQGPPEPV
DLLWLQDAVPLATAPGHGPQRS LHVPGLNKTSSFSCEAHNAKGVTTSTRTATITVLPQ
QPRNLHLVSRQPTELEVAWTPGLSGIYPLTHCTLQAVLSNDGMGIQAGEPDPPEEPLT
SQASVPPHQLRLGSLHPHTPYHIRVACTSSQGPSSWTHWLPVETPEGVPLGPPENISA
10 TRNGSQAFVHWQEPRAPLQGTLLGYRLAYQGQDTPEVLMDIGLRQEVTLELQGDGS
VSNLTVCAAYTAAGDGPWSLPVPLEAWRPGQAQPVHQLVKEPSTPAFSWPWWYV
LLGAVVAAACVLILALFLVHRRKKETRYGEVFPTVERGELVVRVVRKSYSRRTTE
ATLNSLGISEELKEKL RDVMVDRHKVALGKTLGEGEFGAVMEGQLNQDD SILKVAV
KTMKIAICTRSELEDFLSEAVCMKEFDHPNVMRLIGVCFQGSERESFPAPVVILPFMK
15 HGDLSFLLYSRLGDQP VYLPTQMLVKFMADIASGMEYLS TKRFIHRDLAARNCML
NENMSVCVADFGLSKKIYNGDYRQGR IAKMPVKWIAIESLADRVYTSKSDVVSFG
VTMWEIATRGTPTYPGVENSEIYDYLRQGNRLKQPADCLDGLYALMSRCWELNPQ
DRPSFTELREDLENTL KALPPAQEPDEILYVNMDEGGGYPEPPGAAGGADPPTQPDP
KDSCSCLTAAEVHPAGRYVLCPSTTPSPAQPADRGSPAAPGQEDGA

20 **[0216]** In certain aspects, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-associated antigen ERBB-3. Table 11 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to ERBB-3.

Table 11		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Merrimack Seribantumab	EVQLLESGGGLVQPGGSLRLSC AASGFTFSHYVMAWVRQAPG KGLEWVSSISSSGGWTLYADS VKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCTRGLKMATIF DYWGQGT LVT VSS (SEQ ID NO:318) CDR1(SEQ ID NO:319) - GFTFSHY CDR2 (SEQ ID NO:320) - SSSGGW CDR3 (SEQ ID NO:321) - GLKMATIFDY	QSALTQPASVSGSPGQSRRI SCTGTSSDVGSYNVVS WY QQHPGKAPKLIIEVSQRPS GVSNRFSGSKSGNTASLTIS GLQTEDEADYYCCSYAGS SIFVIFGGGTKVTVL (SEQ ID NO:322) CDR1(SEQ ID NO:323) - SSDVGSYNVVS CDR2 (SEQ ID NO:324) - EVSQRPS CDR3 (SEQ ID NO:325) - CSYAGSSIFVI
Amgen Patritumab	QVQLQQWGAGLLKPSETLSLT CAVYGGSFSGYYWSWIRQPPG KGLEWIGEINHSGSTNYPNPSLK SRVTISVETSKNQFSLKLSSVTA ADTAVYYCARDKWTWYFDL WGRGTLVT VSSA (SEQ ID NO:326) CDR1(SEQ ID NO:327) - GGSFSGY CDR2 (SEQ ID NO:328) - NHSGS CDR3 (SEQ ID NO:329) - DKWTWYFDL	DIEMTQSPDSLAVSLGERA TINCRSSQSVLYSSSNRNYL AWYQQNPGQPPKLLIYWA STRESGVPDRFSGSGSGTD FTLTISSLQAEDVAVYYCQ QYYSTPRTFGQGTKVEIKR (SEQ ID NO:330) CDR1(SEQ ID NO:331) - QSVLYSSSNRNYLA CDR2 (SEQ ID NO:332) - WASTRES CDR3 (SEQ ID NO:333) - QYYSTPRT

Genentech Duligotuzumab	EVQLVESGGGLVQPGGSLRLSC AASGFTLSGDWIHWVRQAPGK GLEWVGEISAAGGYTDYADSV KGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARESRVSFEAA MDYWGQGTLVTVSSA (SEQ ID NO:334) CDR1(SEQ ID NO:335) - GFTLSGD CDR2 (SEQ ID NO:336) - SAAGGY CDR3 (SEQ ID NO:337) - ESRVSFEEAMDY	DIQMTQSPSSLSASVGDRV TITCRASQNIATDVAWYQQ KPGKAPKLLIYSASFLYSG VPSRFSGSGSGTDFTLTSS LQPEDFATYYCQQSEPEPY TFGQGTKVEIKR (SEQ ID NO:338) CDR1(SEQ ID NO:339) - QNIATDVA CDR2 (SEQ ID NO:340) - SASFLYS CDR3 (SEQ ID NO:341) - QQSEPEPYT
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[0217] The antigen-binding sites that can bind to ERBB-3 can be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:342.

SEQ ID NO:342

MRANDALQVLGLLFSLARGSEVGNSQAVCPGTLNGLSVTGDAENQYQTLTKLYER
5 CEVVMGNLEIVLTGHNADLSFLQWIREVTGYVLVAMNEFSTLPLPNLRVVRGTQVY
DGKFAIFVMLNYNTNSSHALRQLRLTQLTEILSGGVYIEKNDKLCHMDTIDWRDIVR
DRDAEIVVKDNGRSCPPCHEVCKGRCWGPGSEDCQTLTKTICAPQCNGHCFGNPN
QCCHDECAGGCSGPQDTCFACRHFNDSGACVPRCPQPLVYNKLTFLQLEPNPHTKY
QYGGVCVASCPHNFVVDQTSCVRACPPDKMEVDKNGLMCEPCGGLCPKACEGTG
10 SGSRFQTVDSNIDGFVNCTKILGNLDFLITGLNGDPWHKIPALDPEKLNVFRTVREIT
GYLNIQSWPPMHNFVFSNLTTIGGRSLYNRGFSLLIMKNLNVTSLGFRSLKEISAG
RIYISANRQLCYHHSLNWTKVLRGPTTEERLDIKHNRPRRDCVAEGKVCDPLCSSGGC
WGP GPGQCLSCRNYSRGGVCVTHCNFLNGEPREFAEAEFCFSCHPECQPMEGTATC
NGSGSDTCAQCAHFRDGPCHVSSCPHGVLGAKGPIYKYPDVQNECRPCHENCTQGC
15 KGPELQDCLGQTLVLIGKTHLTMAITVIAGLVVIFMMLGGTFLYWRGRRIQNKRAM
RRYLERGESIEPLDPSEKANKVLARIFKETELRKLKVLGSGVFGTVHKGWVWIEGESI
KIPVCIKVIEDKSGRQSFQAVTDHMLAIGSLDHAHIVRLLGLCPGSSLQLVTQYLPLG
SLLDHVRQHRGALGPQLLLNWGVQIAKGMYYLEEHGMVHRNLAARNVLLKSPSQV

QVADFGVADLLPPDDKQLLYSEAKTPIKWMALESIHFGKYTHQSDVWSYGVTVWE
 LMTFGAEPYAGLRLAEVPDLLEKGERLAQPQICTIDVYVMVMVKCWMIDENIRPTFKE
 LANEFTRMARDPPRYLVIKRESGPGIAPGPEPHGLTNKKLEEVELEPELDLDLDLEAE
 EDNLATTTLGSALSLPVGTLNRPRGSQSLLSPSSGYMPMNQGNLGESCQESAVSGSS
 5 ERCPRPVSLHPMPRGCLASESSEGHVTGSEAELQEKVSMCRSRSRSPRPRGDSAY
 HSQRHSLTPTVPLSPPGLEEEDVNGYVMPDTHLKGTPSSREGTLSSVGLSSVLGTEE
 EDEDEEY EYMNRRRRHSPHPPRPSSLEELGYEYMDVGSDLSASLGSTQSCPLHPVPI
 MPTAGTTPDEDYEYMNRRQDGGGPGGDYAAMGACPASEQGYEEMRAFGQPGHQA
 PHVHYARLKTLSLEATDSAFDNPDYWHSRLFPKANAQRT

- 10 **[0218]** In certain aspects, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-associated antigen EDNRB. Table 12 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to EDNRB.

Table 12		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Genentech RG7636 (US Patent No. 9,464,141)	EVQLVESGGGLVQPGGSL RLSCAASGYTFTSYWMQW VRQAPGKGLEWIGTIYPGD GDTSYAQKFKGRATLSTD KSKNTAYLQMNSLRAEDT AVYYCARWGYAYDIDNW G (SEQ ID NO:343) CDR1(SEQ ID NO:344) - GYTFTSYWMQ CDR2 (SEQ ID NO:345) - TIYPGDGDTSYAQKFK CDR3 (SEQ ID NO:346) - WGYAYDIDN	DIQMTQSPSSLSASVGDRVIT CKSSQSLLDSDGKTYLNWLQ QKPGKAPKRLIYLVSKLDSGV PSRFSGSGSGTDFTLTISSLQPE DFATYYCWQGTHFPYTFGQG TKVEIK (SEQ ID NO:347) CDR1(SEQ ID NO:348) - KSSQSLLDSDGKTYLN CDR2 (SEQ ID NO:349) - LVSKLDS CDR3 (SEQ ID NO:350) - WQGTHFPYT

Rendomab-B49 (PCT Publication No. WO2017220739 A1)	QVQLQQPGAALVKPGASV KLSCKASGYTFISYWMLW VKQRPGRGLEWIGRIDPDS GGTKYNEKFKSKATLTVD KSSSTAYMQLSSLTSEDSA VYYCAREGDYAWFAYWG QGTLVPVSA (SEQ ID NO:351) CDR1(SEQ ID NO:352) - GYTFTSYW CDR2 (SEQ ID NO:353) - IDPDSGGT CDR3 (SEQ ID NO:354) - AREGDYAWFAY	DVLMTQTPLSLPVSLGDQASIS CRSSQSIVHSNGNTYLEWYLQ KPGQSPKLLIYKVSNRFSGVDP RFSGSGSGTDFTLKISRVEAED LGVYYCFQGSHVPWTFGGGT KLEIK (SEQ ID NO:355) CDR1(SEQ ID NO:356) - QSIVHSNGNTY CDR2 (SEQ ID NO:357) - KVS CDR3 (SEQ ID NO:358) - FQGSHVPWT
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[0219] Alternatively, novel antigen-binding sites that can bind to EDNRB can be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:359.

SEQ ID NO:359

5 MQPPPSLCGRALVALVLACGLSRIWGEERGFPDRATPLLQTAEIMTPPTKTLWPKG
SNASLARSLAPAEVPGKDRTAGSPRTISPPPCQGPPIKETFKYINTVVSCLVFLGIIG
NSTLLRIIYKNKCMRNGPNILIASLALGDLLHIVIDIPINVYKLLAEDWPFGAEMCKLV
PFIQKASVGITVLSLCALSIDRYRAVASWSRIKGIGVPKWTAVEIVLIWVVSVVLA VP
EAIGFDIITMDYKGSYLRICLLHPVQKTA FMQFYKTAKDWWLFSFYFCLPLAITAFFY
10 TLMTCEMLRKKSGMQIALNDHLKQRREVAKTVFCLVLVFALCWLPLHL SRILKLTL
YNQNDPNRCELLSFLLVLDYIGINMASLNSCINPIALYL VSKRFKNCFKSCLCWCQS
FEEKQSLEEKQSCLKFKANDHGYDNFRSSNKYSSS

[0220] In certain aspects, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-associated antigen TYRP1. Table 13 lists some exemplary peptide sequences of heavy chain
15 variable domains and light chain variable domains that, in combination, can bind to TYRP1.

Table 13		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
CTA99	EVQLQQSGAELVRPGALV KL SCKTSGFN IKDYFLHWV RQRPDQGLEWIGWINPDN GNTVYDPKFQGTASLTAD TSSNTVYLQLSGLTSEDTA VYFCTRRDYTYEKAALDY WGQGASVIVFSAA (SEQ ID NO:360) CDR1 (SEQ ID NO:361) - GFNIKDY CDR2 (SEQ ID NO:362) - INPDNGN CDR3 (SEQ ID NO:363) - RDYTYEKAALDY	AIQMSQSPASLSASVGETVTIT CRASGNIYNYLAWYQQKQGK SPHLLVYDAKTLADGVPSRFS GSGSGTQYSLKISSLQTEDSGN YYCQHFWSLPFTFGSGTKLEI K (SEQ ID NO:364) CDR1 (SEQ ID NO:365) - RASGNIYNYLA CDR2 (SEQ ID NO:366) - DAKTLAD CDR3 (SEQ ID NO:367) - QHFWSLPFT
Imclone Systems Flanvotumab (clone 20D7S) (US Patent No. 7,951,370)	QVQLVQSGSELKKPGASV KISCKASGYTFTSYAMNW VRQAPGQGLES MGWINTN TGNPTYAQGFTGRFVFSM DTSVSTAYLQISSLKAEDT AIYYCAPRYSSSWYLDYW G QGTLVTVSS (SEQ ID NO:368) CDR1 (SEQ ID NO:369) - SCKASGYTFTSYAMN CDR2 (SEQ ID NO:370) - WINTNTGNPTYAQGFTG	EIVLTQSPATLSLSPGERATLS CRASQSVSSYLAWYQQKPGQ APRLLIYDASN RATGIPARFSG SGSGTDFTLTIS SLEPEDFAVY YCQQRSNWLMYTFGQG TKLE IK (SEQ ID NO:372) CDR1 (SEQ ID NO:373) - RASQSVSSYLA CDR2 (SEQ ID NO:374) - DASN RAT CDR3 (SEQ ID NO:375) - QQRSNWLMYT

	CDR3 (SEQ ID NO:371) - RYSSSWYLDY	
Imclone Systems Flanvotumab (clone 20D7) (US Patent No. 7,951,370)	QVQLVQSGSELKKPGASV KISCKASGYTFTSYAMNW VRQAPGQGLECMGWINTN TGNPTYAQGFTGRFVFSM DTSVSTAYLQISSLKAEDT AIYYCAPRYSSSWYLDYW GQGTLVTVSS (SEQ ID NO:376) CDR1(SEQ ID NO:377) - SCKASGYTFTSYAMN CDR2 (SEQ ID NO:378) - WINTNTGNPTYAQGFTG CDR3 (SEQ ID NO:379) - RYSSSWYLDY	EIVLTQSPATLSLSPGERATLS CRASQSVSSYLAWYQQKPGQ APRLLIYDASNRATGIPARFSG SGSGTDFTLTISSELEPEDFAVY YCQQRSNWLMYTFGQGTKLE IK (SEQ ID NO:380) CDR1(SEQ ID NO:381) - RASQSVSSYLA CDR2 (SEQ ID NO:382) - DASNRAT CDR3 (SEQ ID NO:383) - QQRSNWLMYT

[0221] Alternatively, novel antigen-binding sites that can bind to TYRP1 can be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:384.

SEQ ID NO:384

5 MSAPKLLSLGCIFFPLLLFQQARAQFPRQCATVEALRSGMCCPDLSPVSGPGTDRCGS
SSGRGRCEAVTADSRPHSPQYPHDGRDDREWPLRFFNRTCHCNGNFSGHNCGTCR
PGWRGAACDQRVLIVRRNLLDLSKEEKNHFVRALDMAKRTTHPLFVIATRRSEEILG
PDGNTTPQFENISINYFVWTHYYSVKKTFGLGVGQESFGEVDFSHEGPAFLTWHRYHL
LRLEKDMQEMLQEPSFSLPYWNFATGKNVCDICTDDLMGSRSNFDSLISPNSVFSQ
10 WRVVCDSLEDYDTLGTLCNSTEDGPIRRNPAGNVARPMVQRLPEPQDVAQCLEVGL
FDTPPFYSNSTNSFRNTVEGYSDPTGKYDPAVRSLHNLAHLFLNGTGGQTHLSPNDPI
FVLLHTFTDAVFDEWLRRYNADISTFPLENAPIGHNQRQYNMVPFWPPVTNTEMFVTA
PDNLGYTYEIQWPSREFSVPEIIAIAVVGALLLVALIFGTASYLIRARRSMDEANQPLL
TDQYQCYAEEYEKLQNPNQSVV

[0222] In certain aspects, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-associated antigen OLR1. Table 14 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to OLR1.

Table 14		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Novartis (US Patent No. 9,562,101)	QVQLLES GGGLVQP GGSL RLSCAASGFTFSDYALHW VRQAPGKGLEWVSVISYQ GGFIIYADSVKGRFTISR D NSKNTLYLQMNSLRAEDT AVYYCARSPGLTSYHDSW GQGT LVT VSS (SEQ ID NO:385) CDR1 (SEQ ID NO:386) - DYALH CDR2 (SEQ ID NO:387) - VISYQGGFIYYADSVKG CDR3 (SEQ ID NO:388) - SPGLTSYHDS	QSVLTQPPSVSGAPGQRVTISC SGSSSNTGSNYVSWYQQLPGT APKLLIHSTSHRPSGVPDRFSG SKSGTSASLAITGLQAEDSAD YYCQSWDVESERVVFGGGTK LTVL (SEQ ID NO:389) CDR1 (SEQ ID NO:390) - SGSSSNIGSNYVS CDR2 (SEQ ID NO:391) - STSHRPS CDR3 (SEQ ID NO:392) - QSWDYESERVV
MedImmune (US Patent No. 10,117,889)	QVQLVQSGAEVKKPGAV KVSCKVSGYTLTELSMH WVRQAPGKGLEWMGGF DPEDFKYHHTQKFQGRVT MTEDTSTD TAYMELSSLR SEDTAVYYCALVWGTQG KGVRGWDY YYGMDVWG QGTTVT VSS (SEQ ID NO:393)	QSVVTQPPSVSGAPGQRVTISC TGSSSNIGAGYDVHWYQQLP GTAPKLLIYGNSNRPSGVPDRF SGSKSGTSASLAITGLQAEDEA DYYCQSYDSSL SGWVFGGGT KLTVL (SEQ ID NO:397) CDR1 (SEQ ID NO:398) - TGSSSNIGAGYDVH

	CDR1(SEQ ID NO:394) - ELSMH	CDR2 (SEQ ID NO:399) - GNSNRPS
	CDR2 (SEQ ID NO:395) - GFDPEDFKYHTHQKFQG	CDR3 (SEQ ID NO:400) - QSYDSSLGWV
	CDR3 (SEQ ID NO:396) - VWGTQGKGVRGWDYYY GMDV	

[0223] Alternatively, novel antigen-binding sites that can bind to OLR1 can be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:401 (OLR1 isoform 1), SEQ ID NO:402 (OLR1 isoform 2), or SEQ ID NO:403 (OLR1 isoform 3).

5 SEQ ID NO:401

MTFDDLKIQTVDQPDSEKSNKGKAKGLQFLYSPWWCLAAATLGVLCLGLVVTIMV
LGMQLSQVSDLLTQEQANLTHQKKKLEGQISARQQAEEASQESNELKEMIETLARK
LNEKSKEQMELHHQNLNLQETLKRVANCSAPCPQDWIWHGENCYLFSSGSFNWEKS
QEKCLSLDAKLLKINSTADLDFIQQAISYSSFPFWMGLSRRNPSYPWLWEDGSPLMP
10 HLFVRVGAVSQTYPSGTCAIYIRGAVYAENCILAAFSICQKKANLRAQ

SEQ ID NO:402

MTFDDLKIQTVDQPDSEKSNKGKAKGLQFLYSPWWCLAAATLGVLCLGLVVTIMV
LGMQLSQVSDLLTQEQANLTHQKKKLEGQISARQQAEEASQESNELKEMIETLARK
LNEKSKEQMELHHQNLNLQETLKRVANCSGLHPASNFLFQFSILDGAVSEEPQLPMA
15 LGGRFSFDAPLI

SEQ ID NO:403

MTFDDLKIQTVDQPDSEKSNKGKAKGLQFLYSPWWCLAAATLGVLCLGLVVTIMV
LGMQLSQVSDLLTQEQANLTHQKKKLEGQISARQQAEEASQESNELKEMIETLARK
LNEKSKEQMELHHQNLNLQETLKRVANCSAPCPQDWIWHGENCYLFSSGSFNWEKS
20 QEKCLSLDAKLLKINSTADLI

[0224] In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-associated antigen ADAM12. Exemplary monoclonal antibodies that bind to

ADAM12 can be found in US Patent Publication No. 20160208016, and produced by the hybridoma cell lines 7B8 and 8F8.

[0225] Alternatively, novel antigen-binding sites that can bind to ADAM12 can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:404.

5 SEQ ID NO:404

MAARPLPVSPARALLLALAGALLAPCEARGVSLWNQGRADEVVSASVSGDLWIPV
 KSFDSKNHPEVLNIRLQRESKELIINLERNEGLIASSFTETHYLQDGTDVSLARNYTVI
 LGHCYYHGHVIRGYSDSAVSLSTCSGLRGLIVFENESYVLEPMKSATNRYKLFPAKKL
 KSVRGSCGSHHNTPNLAAKNVFPPTSQTWARRHKRETLKATKYVELVIVADNREFQ
 10 RQGKDLEKVKQRLIEIANHVDKFYRPLNIRIVLVGVEVWNDMDKCSVSQDPFTSLHE
 FLDWRKMKLLPRKSHDNAQLVSGVYFQGTIGMAPIMSMCTADQSGGIVMDHSDN
 PLGAAVTLAHELGHNFGMNHDTLDRGCSCQMAVEKGGCINASTGYPFPMVFSSC
 SRKDLETSLEKGMGVCLFNLPEVRESFGGQKCGNRFVEEGEECDCEPEECMNRC
 NATCTLKPDCAVCAHGLCCEDCQLKPAGTACRDSSNSCDLPEFCTGASPHCPANVY
 15 LHDGHSCQDQDGYCYNGICQTHEQQCVTLWGPGAKPAPGICFERVNSAGDPYGNC
 GKVSKSSFAKCEMRDAKCGKIQCQGGASRPVIGTNAVSIETNIPLQQGGRILCRGTHV
 YLGDDMPDPGLVLAGTKCADGKICLNRCQCNISVFGVHECAMQCHGRGVCNNRKN
 CHCEAHWAPPFCDKFGFGGSTDSGPIRQADNQGLTIGILVTILCLLAAGFVVYLKRKT
 LIRLLFTNKKTIEKLRCVRPSRPPRGFQPCQAHLGHLGKGLMRKPPDSYPPKDNPRR
 20 LLQCQNVDISRPLNGLNVPQPQSTQRVLPPLHRAPRAPSVPARPLPAKPALRQAQGT
 CKPNPPQKPLPADPLARTTRLTHALARTPGQWETGLRLAPLRPAPQYPHQVPRSTHT
 AYIK

[0226] In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-associated antigen PLAUR. Table 15 lists some exemplary sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to PLAUR.

Table 15		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Tactic Pharma ATN-658	EVQLQQSGPELVKTGASVKI SCKASGYSFTSYMHVVKQ	DVVMQTPLTSLVTIGQPAS ISCKSSQSLDSDGKTYLNW

(US Patent No. 8,105,602)	SHGKSLEWIGEINPYNGGAS YNQKIKGRATFTVDTSSRTA YMQFNLSLTSEDSAVYYCARS IYGHSVLDYWGQGTSSVSVSS (SEQ ID NO:405) CDR1(SEQ ID NO:406) - GYSFTSY CDR2 (SEQ ID NO:407) - NPYNGG CDR3 (SEQ ID NO:408) - SIYGHSVLDY	LLQRPQGSPKRLIYLVSKLD SGVPDRFTGSGSGTDFTLKI SRVEAEDLG VYYCWQGTH FPLTFGAGTKLELKR (SEQ ID NO:409) CDR1(SEQ ID NO:410) - QSLDSDGKTYLN CDR2 (SEQ ID NO:411) - LVSKLDS CDR3 (SEQ ID NO:412) - WQGTHFPLT
University of California (US Patent No. 9,029,509)	QVQLQQSGPGLVKPSQTLST TCAISGDSVSSNSAAWNWIR QSPSRGLEWLGRITYYRSKW YNDYAVSVKSRIIINPDTSKN QFSLQLNSVTPEDTAVYYCA RDPGGPLDDSFDIWGQGT MTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSL GTQTYICNVNHKPSNTKVDK KVEPKSC (SEQ ID NO:413) CDR1(SEQ ID NO:414) - MQALQTPFT CDR2 (SEQ ID NO:415) - RTYYRSKWYNDYAVSVKS CDR3 (SEQ ID NO:416) - DPGGPLDDSDFI	LDVVMTQSPLSLPVTGPGEPA SISCRSSQSLLRNNGYNYLD WYLQKPGQSPQLLIYLG SIR ASGVPDRFSGSGSGTDFTLR ISRVEAEDVGVYYCMQALQ TPFTFGQGTKLEIKRTVAAP SVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSICD STYSLSSLTLSKADYEKHK VYACEVTHQGLSSPVTKSF NRGEC (SEQ ID NO:417) CDR1(SEQ ID NO:418) - RSSQSLLRNNGYNYLD CDR2 (SEQ ID NO:419) - LGSIRAS CDR3 (SEQ ID NO:420) - MQALQTPFT

[0227] Alternatively, novel antigen-binding sites that can bind to PLAUR can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:421.

SEQ ID NO:421

MGHPPLLPLLLLLHTCVPASWGLRCMQCKTNGDCRVEECALGQDLCRTTIVRLWEE
 5 GEELELVEKSCTHSEKTNRTLSYRTGLKITSLTEVVCGLDLCNQNSGRAVTYSRSR
 YLECISCGSSDMSCERGRHQSLQCRSPEEQCLDVVTHWIEGEEGRPKDDRHLRGCG
 YLPGCPGSNGFHNNDTFHFLKCCNTTKCNEGPILELENLPQNGRQCYSCCKGNSTHGC
 SSEETFLIDCRGPMNQCLVATGTHEPKNQSYMVRGCATASMCQHAHLGDAFSMNHI
 DVSCCTKSGCNHPDLVDVQYRSGAAPQPGPAHLSLTITLLMTARLWGGTLLWT

- 10 **[0228]** In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-associated antigen CCR6. Table 16 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to CCR6.

Table 16		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Glenmark (US Patent Publication No. 20180086836)	EVQLVESGGGLVQPGGSLRL SCAASGFSFSDYYMYWVRQ APGKGLEWVSYITNGGITYY PDSVKGRFTISRDN SKNTLY LQMNSLRAEDTAVYYCTSP L RGAWFAYWGQGTLVTVSS (SEQ ID NO:422) CDR1(SEQ ID NO:423) - GFSFSDYYMY CDR2 (SEQ ID NO:424) - YITNGGITYYPDSVKG CDR3 (SEQ ID NO:425) - TSPLRGAWFAY	DVVMTQSPLSLPVTLGQPASIS CRSSQSIVHSNGNTYLEWYQQ RPGQSPRLLIYKVSNRFS GVDP RFSGSGSGTDFTLKISRVEAED VGVYYCFQGSHVPLTFGQGT KLEIK (SEQ ID NO:426) CDR1(SEQ ID NO:427) - QSIVHSNGNTYLE CDR2 (SEQ ID NO:428) - KVS NRFS CDR3 (SEQ ID NO:429) - FQGSHVPLT

MSM Protein Technologies (US Patent Publication No. 20150337037)	EVQLLES GGGLVQPGGSLRL SCAASGFTFSSYAMSWVRQ APGKGLEWVSAISGSGGSTY YADSVKGRFTISRDN SKNTL YLQMNSLR AEDTAMYYCAR HGYSATRQGLDYWGQGTLV TVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKR VEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTP PVLDS DGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK (SEQ ID NO:430) CDR1(SEQ ID NO:431) - FTFSSYAMSWVR CDR2 (SEQ ID NO:432) - VSAISGSGGSTYYADS CDR3 (SEQ ID NO:433) - CARHGYSATRQGLDYWGQ	EIVLTQSPGTL SLSPGERATLS CRASQSVSSSYLA WYQQKPG QAPRL LIYGASSRATGIPDRFS GSGSGTDFTLTISRLEPEDFAV YYCQQYSSSPLTFGQGTKVEI KRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSK DSTYSLSS TLTL SKADYEKHK VYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO:434) CDR1(SEQ ID NO:435) - RASQSVSSSYLA CDR2 (SEQ ID NO:436) - GASSRAT CDR3 (SEQ ID NO:437) - CQQYSSSPLTFGQ
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[0229] Alternatively, novel antigen-binding sites that can bind to CCR6 can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:438.

SEQ ID NO:438

MSGESMNFSDVFDSSDYFVSVNTSYYSVDSEMLLC SLQEV RQFSRLFVPIAYSLICV
 FGLLGNILVVITFAFYKKARSMTDVYLLNMAIADILFVLTLPFWAVSHATGAWVFSN
 ATCKLLKGIYAINFNCGMLLLTCISMDRYIAIVQATKSFRLRSRTLPRSKIICLVVWGL
 5 SVIISSTFVFNQKYNTQGS DVCEPKYQTVSEPIRWKLLMLGLELLFGFFIPLMFMIFC
 YTFIVKTLVQAQNSKRHKAIRVIIAVVLVFLACQIPHNMVLLVTAANLGKMNRSCQS
 EKLIGYTKTVTEVLAFLHCCLNPVLYAFIGQKFRNYFLKILKDLWCVRRKYKSSGFS
 CAGRYSENISRQTSETADNDNASSFTM

10 [0230] In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the tumor-associated antigen EPHA4. Table 17 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to EPHA4.

Table 17		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
OncoTherapy Science, Inc. (US Patent No. 8,003,098)	QVQLVQSGAEVKKPGAS VKVSCKVSGYTLTELSM HWVRQAPGKGLEWMGG FDPEDGETIYAQKFQGRV TMTEDTSTD TAYMELSSL RSED TAVYYCATAQPFH WGDDAFDIWGQGTMTV VSS (SEQ ID NO:439) CDR1(SEQ ID NO:440) - ELSMH CDR2 (SEQ ID NO:441) - GFDPEDGETIYAQKFQG CDR3 (SEQ ID NO:442) - AQPFWGDDAFDI	QSVLTQPPSASGTPGQRTISCS GSSSNIGSNTVNWYQPPGTAP KLLIYSNNQRPSGVPDRFSGSK SGTSA SLAISGLQSEDEADYYC AAWDDSLNGPVFGGGTKLTVL G (SEQ ID NO:443) CDR1(SEQ ID NO:444) - SGSSSNIGSNTVN CDR2 (SEQ ID NO:445) - SNNQRPS CDR3 (SEQ ID NO:446) - AAWDDSLNGPV

HKUST (US Patent Publication No. 20170218075)	QVQLQQSGAEVKKPGSS VKVSKAS GYTFTGYY MHWVRQAPGQGLEWM GGIPIFGTANYAQKFQG RVTITADKSTSTAYMELS SLRSEDNAVYYCATAPM VCSSTSCYLRGFDYWGQ GTLVTVSS (SEQ ID NO:447) CDR1(SEQ ID NO:448) - GYTFTGYY CDR2 (SEQ ID NO:449) - IPIFGTA CDR3 (SEQ ID NO:450) - ATAPMVCSSTSCYLRGFD Y	DVVMTQSPLSLPVTGPGEASISC RSSQSLLHSNGYNYLDWYLQK PGQSPQLLIYLGSNRASGVPDR FSGSGSGTDFTLKISRVEAEDV GVYYCMQALQTPITFGQGTRL EIK (SEQ ID NO:451) CDR1(SEQ ID NO:452) - QSLLHSNGYNY CDR2 (SEQ ID NO:453) - LGS CDR3 (SEQ ID NO:454) - MQALQTP
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[0231] Alternatively, novel antigen-binding sites that can bind to EPHA4 can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:455.

SEQ ID NO:455

MAGIFYFALFSCFLFGICDAVTGSRVYPANEVTLLDSRSVQGELGWIASPLEGGWEEV
5 SIMDEKNTPIRTYQVCNVMEPSQNNWLRDWTREGAQRVYIEIKFTLRDCNSLPGV
MGTCKETFNLYYESDNDKERFIRENQFVKIDTIAADESFTQVDIGDRIMKLNTEIRD
VGPLSKKGFYLAQDVGACIALVSVRVFYKKCPLTVRNLAQFPDTITGADTSSLVEV
RGSCVNNSEEKDVPKMYCGADGEWLVPIGNCLCNAGHEERSGECQACKIGYYKAL
STDATCAKCPPHSYSVWEGATSCTCDRGFFRADNDAASMPCTRPPSAPLNLISNVNE
10 TSVNLEWSSPQNTGGRQDISYNVCKKCGAGDPSKCRPCGSGVHYTPQQNGLKTTK
VSITDLLAHTNYTFEIWAVNGVSKYNPNPDQSVSVTVTTNQAAPSSIALVQAKEVTR
YSVALAWLEPDRPNGVILEYEVKYYEKDQNERSYRIVRTAARNTDIKGLNPLTSYVF
HVRARTAAGYGDFSEPLEVTNTVPSRIIGDGANSTVLLVSVSGSVVLVVILIAAFVIS
RRRSKYSKAKQEADEEKHLNQGVRTYVDPFTYEDPNQAVREFAKEIDASCIKIEKVI
15 GVGEFGEVCSGRLKVPKGREICVAIKTLKAGYTDKQRRDFLSEASIMGQFDHPNIIHL

EGVVTCKCPVMIITEYMENGSLDAFLRKNDGRFTVIQLVGMLRGIGSGMKYLS
 YVHRDLAARNILVNSNLVCKVSDFGMSRVLEDDPEAAAYTTRGGKIPRWTAPEAIAY
 RKFTSASDVWSYGIVMWEVMSYGERPYWDMSNQDVIKAIEEGYRLPPPMDCPIALH
 QLMLDCWQKERSDRPKFGQIVNMLDKLIRNPNSLKRTGTESSRPNTALLDPSSPEFSA
 5 VVSVGDWLQAIKMDRYKDNFTAAGYTTLEAVVHVNQEDLARIGITAITHQNKILSSV
 QAMRTQMQQMHGRMPV

[0232] In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the antigen CD14. Exemplary sequences of a heavy chain variable domain and a light chain variable domain that, in combination, bind to CD14 are listed in the US patent publication NO. 20170107294. In certain embodiments of the present invention, the heavy chain variable domain can be at least 95% identical to SEQ ID NO:456, and/or include amino acid sequences identical to the CDR1 (SEQ ID NO:457), CDR2 (SEQ ID NO:458), and CDR3 (SEQ ID NO:459) sequences of SEQ ID NO:456, and the light chain variable domain can be at least 95% identical to SEQ ID NO:460, and/or include amino acid sequences identical to the CDR1 (SEQ ID NO:461), CDR2 (SEQ ID NO:462), and CDR3 (SEQ ID NO:463) sequences of SEQ ID NO:460. Table 18 lists some exemplary peptide sequences of heavy chain variable domains and CDRs, and light chain variable domains and CDRs that, in combination, can bind to CD14.

Table 18		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Nordlandssykehuset Hf (US Patent Publication No. 20170107294)	MGWSCILFLVATATGVH SNIVLTQSPASLAVSLGQ RATISCRASESVDSYGNS FMHWYQQKPGQPPKLLI YLASNLESGVPARFSGSG SRTDFTLTIDPVEADDVA TYYCQQNNGDPYTFGGG TKLEIIR (SEQ ID NO:456)	MGWSCILFLVATATGVHSEVQ LVESGGGLMQPKGSLKLSCAA SGFTFKTYALNWVRQAPGTGL EWVARIRSKSNNYTTYADSV KDRFTISRDDSQNMLYLQMNN LKTEDTAMYYCVRPQSGTSFA YWGQGTLLTVSAA (SEQ ID NO:460)

	CDR1(SEQ ID NO:457) - RASESVDSYGNSFM CDR2 (SEQ ID NO:458) - LASNLES CDR3 (SEQ ID NO:459) - QQNNGDPYT	CDR1(SEQ ID NO:461) - GFTFKTYALN CDR2 (SEQ ID NO:462) - RIRSKSNNYTTYADSVK CDR3 (SEQ ID NO:463) - PQSGTSFAY
Mochida Seiyaku (US Patent Publication No. 20150239982)	CDR1(SEQ ID NO:464) - RYTMG CDR2 (SEQ ID NO:465) - IINSGATYYASWAKG CDR3 (SEQ ID NO:466) - GDA	CDR1(SEQ ID NO:467) - QASQSIGSNLA CDR2 (SEQ ID NO:468) - KASKLAS CDR3 (SEQ ID NO:469) - QCSYTAIGNYGHV

[0233] Alternatively, novel antigen-binding sites that bind to CD14 can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:470.

SEQ ID NO:470

MERASCLLLLLLPLVHVSATTPEPCELDDEDFRCVCNFSEPQPDWSEAFQCVSAVEV
5 EIHAGGLNLEPFLKRVDADADPRQYADTVKALRVRRLTVGAAQVPAQLLVGALRV
LAYSRLKELTLEDLKITGTMPPLEATGLALSSLRLRNVSWATGRSWLAELQQWLK
PGLKVLZIAQAHSPAFCSEQVRAFPALTSLDLSDNPGLGERGLMAALCPHKFPAIQNL
ALRNTGMETPTGVCAALAAAGVQPHSLDLSHNSLRATVNPSAPRCMWSSALNSLNL
SFAGLEQVPKGLPAKLRVLDLSCNRLNRAPQPDELPEVDNLTLDGNPFLVPGTALPH
10 EGSMNSGVVPACARSTLSVGVSGTLVLLQGARGFA

[0234] In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the antigen CD163. Table 19 lists some exemplary sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to CD163.

Table 19		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Cytoguide AS (US Patent Publication No. 20170119790)	QVQLQESGPGGLVKPSETLS LTCTVSGYSITSDYAWN IRQFPGKKLEWMGSIYYS GSTYYNPSLKSRTISVDT SKNQFSLKLSSVTAADTA TYYCVSGTYYFDYWGG TTLTVSS (SEQ ID NO:471) CDR1(SEQ ID NO:472) - GYSITSDY CDR2 (SEQ ID NO:473) - YSG CDR3 (SEQ ID NO:474) - CVSGTYYFDYWG	DIVMTQSPSSLSASVGDRVITCR ASQSVSSDVAWFQQKPGKSPKPL IYYASNRYSGVPSRFSGSGSGTDF TLTISSLQAEDFAVYFCGQDYTSP RTFGGGTKLEIKRA (SEQ ID NO:475) CDR1(SEQ ID NO:476) - ASQSVSSDV CDR2 (SEQ ID NO:477) - YAS CDR3 (SEQ ID NO:478) - QDYTSPRT
Cytoguide AS (US Patent Publication No. 20170119790)	DVQLQESGPGGLVKPSQSL SLTCTVTGYSITSDYAWN WIRQFPGNKLEWMGYITY SGITNYPNPSLKSQISITRDT SKNQFFLQLNSVTTEDTA TYYCVSGTYYFDYWGG TTLTVSS (SEQ ID NO:479) CDR1(SEQ ID NO:480) - GYSITSDY CDR2 (SEQ ID NO:481) - YSG CDR3 (SEQ ID NO:482) - YYFDYWG	SVVMTQTPKSLISIGDRVITICK ASQSVSSDVAWFQQKPGQSPKPL IYYASNRYTGVPDRFTGSGYGTD FTFTISSVQAEDLAVYFCGQDYT SPRTFGGGTKLEIKR (SEQ ID NO:483) CDR1(SEQ ID NO:484) - ASQSVSSDV CDR2 (SEQ ID NO:485) - YAS CDR3 (SEQ ID NO:486) - QDYTSPRT

[0235] Alternatively, novel antigen-binding sites that can bind to CD163 can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:487.

SEQ ID NO:487

MSKLRMVLLLED SG SADFRRHFVNLS PFTITV LLLSACFVTSSLGGTDKELRLVDGE
 5 NKCSGRVEVKVQEEWGTVCNNGWSMEAVSVICNQLGCPTAIKAPGWANSSAGSGR
 IWMDHVSCRGNESALWDCKHDGWGKHSNCTHQQDAGVTCSDGSNLEMRLTRGGN
 MCSGRIEIKFQGRWGTVCDDNFNIDHASVICRQLECGSAVSFSGSSNFGEGSGPIWFD
 DLICNGNESALWNCKHQGWGKHNCDAEDAGVICSKGADLSRLVDGVTECSGRL
 EVRFQGEWGTICDDGWDSYDAAVACKQLGCPTAVTAIGRVNASKGFGHIWLDVS
 10 CQGHEPAIWQCKHHEWGKHHCNHNEDAGVTCSDGSDLELRLRGGGSR CAGTVEVE
 IQRLLGKVC DRGWGLKEADV VCRQLGCGSALKTSYQVYSKIQATNTWFLSSCNGN
 ETSLWDCKNWQWGGLTCDHYEEAKITCSAHREPRLVGGDIPCSGRVEVKHGD TWG
 SICDSDFSLEAASVLCRELQCGTVVSILGGAHFGEGNGQIWAE EFQCEGHESHLSLCP
 VAPRPEGTCSHSRDVG VVCSRYTEIRLVNGKTPCEGRVELKTLGAWGSLCNSHWDIE
 15 DAHVLCQQLKCGVALSTPGGARFGKGNGQIWRHMFHCTGTEQHMGDCPVTALGAS
 LCPSEQVASVICSGNQSQTLSSCNSSSLGPTRPTIPEESAVACIESGQLRLVNGGGRC A
 GRVEIYHEGSWGTICDDSWDLSDAHVVCRQLGCGEAINATGSAHFGE GTGPIWLDE
 MKCNGKESRIWQCHSHGWGQQNCRHKEDAGVICSEFMSLRLTSEASREACAGRLE
 VFYNGAWGTVGKSSMSETTVGVVCRQLGCADKGKINPASLDKAMSIPMWVDNVQ
 20 CPKGPDTLWQCPSSPWEKRLASPSEETWITCDNKIRLQEGPTSCSGRVEIWHGGSWG
 TVCDDSWDLDDAQVVCQQLGCGPALKAFKEAEFGQGTGPIWLNEVKCKGNESSLW
 DCPARRWGHSECGHKEDAAVNCTDISVQKTPQKATTGRSSRQSSFI AVGILGVVLLA
 IFVALFFLTKKRRQRQLAVSSRGENLVHQIQYREMNSCLNADDLDLMNSSENSHES
 ADFSAAELISVSKFLPISGMEKEAILSHTTEKENG NL

25 **[0236]** In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the antigen CSF3R. Table 20 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to CSF3R.

Table 20		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
CSL Ltd. (US Patent Publication No. 20170226214)	EVQLLES GGGLVQP GGSRLR SCAASGFTFSLYWMGWVRQ APGKGLEWVSSISSSGGVTP YADSVKGRFTISRDN SKNTL YLQMNSLRAEDTAVYYCAM LGELGWFD PWGQGLTVTS S (SEQ ID NO:488) CDR1(SEQ ID NO:489) - LYWMG CDR2 (SEQ ID NO:490) - SISSSGGVTPYADSVKG CDR3 (SEQ ID NO:491) - LGELGWFD P	DIQMTQSPSALSASVGDRVIT CRASQGISSYLNWYQQKPGK APKLLIYYASNLQNGIPSRFSG SGSGTDFTLTISSLQPEDFATY HCQQSYSTPLTFGGGTNVEIR (SEQ ID NO:492) CDR1(SEQ ID NO:493) - RASQGISSYLN CDR2 (SEQ ID NO:494) - ASNLQN CDR3 (SEQ ID NO:495) - QQSYSTPLT

[0237] Alternatively, novel antigen-binding sites that can bind to CSF3R can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:496. SEQ ID NO:496

MARLGNC SLTWAALIILLPGSLEECGHISVSAPIVHLGDPITASCIKQNC SHLDPEPQ
 5 ILWRLGAELQPGGRQQRLSDGTQESIITLPHLNHTQAFLSCCLNWGNSLQILDQVELR
 AGYPPAIPHNLSCLMNLTTSSLICQWEPGPETHLPTSFTLKSFKSRGNCQTQGDSILDC
 VPKDGQSHCCIPRKHLLLYQNMGIWVQAENALGTSMSPQLCLDPM DVVKLEPPMLR
 TMDPSPEAAPPQAGCLQLCWEPWQPGLHINQKCELRHKPQRGEASWALVGPLPLEA
 LQYELCGLLPATAYTLQIRCIRWPLPGHWS DWSPSLELR TTERAPT VRLDTWWRQR
 10 QLDPRTVQLFWKVPLEEDSGRIQGYV VSWRPSGQAGAILPLCNTTEL SCTFHLPSEA
 QEVALVAYNSAGTSRPTPVVFSESRGPALTRLHAMARDPHSLWVGWEPPNPWPQG
 YVIEWGLGPPSASNSNKTWRMEQNGRATGFLLENIRPFQLYEIIVTPLYQDTMGPS
 QHVVAYSQEMAPSHAPELHLKHIGKTWAQLEWVPEPPELGKSPLTHYTIFWTNAQN
 QSFSAILNASSRGFVLHGLEPASLYHIHLMAASQAGATNSTVLTLMTLTPEGSELHIL

GLFGLLLLLTCLCGTAWLCCSPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPG
 LGTPPITKLTVLEEDEKKPVPWESHNSSETCGLPTLVQTYVLQGDPRVSTQPQSQSG
 TSDQVLYGQLLGSPTSPGPGHYLRCDSTQPLLAGLTPSPKSYENLWFQASPLGTLVTP
 APSQEDDCVFGPLLNFPLLQGIRVHGMEALGSF

- 5 **[0238]** In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the antigen Siglec-9. Table 21 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to Siglec-9.

Table 21		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Innate Pharma (PCT Publication No. WO2017153433)	DVQLQESGPGLVKPSQSL SLTCTVTGYSITGGFAWN WIRQFPGNTLEWMGYIGY GGSTSYNPSLNSRISITRDT SKNHFFLQFNSVTDDSA TYYCARGDYLFAYWGQG TLVTVSA (SEQ ID NO:497) CDR1 (SEQ ID NO:498) - GGFAWN CDR2 (SEQ ID NO:499) - YIGYGGSTSYNPSLNS CDR3 (SEQ ID NO:500) - GDYLFAY	DIVMTQSHKFMSTSVGDRVSI TCKASQDVNTAVAWYQQKPG QSPKLLIYSASYRYTGVDPDRFT GSGSGTDFTFTISSVQAEDLAV YYCQQHYSTPRTFGGGTKLEI K (SEQ ID NO:501) CDR1(SEQ ID NO:502) - KASQDVNTAVA CDR2 (SEQ ID NO:503) - SASYRYT CDR3 (SEQ ID NO:504) - QQHYSTPRT
Alector LLC (PCT Publication No. WO2017075432)	EVKLVESGGALVKPGGSL KLSCAASGFTFSNYAMSW VRQTPEKRLEWVATINNG GSYTYYSDSVKGRFAISR DNAKNTLYLQMSNLRSE	DIQMTQSPSSLSASLGERVSLT CRASQEISGYLGWLQKPDGT IKRLIFSTSTLDSGVPKRFSGSR SGSDYSLTISSESEDFADYYC LQYASYPPTFGGGTKLEIK

	DTALYYCVRRDYGTSDF DYWGQGTTTLTVSS (SEQ ID NO:505) CDR1(SEQ ID NO:506) - FTFSNYAMS CDR2 (SEQ ID NO:507) - VATINNGGSYTYYS CDR3 (SEQ ID NO:508) - VRRDYGTSDFDY	(SEQ ID NO:509) CDR1(SEQ ID NO:510) - RASQEISGYLG CDR2 (SEQ ID NO:511) - STSTLDS CDR3 (SEQ ID NO:512) - LQYASYPPT
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[0239] Alternatively, novel antigen-binding sites that can bind to Siglec-9 can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:513.

SEQ ID NO:513

MLLLLLPLLWGRERAEGQTSKLLTMQSSVTVQEGLCVHVPCSFYPSHGWYIPGPVV
5 HGYWFREGANTDQDAPVATNNPARAVWEETDRFHLGDPHTKNCTLSIRDARRS
DAGRYFFRMEKGSIKWNYKHHRLSVNVTALTHRPNILIPGTLESGCPQNLTCSPWA
CEQGTPPMISWIGTSVSPLDPSTTRSSVLTLIPQPQDHGTS LTCQVTFPGASVTTNKTV
HLNVSYPQNLMTVFQGDGTSTVLGNGSSLSLPEGQSLRLVCAVDAVDSNPPARL
SLSWRGLTLCPSQPSNPGVLELPWVHLRDAAEFTCRAQNPLGSQQVYLVNLSLQSKA
10 TSGVTQGVVGGAGATALVFLSFCVIFVVVRSCRKKSARPAAGVGDGTGIEDANAVRG
SASQILNHFIFGPTFLGLGFEFLNLRDLCCHPDSEFYVYHFHFRLIKNIAGEIVWSLE
GKILWLLDVSDFFHWFFLICVG

[0240] In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the
15 antigen ITGAM. Table 22 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to ITGAM.

Table 22		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
The General Hospital Corporation (PCT Publication No. WO2016138538)	VQLVESGGGLVQPGGSLR LSCAASGFNIKDTYIHWV RQAPGKGLEWVARIDPAN DKTRYADSVKGRFTISAD TSKNTAYLQMNSLRAEDT AVYYCSSEGHYGYDGYA MDYWGQGTLLTVSS (SEQ ID NO:514) CDR1(SEQ ID NO:515) - GFNIKD CDR2 (SEQ ID NO:516) - DPANDK CDR3 (SEQ ID NO:517) - HYGYDGYA	DIVMSQSPDSLAVSLGERVTL NCKSSQLLYSSNQKNYLAWY QKPGQSPKLLIYWASARESG VPDRFSGSGSGTDFTLTISVQ AEDVAVYYCQQYYSYPLTFG AGTKLELK (SEQ ID NO:518) CDR1(SEQ ID NO:519) - YSSNQKNY CDR2 (SEQ ID NO:520) - WAS CDR3 (SEQ ID NO:521) - YYSYPL

[0241] Alternatively, novel antigen-binding sites that can bind to ITGAM can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:522.

SEQ ID NO:522

MALRVLLLTALTLCHGFNLDTENAMTFQENARGFGQSVVQLQGSRVVVGAPQEIVA
 5 ANQRGSLYQCDYSTGSCEPIRLQVPVEAVNMSLGLSLAATTSPQLLACGPTVHQTC
 SENTYVKGLCFLFGSNLRQQPQKFPEALRGCPQEDSDIAFLIDGSGSIIPHDFRRMKEF
 VSTVMEQLKKSKTLFSLMQYSEEFRIHFTFKEFQNNPNPRSLVKPITQLLGRTHATGI
 RKVVRELFNITNGARKNAFKILVVITDGEKFGDPLGYEDVIPEADREGVIRYVIGVGD
 AFRSEKSRQELNTIASKPPRDHVFQVNNFEALKTIQNQLREKIFAIEGTQTGSSSSFEH
 10 EMSQEGFSAAITSNGPLLSTVGSYDWAGGVFLYTSKEKSTFINMTRVDSMDNDAYL
 GYAAAILRNRVQSLVLGAPRYQHIGLVAMFRQNTGMWESNANVKGTQIGAYFGAS
 LCSVDVDSNGSTDLVLIGAPHYYEQTRGGQVSVCPPLRGQRARWQCDAVLYGEQG
 QPWGRFGAALTVLGDVNGDKLTDVAIGAPGEEDNRGAVYLFHGTSGSGISPSHSQRI

AGSKLSPRLQYFGQSLSGGQDLTMDGLVDLTVGAQGHVLLLRSPVLRVKAIMFEN
 PREVARNVFECNDQVVKGKEAGEVRVCLHVQKSTRDRLREGQIQSVVITYDLALDSG
 RPHSRAVFNETKNSTRRQTQVLGLTQTCETLKLQLPNCIEDPVSPIVLRLNFSLVGTPL
 SAFGNLRPVLAEDAQRLFTALFPFEKNCGNDNICQDDLSITFSFMSLDCLVVGGPREF
 5 NVTVTVRNDGEDSYRTQVTFFFPLDLSYRKVSTLQNQRSQRSWRLACESASSTEVSG
 ALKSTSCSINHPIFPENSEVTFNITFDVDSKASLGNKLLKANVTSENNMPRTNKTEFQ
 LELPVKYAVYMMVVTSHGVSTKYLNFTASENTSRVMQHQYQVSNLGQRSLPISLVFL
 VPVRLNQTVIWDPRQVTFSENLSSTCHTKERLPSSHDFLAELRKAPVVNCSIAVCQRI
 QCDIPFFGIQEEFNATLKGNL SFDWYIKTSHNHLLIVSTAEILFNDSVFTLLPGQGAFV
 10 RSQTETKVEPFEPNPLPLIVGSSVGGLLLLALITAALYKLGFFKRQYKDMMSSEGGPP
 GAEPQ

[0242] In certain embodiments, the present disclosure provides multi-specific binding
 proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the
 antigen CCR1. Table 23 lists some exemplary peptide sequences of heavy chain variable
 15 domains and light chain variable domains that, in combination, can bind to CCR1.

Table 23		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
(PCT Publication No. WO2017126587)	DVKLVESGEGLVKPGGS LKLSCAASGFTFSRNAMS WVRQTPEKRLEWVAYIS SGSDYIYYADTVKGRFTV SRDNARNTLYLQMTSLR SEDAMYFCTRFSYGYG KNAPDYWGQGTSVTVSS (SEQ ID NO:523) CDR1(SEQ ID NO:524) - RNAMS CDR2 (SEQ ID NO:525) - YISSGSDYIYYADTVK	DIQMTQSPSSLSASLGGKVTIT CKASQDINKYIAWYQHKGPGQ GPRLLIHYTSSLQPGIPSRFSGS GSGRDYSFSISNLEPEDIATYY CLQYDYTMTFGGGGTKLEIR (SEQ ID NO:527) CDR1(SEQ ID NO:528) - KASQDINKYIA CDR2 (SEQ ID NO:529) - YTSSLQP CDR3 (SEQ ID NO:530) - LQYDYTMT

	CDR3 (SEQ ID NO:526) - FSYGYGKNAPDY	
(PCT Publication No. WO2017126587)	QVQLKQSGPGLVQPSQSL SITCTVSGFSLNNYGVHW VRQPPGKGLEWLGVIWS AGTTVYNAAFISRLSISKD DSKSQVFFKMNSLQAGD TAIYYCAKDGSRYYTAM DYWGQGTSVTVSS (SEQ ID NO:531) CDR1(SEQ ID NO:532) - NYGVH CDR2 (SEQ ID NO:533) - VIWSAGTTVYNAAFIS CDR3 (SEQ ID NO:534) - DGSRYYTAMDY	DVVMQTQTPRSLPVSLGDQASI SCRSRQSLIHSNGITFLHWYLQ KAGQSPKLLIYKVSNRFSGVP DRFSGSGSGTDFTLRISRVEAE DLGVYFCSQGTHVPPTFGGGT KLEIK (SEQ ID NO:535) CDR1(SEQ ID NO:536) - RSRQSLIHSNGITFLH CDR2 (SEQ ID NO:537) - KVSNRFS CDR3 (SEQ ID NO:538) - SQGTHVPPT

[0243] Alternatively, novel antigen-binding sites that can bind to CCR1 can be identified by screening for binding to the amino acid sequence defined by SEQ ID NO:539.

SEQ ID NO:539

5 METPNTTEDYDTTTEFDYGDATPCQKVNERAFGAQLLPPLYSLVFVIGLVGNILVVL
VLVQYKRLKNMTSIYLLNLAISDLLFLFTLPFWIDYKLKDDWVFGDAMCKILSGFY
TGLYSEIFFIILLTIDRYLAIVHAVFALRARTVTFGVITSIIIWALAILASMPGLYFSKTQ
WEFTHHTCSLHFPHESLREWKLQALKLNLFGLVLPLLVMIICTGIIKILLRRPNEKK
SKAVRLIFVIMIIFFLFWTPYNLTILISVFQDFLFTHECEQSRHLDLAVQVTEVIAYTHC
10 CVNPVIYAFVGERFRKYLRQLFHRRVAVHLVKWLPFLSVDRLERVSSTSPSTGEHEL
SAGF

[0244] In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the antigen LRRC25. Antigen-binding sites that can bind to LRRC25 can be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:540.

SEQ ID NO:540

MGGTLAWTLLLPLLLRESDSLEPSCTVSSADVDWNAEFSATCLNFSGLSLSLPHNQS
 LRASNVILLDLSGNGLRELPTFFAHLQKLEVLNVLRNPLSRVDGALAARCDLQDLQA
 DCNCALESWHDIRRDNC SGQKPLLCWDTTSSQHNLSAFLEVSCAPGLASATIGAVVV
 5 SGCLLLGLAIAGPVLAWRLWRCRVARSRLENKPWAAQDGPKPGLGLQPRYGSRSAP
 KPQVAVPSCPSTPDYENMFVGQPAAEHQWDEQGAHPSEDNDFYINYKDIDLASQPV
 YCNLQSLGQAPMDEEEYVIPGH

[0245] In certain embodiments, the present disclosure provides multi-specific binding
 proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the
 10 antigen PTAFR. The antigen-binding sites that can bind to PTAFR can be identified, for
 example, by screening for binding to the amino acid sequence defined by SEQ ID NO:541.

SEQ ID NO:541

MEPHDSSHMDSEFRYTLFPIVYSIIFVLGVIANGYVLWVFARLYPCKKFNEIKIFMVN
 LTMADMLFLITLPLWIVYYQNQGNWILPKFLCNVAGCLFFINTYCSVAFLGVITYNRF
 15 QAVTRPIKTAQANTRKRGISLSLVIWVAIVGAASYFLILDSTNTVPDSAGSGNVTRCF
 EHYEKGSPVPLIIHIFIVFSFFLVFLIILFCNLVIIRTLLMQPVQQQRNAEVKRRALWMV
 CTVLAVFIICFVPHHVQLPWTLAELGFQDSKFHQAINDAHQVTLCLLSTNCVLDPVI
 YCFLTKKFRKHLTEKFYSMRSSRKCSRATTDTVTEVVVPFNQIPGNSLKN

[0246] In certain embodiments, the present disclosure provides multi-specific binding
 20 proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the
 antigen SIRPB1. The antigen-binding sites that can bind to SIRPB1 can be identified, for
 example, by screening for binding to the amino acid sequence defined by SEQ ID NO:542
 (SIRPB1 isoform 1), or SEQ ID NO:543 (SIRPB1 isoform 3)

SEQ ID NO:542

MPVPASWPHLPSPFLLMTLLLGRLTG VAGED ELQVIQPEKSVSVAAGESATLRCAMT
 SLIPVGPIMWFRGAGAGRELIYNQKEGHFPRVTTVSELTKRNNLDFSISISNITPADAG
 TYYCVKFRKGSPPDVEFKSGAGTELSVR AKPSAPVVSGPAVRATPEHTVSFTCESHG
 FSPRDITLKWFKNGNELSDFQTNVDPAGDSVSYSIHSTARVVLTRGDVHSQVICEIAH
 ITLQGDPLRG TANLSEAIRVPPTLEV TQQPMRAENQANVTCQVS NFYPRGLQLTWLE
 30 NGNVSRTETASTLIENKDGTYNWMSWLLVNTCAHRDDVVLTCQVEHDGQQAVSKS
 YALEISAHQKEHGSDITHEAALAPTAPLLVALLLGPKLLL VVGVS AIYICWKQKA

SEQ ID NO:543

MPVPASWPHLPSPFLLMTLLLGRLTGVAGEEELQVIQPDKSISVAAGESATLHCTVTS
 LIPVGPIQWFRGAGPGRELIYNQKEGHFPRVTTVSDLTKRNNMDFSIRISNITPADAGT
 YYCVKFRKGSPDHVEFKSGAGTELSVRAKPSAPVVS GPAARATPQHTVSFTCESHGF
 5 SPRDITLKWFKNGNELSDFQTNVDPAGDSVSYSIHSTAKVVLTREDVHSQVICEVAH
 VTLQGDPLRGTANLSETIRVPPTLEVTTQQPVRAENQVNVTCQVRKFYPQRLQLTWLE
 NGNVSRTETASTLTENKDGTYNWMWSLLVNVSAHRDDVKLTCQVEHDGQPAVSKS
 HDLKVSAHPKEQGSNTAPGPALASAAPLLIAFLLGPKVLLVVGVSVIYVYWKQKA

[0247] In certain embodiments, the present disclosure provides multi-specific binding
 10 proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the
 antigen TLR2. Table 24 lists some exemplary peptide sequences of heavy chain variable
 domains and light chain variable domains that, in combination, can bind to TLR2.

Table 24		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Amgen (US Patent No. 9,458,234)	MSSPQSLKTLILTMGWSWIF LFLLSGTAGVHSQVQLQQS GPELVNPGASVKLSCKASG FTFTTYGINWVKQGPQGGL EWIGWIYPRDGSTNFNENF KDKAALTVDTSSSTAYMEL HSLTSEDSAVYFCARLTGG TFLDYWGQGTTTLTVSSAKT TPPSVYPLAPGSAA (SEQ ID NO:544) CDR1(SEQ ID NO:545) - GFTFTTYG CDR2 (SEQ ID NO:546) - IYPRDGST	MESDTLLLWVLLLWVPGST GDIVLTQSPASLAVSLGQRA TISCRASESVEYYGTSLMQ WYQQKPGQPPLKLLIFGASN VESGVPVRFSGSGSGTDFSL NIHPVEEDDIVMYFCQQSRK LPWTFGGGTKLEIKRADAA PTVSIFPPSSE (SEQ ID NO:548) CDR1(SEQ ID NO:549) - ESVEYYGTS CDR2 (SEQ ID NO:550) - GAS CDR3 (SEQ ID NO:551) - QQSRKLPWT

	CDR3 (SEQ ID NO:547) - ARLTGGTFLDY	
OSPONA Therapeutic (PCT Publication No. WO2011003925)	QVQLVQSGSELKKPGASVK LSCKASGFTFTTYGINWVR QAPGQGLEWIGWIYPRDGS TNFNENFKDRATITVDTSAS TAYMELSSLRSEDNAVYFC ARLTGGTFLDYWGQGTTVT VSSA (SEQ ID NO:552) CDR1(SEQ ID NO:553) - GFTFTTY CDR2 (SEQ ID NO:554) - YPRDGS CDR3 (SEQ ID NO:555) - LTGGTFLDY	DIVLTQSPATLSLSPGERATL SCRASESVEYYGTSLMQWY QQKPGQPPKLLIFGASNVES GVPDRFSGSGSGTDFTLKIS RVEAEDVGMVFCQQSRKLP WTFGGGTKVEIKR (SEQ ID NO:556) CDR1(SEQ ID NO:557) - ESVEYYGTSLMQ CDR2 (SEQ ID NO:558) - GASNVES CDR3 (SEQ ID NO:559) - QQSRKLPWT

[0248] Alternatively, novel antigen-binding sites that can bind to TLR2 can be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:560.

SEQ ID NO:560

5 MPHTLWMVWVLGVIISLSKEESSNQASLSCDRNGICKGSSGSLNSIPSGLTEAVKSLD
LSNNRITYISNSDLQRCVNLQALVLTSNGINTIEEDSFSSLGSLEHLDLSYNYLSNLSSS
WFKPLSSLTFLNLLGNPYKTLGETSLFSLTKLQILRVGNMDTFTKIQRKDFAGLTFL
EELEIDASDLQSYEPKSLKSIQNVSHLILHMKQHILLLEIFVDVTSSVECLELRDLDLD
TFHFSELSTGETNSLIKKFTFRNVKITDESLFQVMKLLNQISGLLELEFDDCTLNGVGN
10 FRASDNDNRVIDPGKVETLTIRRLHIPRFYLFYDLSTLYSLTERVKRITVENSKVFLVPC
LLSQHLKSLEYLDLSENLMVEEYLKNSACEDAWPSLQTLILRQNHLASLEKTGETLL
TLKNLTNIDISKNSFHSMPEQCQWPEKMKYLNLSSTRIHSVTGCIPKTEILDVSNNNL
NLFSNLNPQLKELYISRNKLMTPDASLLPMLLVKISRNAITTFSSKEQLDSFHTLTKTL
EAGGNFICSCEFLSFTQEQQALAKVLIDWPANYLCDSPSHVRGQQVQDVRLSVSEC
15 HRTALVSGMCCALFLLILLTGVLCHRFHGLWYMKMMWAWLQAKRKPRKAPSRNIC

YDAFVSYSERDAYWVENLMVQELENFNPPFKLCLHKRDFIPGKWIIDNIIDSIEKSHK
TVFVLSENFVKSEWCKYELDFSHFRLFDENNDAAAILILLEPIEKKAIPQRFCCKLRKIMN
TKTYLEWPMDEAQREGFWVNLRAAIKS

[0249] In certain embodiments, the present disclosure provides multi-specific binding
5 proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the
antigen TLR4. Table 25 lists some exemplary peptide sequences of heavy chain variable
domains and light chain variable domains that, in combination, can bind to TLR4.

Table 25		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Noviummne SA (US Patent No. 10,023,646)	QVTLKESGPGILQPSQTLSL TCSFSGFSLTTYNIGVGWIR QPSGKGLEWLAHIWWND NIYYNTVLKSRLTFSKDT NNQVFLKIASVDIADTATY YCIRMAEGRYDAMDYWG QGTSVTVSS (SEQ ID NO:561) CDR1(SEQ ID NO:562) - TYNIGVG CDR2 (SEQ ID NO:563) - HIWWNDNIYYNTVLKS CDR3 (SEQ ID NO:564) - MAEGRYDAMDY	AIQMTQSTSSLSASLGDRVTIN CRASQDITNYLNWYQQKPDG TVRLLIYYTSKLHSGAPSRFSG RSGGTDYSLTISNLEQEDIATY FCQQGNTFPWTFGGGTKLEIK R (SEQ ID NO:565) CDR1(SEQ ID NO:566) - RASQDITNYLN CDR2 (SEQ ID NO:567) - YTSKLHS CDR3 (SEQ ID NO:568) - QQGNTFPWT
Astellas (US Patent Publication No. 20170306053)	EVQLVESGGGLVQPGGSL RLSCAASGFTFDTYAMHW VRQAPGKGLEWVAGISWN SGNIGYADSVKGRFTISR NSKNTLYLQMNSLRAEDT	DIQMTQSPSSLSASVGDRVTIT CRASQSISSWLAWYQQKPGK APKLLIYKASSLESGVPSRFSG SGSGTDFLTISLQPEDFATY YCQQYSSYSWTFGQGGTKVEIK R

	AVYYCAKDWDNWNLFY WGQGTLLTVSS (SEQ ID NO:569) CDR1(SEQ ID NO:570) - GFTFDTYA CDR2 (SEQ ID NO:571) - ISWNSGNIGYADSVKG CDR3 (SEQ ID NO:572) - DWDNWNLFY	(SEQ ID NO:573) CDR1(SEQ ID NO:574) - RASQSISSWLA CDR2 (SEQ ID NO:575) - KASSLES CDR3 (SEQ ID NO:576) - QQYSSYSWT
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[0250] Alternatively, novel antigen-binding sites that can bind to TLR4 can be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:577.

SEQ ID NO:577

5 MMSASRLAGTLIPAMAFSLCVRPESWEPCVEVVPNITYQCMELNFYKIPDNLPFSTK
NLDLSFNPLRHLGSYSFFSFPELQVLDLSRCEIQTIEDGAYQSLSHLSTLILTGNPIQSL
ALGAFSGLSSLQKLVAVETNLASLENFPIGHLKTLKELNVAHNLIQSFKLPEYFSNLT
NLEHLDLSSNKIQSIYCTDLRVLHQMPLLNLSLDLSLNP MNFIQPGAFKEIRLHKLTLR
NNFDSLNMVKTICQGLAGLEVHRLVLGEFRNEGNLEKFDKSALEGLCNLTIEEFRLA
10 YLDYYLDDIIDLFNCLTNVSSFSLSVSVTIERVKDFSYNFGWQHLELVNCKFGQFPTLK
LKSLKRLTFTSNKGGNAFSEVDLPSLEFLDLSRNGLSFKGCCSQSDFGTTSLKYLDLS
FNGVITMSSNFLGLEQLEHLDLFQHSNLKQMSEFSVFLSLRNLIYLDISHTHTRVAFNGI
FNGLSSLEVLKMAGNSFQENFLPDIFTELRLNLTFLDLSQCQLEQLSPTAFNSLSSLQVL
NMSHNNFFSLDTPYKCLNSLQVLDYSLNHIMTSKKQELQHFPSSLAFLNLTQNDF
15 CTCEHQSFLQWIKDQRQLLEVERMECATPSDKQGMPLVSLNITCQMNKTIIGVSVL
SVLVVSVVAVLVYKFYFHLMLLAGCIKYGRGENIYDAFVIYSSQDEDWVRNELVKN
LEEGVPPFQLCLHYRDFIPGVAIAANIHEGFHKSARKVIVVVSQHFIQSRWCIFEYEIAQ
TWQFLSSRAGIIFIVLQKVEKTLLRQQVELYRLLSRNTYLEWEDSVLGRHIFWRRLRK
ALLDGKSWNPEGTVGTGCNWQEATSI

20 **[0251]** In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the

antigen CD300LB. The antigen-binding sites that can bind to CD300LB can be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:578.

SEQ ID NO:578

MWLPPALLLLSLSGCFSIQGPESVRAPEQGS�TVQCHYKQGWETYIKWWCRGVRW
 5 DTCKILIETRGSEQGEKSDRVSIKDNQKDRTFTVTMEGLRRDDADVWCGIERRGPD
 LGTQVKVIVDPEGAASTASSPTNSNMAVFIGSHKRNHYMLLVFVKVPILLILVTAIL
 WLKGSQRVPEEPGEQPIYMNSEPLTKDMAT

[0252] In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the
 10 antigen ATP1A3. The antigen-binding sites that can bind to ATP1A3 can be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:579 (ATP1A3 isoform 2), or SEQ ID NO:580 (ATP1A3 isoform 3).

SEQ ID NO:579

MGGWEEERNRRATDKKDDKDSPKKNKGKERRDLDDLKKEVAMTEHKMSVEEVCR
 15 KYNTDCVQGLTHSKAQEILARDGPNALTPPPTTPEWVKFCRQLFGGFSILLWIGAILC
 FLAYGIQAGTEDDPSGDNLYLGIVLAAVVIITGCFSSYYQEAKSSKIMESFKNMVPQQA
 LVIREGEKMQVNAEEVVVGDLVEIKGGDRVPADLRIISAHGCKVDNSSLTGESEPQT
 RSPDCTHDNPLETRNITFFSTNCVEGTARGVVVATGDRTVMGRIATLASGLEVGKTP
 20 AIEIEHFIQLITGVAVFLGVSSFILSLILGYTWLEAVIFLIGIIVANVPEGLLATVTVCLTL
 TAKRMARKNCLVKNLEAVETLGSTSTICSDKTGTLTQNRMTVAHMFWDNQIHEAD
 TTEDQSGTSFDKSSHTWVALSHIAGLCNRAVFKGGQDNIPVLKRDVAGDASESALLK
 CIELSSGSVKLMRERNKKVAEIPFNSTNKYQLSIHETEDPNDNRYLLVMKGAPERILD
 RCSTILLQGKEQPLDEEMKEAFQNAYLELGGLGERVLGFCHYYLPEEQFPKGFAFDC
 DDVNFTTDNLCFVGLMSMIDPPRAAVPDAVGKCRSAGIKVIMVTGDHPITAKAIAKG
 25 VGIISEGNETVEDIAARLNIPVSQVNPRDAKACVIHGTDLKDFTSEQIDEILQNHTEIVF
 ARTSPQQKLIIVEGCQRQGAIVAVTGDGVNDSPALKKADIGVAMGIAGSDVSKQAA
 DMILLDDNFASIVTGVEEGRILFDNLKKSIAYTLTSTNIPEITPFLFIMANIPLPLGTITIL
 CIDLGTDMPAISLAYEAAESDIMKRQPRNPRTDKLVNERLISMAYGQIGMIQALGG
 FFSYFVILAENGFLPGNLVGIRLNWDDRTVNDLEDSSYGQQWTYEQRKVVEFTCHTA
 30 FFVSIVVVQWADLIICKTRNSVFQQGMKNKILIFGLFEETALAAFLSYCPGMDVALR
 MYPLKPSWWFCAFPYSFLIFVYDEIRKLILRRNPGGWVEKETYY

SEQ ID NO:580

MGS GGSDSYRIATSQDKKDDKDSPKKNKGKERRDLDDLKKEVAMTEHKMSVEEVC
 RKYNTDCVQGLTHSKAQEILARDGPNALTPPPTTPEWVKFCRQLFGGFSILLWIGAIL
 CFLAYGIQAGTEDDPSGDNLV LGIVLAAVVIITGCF SY YQEAKSSKIMESFKNMVPQQ
 5 ALVIREGEKMQVNAAEEVVVGDLVEIKGGDRVPADLRISAHGCKVDNSSLTGESEPQ
 TRSPDCTHDNPLETRNITFFSTNCVEGTARGVVVATGDRTVMGRIATLASGLEVGKT
 PIAIEIEHFIQLITGVA VFLGV SFFILSLILGYTWLEAVIFLIGHIVANVPEGLLATVTVCL
 TLTA KR MARKNCLVKNLEAVETLGSTSTICSDKTGTLTQNRMTVAHMFWDNQIHEA
 DTTEDQSGTSFDKSSHTWVALSHIAGLCNRAVFKGGQDNIPVLKRDVAGDASESALL
 10 KCIELSSGSVKLMRERNKKVAEIPFNSTNKYQLSIHETEDPNDNRYLLVMKGAPERIL
 DRCSTILLQGKEQPLDEEMKEAFQNAYLELGGLGERVLGFCHYYLP EEQFPKGFAFD
 CDDVNFTTDNL CFVGLMSMIDPPRAAVPDAVGKCRSAGIKVIMVTGDHPITAKAIAK
 GVGIHSEGNETVEDIAARLNIPVSQVNPRDAKACVIHGTDLKDFTSEQIDEILQNHTEIV
 FARTSPQQKLIIVEGCQRQGAIVAVTGDGVNDSPALKKADIGVAMGIAGSDVSKQAA
 15 DMILLDDNFASIVTGVEEGRLIFDNLKKSIA YTLTSNIPEITPFLLFIMANIPLPLGTITIL
 CIDLGTDMPAISLAYEAAESDIMKRQPRNPRTDKLVNERLISMAYGQIGMIQALGG
 FFSYFVILAENGFLPGNLVGIRLNWDDRTVNDLED SYGQQW TYEQRKVVEFTCHTA
 FFVSIVVVQWADLIICKTRRNSVFQQGMKNKILIFGLFEETALAAFLSYCPGMDVALR
 MYPLKPSWWFCAFPYSFLIFVYDEIRKLILRRNPGGWVEKETYY

- 20 **[0253]** In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the antigen CCR5. Table 26 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to CCR5.

Table 26		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
AbbVie (US Patent No. 7,851,600)	MEWSGVFIFLLSVTAGVHS EVQLVESGGGLVKPGGSL RLSCAASGYTFSNYWIGW VRQAPGKGLEWIGDIYPGG NYIRNNEKFKDKTTLSADT SKNTAYLQMNSLKTEDTA	MKLPVRLLVLMFWIPASSDI VMTQSPLSLPVTGPGEPAISCR SSQRLSSYGHTYLHWY LQKP GQSPQLLIYEVS NRFSGVPDRF SGSGSGTDFTLKISRVEAEDVG

	VYYCGSSFGSNYVFAWFT YWGQGLTVTVSS (SEQ ID NO:581) CDR1(SEQ ID NO:582) - GYTFSNYWIG CDR2 (SEQ ID NO:583) - DIYPGGNYIRNNEKFKD CDR3 (SEQ ID NO:584) - SFGSNYVFAWFTY	VYYCSQSTHVPLTFGQGTKVE IK (SEQ ID NO:585) CDR1(SEQ ID NO:586) - RSSQRLSSYGHTYLH CDR2 (SEQ ID NO:587) - EVSNRFS CDR3 (SEQ ID NO:588) - SQSTHVPLT
CYTODYN/ AbbVie (US Patent No. 7,851,600)	MEWSGVFIFLLSVTAGVHS QVQLVQSGPDVKKPGTSM KMSCKTSGYTFSNYWIGW VRQAPGQGLEWIGDIYPGG NYIRNNEKFKDKTTLTADT STSTAYMQLGSLRSEDVAV YYCGSSFGSNYVFAWFTY WGQGLTVTVSS (SEQ ID NO:589) CDR1(SEQ ID NO:590) - GYTFSNYWIG CDR2 (SEQ ID NO:591) - DIYPGGNYIRNNEKFKD CDR3 (SEQ ID NO:592) - SFGSNYVFAWFTY	MKLPVRLLVLMFWIPASSDI VMTQSPLSLPVTGPGEASISCR SSQRLSSYGHTYLHWYLQKP GQSPQLLIYEVSNRFSGVPDRF SGSGSGTDFTLKISRVEAEDVG VYYCSQSTHVPLTFGQGTKVE IK (SEQ ID NO:593) CDR1(SEQ ID NO:594) - RSSQRLSSYGHTYLH CDR2 (SEQ ID NO:595) - EVSNRFS CDR3 (SEQ ID NO:596) - SQSTHVPLT

[0254] Alternatively, novel antigen-binding sites that can bind to CCR5 can be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:597.

SEQ ID NO:597

MDYQVSSPIYDINYYTSEPCQKINVKQIAARLLPPLYSLVFIFGFVGNMLVILILINCKR
 LKSMTDIYLLNLAISDLFFLLTVPFWAHYAAAQWDFGNTMCQLLTGLYFIGFFSGIFF
 IILLTIDRYLAVVHAVFALKARTVTFGVVTSVITWVAVFASLPGIIFTRSQKEGLHYT
 5 CSSHFPYSQYQFWKNFQTLKIVILGLVLPLLVMVICYSGILKTLLRCRNEKKRHRAVR
 LIFTIMIVYFLFWAPYNIVLLLNTFQEFGNLCSSSNRLDQAMQVTETLGMTHCCIN
 PIIYAFVGEKFRNYLLVFFQKHIAKRFCKCCSIFQQEAPERASSVYTRSTGEQEISVGL

[0255] In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the antigen B7-H4 or VTCN1 (V-Set Domain Containing T Cell Activation Inhibitor 1). Table 27 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in combination, can bind to B7-H4.

Table 27		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
Milennium Pharmaceuticals (US Patent No. 8,759,490)	GVQLVESGGGLVQPGGSLR LSCAASGFTISRNDMHWVR QATGKGLEWVSAIGTGGDT YYPGSVKGRFTISRRENAKNS LYLQMNSLRAGDTAVYYC ARGHMTTFGGFIVIGNGMD VWGQGTTVTVSS (SEQ ID NO:598) CDR1: GFTISRN (SEQ ID NO:599) CDR2: GTGGD (SEQ ID NO:600) CDR3: GHMTTFGGFIVIGNGMDV (SEQ ID NO:601)	EIVMTQSPATLSVSPGERATL SCRASQSVRSNLAWYQQKP GQAPRLLIYGASTRATGIPAR FSGSGSGTEFTLTISSLQSEDF AVYSCQQYNNWPWTFGQ GTKVEIKR (SEQ ID NO:602) CDR1: QSVRSNLA (SEQ ID NO:603) CDR2: GASTRAT (SEQ ID NO:604) CDR3: QQYNNWPWT (SEQ ID NO:605)

ER Squibb and Sona LLC (US Patent No. 9,988,453)	QVQLQQWGAGLLKPSETLS LTCAVYGGSFSDYFWTWIR QPPGKGLEWIGEINHSGTTN YNPSLKS RV TISADTSKNQF SRLSSVTAADTAVYYCARL SSWSNWAFEYWGQGLVT VSS (SEQ ID NO:606) CDRH1: DYFWT (SEQ ID NO:607) CDRH2: EINHSGTTN YNPSLKS (SEQ ID NO:608) CDRH3: LSSWSNWAFEY (SEQ ID NO:609)	EIVLTQFPGLSLSPGERATL SCRASQSVSSTYLAWYQQKP GQAPRVLIYGASRRATGIPD RFSGSGSGTDFTLTISRLEPE DFAVYYCQQYGSSPLTFGGG TKVEIK (SEQ ID NO:610) CDRL1: RASQSVSSTYLA (SEQ ID NO:611) CDRL2: GASRRAT (SEQ ID NO:612) CDRL3: QQYGSSPLT (SEQ ID NO:613)
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[0256] Alternatively, novel antigen-binding sites that can bind to B7-H4 can be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:614.

SEQ ID NO:614

5 MASLGQILFWSIISIIILAGAIALIIGFGISGRHSITVTTVASAGNIGEDGILSCTFE
PDIKLSDIVIQWLKEGVLGLVHEFKEGKDELSEQDEMFRGRTAVFADQVIVG
NASLRLKNVQLTDAGTYKCYIITSKGKGNANLEYKTGAFSMPEVNVDYNASS
ETLRCEAPRWFPQPTVVWASQVDQGANFSEVSNTSFELNSENVTMKVVSVL
10 YNVTINNTYSCMIENDIAKATGDIKVTSEIKRRSHLQLLNSKASLCVSSFFAIS
WALLPLSPYLMLK

[0257] In certain embodiments, the present disclosure provides multi-specific binding proteins that bind to the NKG2D receptor and CD16 receptor on natural killer cells, and the antigen V-domain Ig suppressor of T cell activation (VISTA). Table 28 lists some exemplary peptide sequences of heavy chain variable domains and light chain variable domains that, in
15 combination, can bind to VISTA.

Table 28		
Source	Heavy chain variable domain amino acid sequence	Light chain variable domain amino acid sequence
MedImmune (US Patent Publication No. US20160096891)	QVQLQQSGAELVKPGAS VKLSCKASGYTFTSHDIN WVRQRPELGLEWIGWIFP GDGSTKFNEKFKGKATL TTDKSSSTAYIQLSRLTSE DSAVYFCARNSFYSDY WGQGTSTVTVSS (SEQ ID NO:615) CDH1: GYTFTSHD (SEQ ID NO:616) CDH2: IFPGDGST (SEQ ID NO:617) CDH3: ARNSFYSDY (SEQ ID NO:618)	DIVMSQSPSSLAVSAGEKVT MSCKSSQSLLNSRTRKNQ LA WYQQKPGQSPKLLIYWAFIRE SGVPDRFTGSGSGTDFTLT ISSVQAEDLAVYYC KQSYNL RT FGGGIKLEIK (SEQ ID NO:619) CDRL1: QSLLNSRTRKNQ (SEQ ID NO:620) CDRL2: WAF (SEQ ID NO:621) CDRL3: KQSYNLRT (SEQ ID NO:622)
Pierre Fabre Medicament SA (US Patent No. 10,100,123)	EVQLLQSGPELEKPGASV KISCKASGYSTGYNMN WVKQSNGKSLEWIGNIDP YYDYTSYNLKFCDKATL TVDKSSSTAYMQLKSLTS EDSAVYYCATSTMITPFD YWGQGTTLTVSS (SEQ ID NO:623) CDH1: GYSTGYNMN (SEQ ID NO:624) CDH2: NIDPYDYTSYNLKFCD (SEQ ID NO:625)	DVLMTQTPLSLPVSLGDQASIS CRSSQSIVHSNGNTYLEWYLQ KPGQSPKLLIYKVSNRFSGVPD RFSGSGSGTDFTLKINRVEAED LGVYYCFQGSHVPWTFGGGT KLEIK (SEQ ID NO:627) CDR1: RSSQ SIVHSNGNTYLE (SEQ ID NO:628) CDR2: KVSNRFS (SEQ ID NO:629) CDR3: FQGSHVPWT (SEQ ID NO:630)

	CDH3: STMITPFDY (SEQ ID NO:626)	
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[0258] Alternatively, novel antigen-binding sites that can bind to VISTA can be identified, for example, by screening for binding to the amino acid sequence defined by SEQ ID NO:631.

SEQ ID NO: 631

5 MGVPTALEAGSWRWGSLFLAASLGPVAAFQVATPYSLYVCPEGQNVTLTCRL
LGPVDKGHDVTFYKWTYRSSRGEVQTCSERRPIRNLTFQDLHLHHGGHQAANTSHD
LAQRHGLEASDHHGNFSITMRNLTLDSGLYCCLVVEIRHHHSEHRVHGAMELQV
QTGKDAPSNCVVYPSSSQDSENITAAALATGACIVGILCLPLILLVYKQRQAASNRR
AQELVRMDSNIQGIENPGFEASPPAQGIPEAKVRHPLSYVAQRQPSESGRHLLSEPSTP
10 LSPPGPGDVFFPSLDPVPDSPNFEVI

[0259] Within the Fc domain, CD16 binding is mediated by the hinge region and the CH2 domain. For example, within human IgG1, the interaction with CD16 is primarily focused on amino acid residues Asp 265 – Glu 269, Asn 297 – Thr 299, Ala 327 – Ile 332, Leu 234 – Ser 239, and carbohydrate residue N-acetyl-D-glucosamine in the CH2 domain (*see, e.g.,*
15 *Sondermann P. et al. (2000) Nature;406(6793):267-273.*). Based on the known domains, mutations can be selected to enhance or reduce the binding affinity to CD16, such as by using phage-displayed libraries or yeast surface-displayed cDNA libraries, or can be designed based on the known three-dimensional structure of the interaction.

[0260] The assembly of heterodimeric antibody heavy chains can be accomplished by
20 expressing two different antibody heavy chain sequences in the same cell, which may lead to the assembly of homodimers of each antibody heavy chain as well as assembly of heterodimers. Promoting the preferential assembly of heterodimers can be accomplished by incorporating different mutations in the CH3 domain of each antibody heavy chain constant region as shown in US13/494,870, US16/028850, US11/533,709, US12/875,015,
25 US13/289,934, US14/773,418, US12/811,207, US13/866,756, US14/647,480, and US14/830,336. For example, mutations can be made in the CH3 domain based on human IgG1 and incorporating distinct pairs of amino acid substitutions within a first polypeptide and a second polypeptide that allow these two chains to selectively heterodimerize with each

other. The positions of amino acid substitutions illustrated below are all numbered according to the EU index as in Kabat.

[0261] In one scenario, an amino acid substitution in the first polypeptide replaces the original amino acid with a larger amino acid, selected from arginine (R), phenylalanine (F), tyrosine (Y) or tryptophan (W), and at least one amino acid substitution in the second polypeptide replaces the original amino acid(s) with a smaller amino acid(s), chosen from alanine (A), serine (S), threonine (T), or valine (V), such that the larger amino acid substitution (a protuberance) fits into the surface of the smaller amino acid substitutions (a cavity). For example, one polypeptide can incorporate a T366W substitution, and the other can incorporate three substitutions including T366S, L368A, and Y407V.

[0262] An antibody heavy chain variable domain of the invention can optionally be coupled to an amino acid sequence at least 90% identical to an antibody constant region, such as an IgG constant region including hinge, CH2 and CH3 domains with or without CH1 domain. In some embodiments, the amino acid sequence of the constant region is at least 90% identical to a human antibody constant region, such as a human IgG1 constant region, an IgG2 constant region, IgG3 constant region, or IgG4 constant region. In some other embodiments, the amino acid sequence of the constant region is at least 90% identical to an antibody constant region from another mammal, such as rabbit, dog, cat, mouse, or horse. One or more mutations can be incorporated into the constant region as compared to human IgG1 constant region, for example at Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411 and/or K439. Exemplary substitutions include, for example, Q347E, Q347R, Y349S, Y349K, Y349T, Y349D, Y349E, Y349C, T350V, L351K, L351D, L351Y, S354C, E356K, E357Q, E357L, E357W, K360E, K360W, Q362E, S364K, S364E, S364H, S364D, T366V, T366I, T366L, T366M, T366K, T366W, T366S, L368E, L368A, L368D, K370S, N390D, N390E, K392L, K392M, K392V, K392F, K392D, K392E, T394F, T394W, D399R, D399K, D399V, S400K, S400R, D401K, F405A, F405T, Y407A, Y407I, Y407V, K409F, K409W, K409D, T411D, T411E, K439D, and K439E.

[0263] In certain embodiments, mutations that can be incorporated into the CH1 of a human IgG1 constant region may be at amino acid V125, F126, P127, T135, T139, A140, F170, P171, and/or V173. In certain embodiments, mutations that can be incorporated into the C κ of a human IgG1 constant region may be at amino acid E123, F116, S176, V163, S174, and/or T164.

[0264] Alternatively, amino acid substitutions could be selected from the following sets of substitutions shown in Table 29.

Table 29		
	First Polypeptide	Second Polypeptide
Set 1	S364E/F405A	Y349K/T394F
Set 2	S364H/D401K	Y349T/T411E
Set 3	S364H/T394F	Y349T/F405A
Set 4	S364E/T394F	Y349K/F405A
Set 5	S364E/T411E	Y349K/D401K
Set 6	S364D/T394F	Y349K/F405A
Set 7	S364H/F405A	Y349T/T394F
Set 8	S364K/E357Q	L368D/K370S
Set 9	L368D/K370S	S364K
Set 10	L368E/K370S	S364K
Set 11	K360E/Q362E	D401K
Set 12	L368D/K370S	S364K/E357L
Set 13	K370S	S364K/E357Q
Set 14	F405L	K409R
Set 15	K409R	F405L

[0265] Alternatively, amino acid substitutions could be selected from the following sets of substitutions shown in Table 30.

Table 30		
	First Polypeptide	Second Polypeptide
Set 1	K409W	D399V/F405T
Set 2	Y349S	E357W

Set 3	K360E	Q347R
Set 4	K360E/K409W	Q347R/D399V/F405T
Set 5	Q347E/K360E/K409W	Q347R/D399V/F405T
Set 6	Y349S/K409W	E357W/D399V/F405T

[0266] Alternatively, amino acid substitutions could be selected from the following set of substitutions shown in Table 31.

Table 31		
	First Polypeptide	Second Polypeptide
Set 1	T366K/L351K	L351D/L368E
Set 2	T366K/L351K	L351D/Y349E
Set 3	T366K/L351K	L351D/Y349D
Set 4	T366K/L351K	L351D/Y349E/L368E
Set 5	T366K/L351K	L351D/Y349D/L368E
Set 6	E356K/D399K	K392D/K409D

[0267] Alternatively, at least one amino acid substitution in each polypeptide chain could be selected from Table 32.

Table 32	
First Polypeptide	Second Polypeptide
L351Y, D399R, D399K, S400K, S400R, Y407A, Y407I, Y407V	T366V, T366I, T366L, T366M, N390D, N390E, K392L, K392M, K392V, K392F, K392D, K392E, K409F, K409W, T411D and T411E

- 5 **[0268]** Alternatively, at least one amino acid substitution could be selected from the following set of substitutions in Table 33, where the position(s) indicated in the First Polypeptide column is replaced by any known negatively-charged amino acid, and the

position(s) indicated in the Second Polypeptide Column is replaced by any known positively-charged amino acid.

Table 33	
First Polypeptide	Second Polypeptide
K392, K370, K409, or K439	D399, E356, or E357

[0269] Alternatively, at least one amino acid substitution could be selected from the following set of substitutions in Table 34, where the position(s) indicated in the First Polypeptide column is replaced by any known positively-charged amino acid, and the position(s) indicated in the Second Polypeptide Column is replaced by any known negatively-charged amino acid.

Table 34	
First Polypeptide	Second Polypeptide
D399, E356, or E357	K409, K439, K370, or K392

[0270] Alternatively, amino acid substitutions could be selected from the following set in Table 35.

Table 35	
First Polypeptide	Second Polypeptide
T350V, L351Y, F405A, and Y407V	T350V, T366L, K392L, and T394W

[0271] Alternatively, or in addition, the structural stability of a hetero-multimeric protein may be increased by introducing S354C on either of the first or second polypeptide chain, and Y349C on the opposing polypeptide chain, which forms an artificial disulfide bridge within the interface of the two polypeptides.

[0272] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at position T366, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of T366, L368 and Y407.

[0273] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of T366, L368 and Y407, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at position T366.

[0274] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of E357, K360, Q362, S364, L368, K370, T394, D401, F405, and T411 and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Y349, E357, S364, L368, K370, T394, D401, F405 and T411.

[0275] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Y349, E357, S364, L368, K370, T394, D401, F405 and T411 and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of E357, K360, Q362, S364, L368, K370, T394, D401, F405, and T411.

[0276] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of L351, D399, S400 and Y407 and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of T366, N390, K392, K409 and T411.

[0277] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of T366, N390, K392, K409 and T411 and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of L351, D399, S400 and Y407.

[0278] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Q347, Y349, K360, and K409, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Q347, E357, D399 and F405.

[0279] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Q347, E357, D399 and F405, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Y349, K360, Q347 and K409.

[0280] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of K370, K392, K409 and K439, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of D356, E357 and D399.

[0281] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of D356, E357 and D399, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of K370, K392, K409 and K439.

[0282] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of L351, E356, T366 and D399, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Y349, L351, L368, K392 and K409.

[0283] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at

one or more positions selected from the group consisting of Y349, L351, L368, K392 and K409, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of L351, E356, T366 and D399.

- 5 **[0284]** In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by an S354C substitution and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by a Y349C substitution.
- 10 **[0285]** In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by a Y349C substitution and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by an S354C substitution.
- 15 **[0286]** In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by K360E and K409W substitutions and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by Q347R, D399V and F405T substitutions.
- 20 **[0287]** In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by Q347R, D399V and F405T substitutions and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by K360E and K409W substitutions.
- 25 **[0288]** In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by a T366W substitution and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T366S, T368A, and Y407V substitutions.
- 30 **[0289]** In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T366S, T368A, and Y407V substitutions and wherein the amino acid sequence of the other

polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by a T366W substitution.

[0290] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, L351Y, F405A, and Y407V substitutions and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, T366L, K392L, and T394W substitutions.

[0291] In some embodiments, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, T366L, K392L, and T394W substitutions and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, L351Y, F405A, and Y407V substitutions.

[0292] The multi-specific binding proteins described above can be made using recombinant DNA technology well known to a skilled person in the art. For example, a first nucleic acid sequence encoding the first immunoglobulin heavy chain can be cloned into a first expression vector; a second nucleic acid sequence encoding the second immunoglobulin heavy chain can be cloned into a second expression vector; a third nucleic acid sequence encoding the immunoglobulin light chain can be cloned into a third expression vector; and the first, second, and third expression vectors can be stably transfected together into host cells to produce the multimeric proteins.

[0293] To achieve the highest yield of the multi-specific binding protein, different ratios of the first, second, and third expression vector can be explored to determine the optimal ratio for transfection into the host cells. After transfection, single clones can be isolated for cell bank generation using methods known in the art, such as limited dilution, ELISA, flow cytometry, microscopy, or Clonepix.

[0294] Clones can be cultured under conditions suitable for bio-reactor scale-up and maintained expression of the multi-specific binding protein. The multi-specific binding proteins can be isolated and purified using methods known in the art including centrifugation, depth filtration, cell lysis, homogenization, freeze-thawing, affinity purification, gel filtration, ion exchange chromatography, hydrophobic interaction exchange chromatography, and mixed-mode chromatography.

II. CHARACTERISTICS OF MULTI-SPECIFIC BINDING PROTEINS

[0295] In certain embodiments, the multi-specific binding proteins described herein, which include an NKG2D-binding domain and a binding domain for DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4, bind to cells expressing human NKG2D. In certain embodiments, the multi-specific binding proteins bind to cells expressing NKG2D and/or CD16, such as NK cells, and tumor cells expressing DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4, simultaneously. Binding of the multi-specific binding proteins to NK cells can enhance the cytotoxic activity of NK cells leading to destruction of the tumor cells.

[0296] In certain embodiments, the multi-specific binding proteins described herein bind to a tumor-associated antigen at a comparable level to that of a corresponding monoclonal antibody having the same tumor-associated antigen binding site. In certain embodiments, the multi-specific binding proteins described herein may be more effective in reducing tumor growth and killing cancer cells expressing a tumor-associated antigen selected from DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4, than a corresponding monoclonal antibody having the same tumor-associated antigen binding site.

[0297] In certain embodiments, the multi-specific binding proteins described herein, which include an NKG2D-binding domain and a tumor-associated antigen binding domain for DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4, can activate primary human NK cells when co-cultured with tumor cells expressing DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4. NK cell activation is marked by the increase in CD107a expression, degranulation, and IFN γ cytokine production. Furthermore, compared to a corresponding monoclonal antibody having the same tumor-associated antigen binding site, the multi-specific binding proteins described herein show superior activation of human NK cells in the presence of tumor cells expressing DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4.

[0298] In certain embodiments, the multi-specific binding proteins described herein, which include an NKG2D-binding domain and a binding domain for DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4, can enhance the activation of resting and IL-2-activated human NK cells in the presence of tumor cells expressing DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4.

[0299] In certain embodiments, the multi-specific binding proteins described herein, which include an NKG2D-binding domain and a tumor-associated antigen binding domain for DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4, can enhance the cytotoxic activity of resting and IL-2-activated human NK cells in the presence of tumor cells expressing DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4. In certain embodiments, compared to corresponding monoclonal antibodies having the same tumor-associated antigen binding site, the multi-specific binding proteins described herein can have greater cytotoxic activity against tumor cells having medium and low expression of DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4.

[0300] In certain embodiments, the multi-specific binding proteins described herein can be advantageous in treating cancers with high expression of Fc receptor (FcR), or cancers residing in a tumor microenvironment with high levels of FcR expression, compared to corresponding DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4 monoclonal antibodies having the same tumor-associated antigen binding site. Monoclonal antibodies exert their effects on tumor growth through multiple mechanisms including, for example, initiation of antibody-dependent cellular cytotoxicity (ADCC), cell-dependent cytotoxicity (CDC), phagocytosis, and signal blockade, amongst others. Amongst FcγRs, CD16 has the lowest affinity for IgG Fc; FcγRI (CD64) is the high-affinity FcR, which binds about 1000 times more strongly to IgG Fc than CD16. CD64 is normally expressed on cells of many hematopoietic lineages, such as the myeloid lineage, and can also be expressed on cancer cells derived from these lineages, such as in acute myeloid leukemia (AML). Immune cells infiltrating into a tumor, such as MDSCs and monocytes, also express CD64 and are

known to contribute to the tumor microenvironment. Expression of CD64 by tumor cells or by cells in the tumor microenvironment can have a detrimental effect on monoclonal antibody therapy. Expression of CD64 in the tumor microenvironment makes it difficult for monoclonal antibodies to engage CD16 on the surface of NK cells, as they preferentially bind to the high-affinity CD64 Fc-receptor. By targeting two activating receptors on the surface of NK cells, the multi-specific binding proteins of the present invention can overcome the detrimental effect of CD64 expression (either on tumors or in the tumor microenvironment). Regardless of CD64 expression on the tumor cells, the multi-specific binding proteins described herein are able to induce human NK cell responses against tumor cells, because dual targeting of two activating receptors (*i.e.*, NKG2D and CD16) on NK cells provides stronger specific binding to and activation of NK cells.

[0301] In some embodiments, the multi-specific binding proteins described herein can provide a better safety profile through reduced on-target, off-tumor, side effects. Natural killer cells and CD8 T cells are both able to directly lyse tumor cells, although the mechanisms through which NK cells and CD8 T cells recognize normal, healthy, cells from tumor cells differ. The activity of NK cells is regulated by the balance of signals from activating (*e.g.*, NCRs, NKG2D, CD16, *etc.*) and inhibitory (*e.g.*, KIRs, NKG2A, *etc.*) receptors. The presence of stressed, virally-infected, or transformed self-cells stimulates activating receptors and shifts the balance towards NK cell activation. In contrast, normal, healthy, self-cells activate inhibitory receptors which shift the balance towards NK cell tolerance, thereby protecting normal, healthy tissues from potentially damaging NK cell activity. By coupling NK cell activation with tumor-associated antigen binding, or binding of antigens in the tumor microenvironment, multi-specific binding proteins described herein can avoid off-tumor side effects, and/or have an increased therapeutic window.

[0302] Unlike NK cells, T cells require recognition of a specific peptide presented by MHC/HLA molecules for activation and effector functions. T cells have been the primary target of current immunotherapies, and many strategies have been developed to redirect T cell responses against the tumor. T cell bispecifics, checkpoint inhibitors, and CAR-T cells have all been approved by the FDA, but these approaches often suffer from dose-limiting toxicities. T cell bispecifics and CAR-T cell technologies are based upon the TCR-MHC recognition system and use binding domains to target antigens on the surface of tumor cells, and engineered signaling domains to transduce the activation signals into an effector cell. Although effective at eliciting an anti-tumor immune response, these therapies are often

coupled with cytokine release syndrome (CRS), and on-target, off-tumor, side effects. In contrast, the multi-specific binding proteins of the present invention will not “override” the natural activating and inhibiting systems of NK cells but will instead provide additional activation signals to NK cells, while maintaining NK tolerance to normal, healthy self cells.

5 **[0303]** In some embodiments, a multi-specific binding protein described herein can delay progression of a tumor more effectively than a corresponding monoclonal antibody having the same tumor-associated antigen-binding domain. In some embodiments, a multi-specific binding protein described herein is more effective at inhibiting cancer metastasis than a corresponding monoclonal antibody having the same tumor-associated antigen-binding domain.

10 **[0304]** In certain other embodiments, the multi-specific binding proteins described herein include an NKG2D-binding site, a CD16-binding site, and a binding site for CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, or CCR5. In some embodiments, the multi-specific binding proteins further include an additional binding site for a tumor-associated antigen. In some embodiments, the multi-specific binding proteins bind to cells expressing NKG2D and/or CD16, such as NK cells, and cells expressing any one of the above antigens, such as MDSCs and/or TAMs simultaneously. In some other embodiments, the multi-specific binding proteins bind to cells expressing NKG2D and/or CD16, such as NK cells; cells expressing any one of the above antigens, such as MDSCs and/or TAMs; and tumor cells expressing a tumor-associated antigen, simultaneously. Binding of the multi-specific binding proteins to NK cells can enhance the activity of the NK cells toward destruction of the MDSCs and/or TAMs in a tumor environment, and promote an immune response against the tumor cells in the same tumor environment. In some embodiments, the multi-specific binding proteins that include an additional tumor-associated antigen-binding site enhance the activity of the NK cells toward destruction of the tumor cells that express the tumor-associated antigen as well as the MDSCs and/or TAMs in the tumor environment.

20 **[0305]** In some embodiments, the multi-specific binding proteins of the present invention, which include an additional tumor-associated antigen-binding site, bind to the tumor cells expressing the tumor-associated antigen with a similar affinity to that of a monoclonal antibody having the same antigen-binding site. The multi-specific binding proteins can be more effective in killing tumor cells than the corresponding monoclonal antibodies having the same antigen-binding site.

[0306] In certain embodiments, the multi-specific binding proteins described herein bind to the antigen CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, or CCR5 with a similar affinity to that of a corresponding monoclonal antibody having the same antigen-binding site. In some
5 embodiments, the multi-specific binding proteins of the present invention are more effective at de-repressing/enhancing the immune response within a tumor microenvironment, and in killing the tumor cells residing therein than the corresponding monoclonal antibodies having the same antigen-binding site.

[0307] In certain embodiments, the multi-specific binding proteins described herein,
10 which include an NKG2D-binding site and a binding site for CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, or CCR5, activate primary human NK cells when co-cultured with cells expressing CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, or CCR5. NK cell activation is marked by an
15 increase in CD107a expression, degranulation, and IFN- γ cytokine production. Furthermore, compared to a corresponding monoclonal antibody having the same antigen-binding site, the multi-specific binding proteins disclosed herein may elicit superior activation of human NK cells in the presence of cells expressing the antigen CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB,
20 ATP1A3, or CCR5. In some embodiments, the cells expressing one or more of these antigens are MDSCs and/or TAMs.

[0308] In certain embodiments, the multi-specific binding proteins described herein, which include an NKG2D-binding site and a binding site for CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB,
25 ATP1A3, or CCR5, enhance the activation of resting and IL-2-activated human NK cells co-cultured with cells expressing CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, or CCR5. In some embodiments, the cells expressing one or more of these antigens are MDSCs and/or TAMs.

[0309] In certain embodiments, compared to a corresponding monoclonal antibody that
30 binds to CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, or CCR5, the multi-specific binding proteins disclosed herein can have greater cytotoxic activity against cells having medium and low expression of CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1,

LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, or CCR5. In some embodiments, the cells expressing medium and low levels of CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, or CCR5 are MDSCs. In some embodiments, the cells expressing medium and low levels of CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, or CCR5 are TAMs.

III. THERAPEUTIC APPLICATIONS

[0310] The invention provides methods for treating cancer using a multi-specific binding protein described herein and/or a pharmaceutical composition described herein. The methods may be used to treat a variety of cancers which express DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4 by administering to a patient in need thereof a therapeutically effective amount of a multi-specific binding protein described herein.

[0311] The cancer to be treated can be characterized according to the presence of a particular antigen expressed on the surface of the cancer cell. In certain embodiments, the cancer cell can express one or more of the following in addition to DLL3, MUC1 (or MUC1-C), Plexin-A1, TNFRSF10B, STEAP1, CDCP1, PTK7, AXL, ERBB-3, EDNRB, TYRP1, OLR1, ADAM12, PLAUR, CCR6, or EPHA4: CD2, CD19, CD20, CD30, CD38, CD40, CD52, CD70, EGFR/ERBB-1, IGF1R, HER3/ERBB-3, HER4/ERBB-4, MUC1, TROP2, cMET, SLAMF7, PSCA, MICA, MICB, TRAILR1, TRAILR2, MAGE-A3, B7.1, B7.2, CTLA4, and PD1.

[0312] The methods may also be used to treat a variety of cancers, which co-exist with CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, or CCR5-expressing MDSCs and/or TAMs in the tumor microenvironment.

[0313] The therapeutic method can be characterized according to the cancer to be treated. Exemplary cancers to be treated may be acral lentiginous melanoma, actinic keratoses, acute lymphoblastic leukemia, acute lymphocytic leukemia, acute myeloid leukemia, acute myeloid leukemia, adenocarcinoma, adenoid cystic carcinoma, adenocarcinoma, adenocarcinoma, anal canal cancer, anaplastic large cell lymphoma, angioimmunoblastic T-cell lymphoma, angiosarcoma, anorectal cancer, astrocytic tumor, Bartholin's gland carcinoma, basocellular carcinomas (*e.g.*, skin), B-cell lymphoma, biliary tract cancer, bladder cancer,

bone cancer, bone marrow cancer, brain cancer, breast cancer, bronchial cancer, bronchial gland carcinoma, Burkitt lymphoma, carcinoid, cervical cancer, cholangiocarcinoma, chondrosarcoma, choroid plexus papilloma/carcinoma, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic neutrophilic leukemia, clear cell carcinoma, colon cancer, colorectal cancer, connective tissue cancer, cutaneous T-cell lymphoma, cystadenoma, diffuse large B-cell lymphoma, digestive system cancer, duodenum cancer, endocrine system cancer, endodermal sinus tumor, endometrial cancer/hyperplasia, endometrial stromal sarcoma, endometrioid adenocarcinoma, endothelial cell cancer, enteropathy type T-cell lymphoma, ependymal cancer, epithelial cell cancer, esophageal cancer, Ewing sarcoma, extranodal marginal zone B-cell lymphoma, extranodal natural killer/T-cell lymphoma, eye and orbit cancer, female genital cancer, focal nodular hyperplasia, follicular lymphoma, gall bladder cancer, gastric antrum cancer, gastric cancer, gastric fundus cancer, gastrinoma, glioblastoma, glioma, glucagonoma, hairy cell leukemia, head and neck cancer, heart cancer, hemangioblastoma, hemangioendothelioma, hemangiomas, hematological tumors, hepatic adenoma, hepatic adenomatosis, hepatocellular carcinoma, hepatobiliary cancer, Hodgkin's disease, ileum cancer, insulinoma, intraepithelial neoplasia, intraepithelial squamous cell neoplasia, intrahepatic bile duct cancer, invasive squamous cell carcinoma, jejunum cancer, joint cancer, Kaposi's sarcoma, kidney cancer, large cell carcinoma, large intestine cancer, leiomyosarcoma, lentigo maligna melanomas, leukemia, liver cancer, lung cancer, lymphoma, lymphoplasmacytic lymphoma, male genital cancer, malignant melanoma, malignant mesothelial tumors, mantle cell lymphoma, marginal zone B-cell lymphoma, medulloblastoma, medulloepithelioma, melanoma, meningeal cancer, mesothelial cancer, mesothelioma, metastatic carcinoma, mouth cancer, mucoepidermoid carcinoma, multiple myeloma, muscle cancer, myelodysplastic neoplasms, myeloproliferative neoplasms, nasal tract cancer, nervous system cancer, neuroblastoma, neuroepithelial adenocarcinoma, nodal marginal zone B-cell lymphoma, nodular melanoma, non-epithelial skin cancer, non-Hodgkin's lymphoma, oat cell carcinoma, oligodendroglial cancer, oral cavity cancer, osteosarcoma, ovarian cancer, pancreatic cancer, papillary serous adenocarcinoma, parotid, pelvic cancer, penile cancer, peripheral T-cell lymphoma, pharynx cancer, pituitary tumors, plasmacytoma, precursor T-lymphoblastic lymphoma, primary central nervous system lymphoma, primary mediastinal B-cell lymphoma, prostate cancer, pseudosarcoma, pulmonary blastoma, rectal cancer, renal cancer, renal cell carcinoma, respiratory system cancer, retinoblastoma, rhabdomyosarcoma, sarcoma, serous carcinoma, sinus cancer, skin cancer, small cell carcinoma, small intestine cancer, small lymphocytic lymphoma, smooth

muscle cancer, soft tissue cancer, somatostatin-secreting tumor, spine cancer, splenic marginal zone B-cell lymphoma, squamous cell carcinoma (*e.g.*, skin), striated muscle cancer, subcutaneous panniculitis-like t-cell lymphoma, submesothelial cancer, superficial spreading melanoma, T cell leukemia, T cell lymphoma, testicular cancer, thyroid cancer, tongue cancer, undifferentiated carcinoma, ureter cancer, urethra cancer, urinary bladder cancer, uterine cancer, uterine corpus cancer, uveal melanoma, vaginal cancer, verrucous carcinoma, VIPoma, vulva cancer, well-differentiated carcinoma, or Wilms tumor.

IV. COMBINATION THERAPY

[0314] Another aspect of the invention provides for combination therapy. Multi-specific binding proteins described herein can be used in combination with additional therapeutic agents to treat a cancer.

[0315] Exemplary therapeutic agents that may be used as part of a combination therapy in treating cancer, include, for example, radiation, mitomycin, tretinoin, ribomustin, gemcitabine, vincristine, etoposide, cladribine, mitobronitol, methotrexate, doxorubicin, carboquone, pentostatin, nitracrine, zinostatin, cetorelix, letrozole, raltitrexed, daunorubicin, fadrozole, fotemustine, thymalfasin, sobuzoxane, nedaplatin, cytarabine, bicalutamide, vinorelbine, vesnarinone, aminoglutethimide, amsacrine, proglumide, elliptinium acetate, ketanserin, doxifluridine, etretinate, isotretinoin, streptozocin, nimustine, vindesine, flutamide, drogenil, butocin, carmofur, razoxane, sizofilan, carboplatin, mitolactol, tegafur, ifosfamide, prednimustine, picibanil, levamisole, teniposide, improsulfan, enocitabine, lisuride, oxymetholone, tamoxifen, progesterone, mepitiostane, epitiostanol, formestane, interferon-alpha, interferon-2 alpha, interferon-beta, interferon-gamma, colony stimulating factor-1, colony stimulating factor-2, denileukin diftitox, interleukin-2, luteinizing hormone releasing factor and variations of the aforementioned agents that may exhibit differential binding to its cognate receptor, and increased or decreased serum half-life.

[0316] An additional class of agents that may be used as part of a combination therapy in treating cancer is immune checkpoint inhibitors. Exemplary immune checkpoint inhibitors include agents that inhibit one or more of (i) cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), (ii) programmed cell death protein 1 (PD1), (iii) PDL1, (iv) LAG3, (v) B7-H3, (vi) B7-H4, and (vii) TIM3. The CTLA4 inhibitor ipilimumab has been approved by the United States Food and Drug Administration for treating melanoma.

[0317] Yet other agents that may be used as part of a combination therapy in treating cancer are monoclonal antibody agents that target non-checkpoint targets (*e.g.*, herceptin) and non-cytotoxic agents (*e.g.*, tyrosine-kinase inhibitors).

[0318] Yet other categories of anti-cancer agents include, for example: (i) an inhibitor selected from an ALK inhibitor, an ATR inhibitor, an A2A antagonist, a base excision repair inhibitor, a Bcr-Abl tyrosine kinase inhibitor, a Bruton's tyrosine kinase inhibitor, a CDC7 inhibitor, a CHK1 inhibitor, a Cyclin-dependent kinase inhibitor, a DNA-PK inhibitor, an inhibitor of both DNA-PK and mTOR, a DNMT1 inhibitor, a DNMT1 inhibitor plus 2-chloro-deoxyadenosine, an HDAC inhibitor, a Hedgehog signaling pathway inhibitor, an IDO inhibitor, a JAK inhibitor, an mTOR inhibitor, a MEK inhibitor, a MELK inhibitor, a MTH1 inhibitor, a PARP inhibitor, a phosphoinositide 3-kinase inhibitor, an inhibitor of both PARP1 and DHODH, a proteasome inhibitor, a topoisomerase-II inhibitor, a tyrosine kinase inhibitor, a VEGFR inhibitor, and a WEE1 inhibitor; (ii) an agonist of OX40, CD137, CD40, GITR, CD27, HVEM, TNFRSF25, or ICOS; and (iii) a cytokine selected from IL-12, IL-15, GM-CSF, and G-CSF.

[0319] Proteins of the invention can also be used as an adjunct to surgical removal of the primary lesion.

[0320] The amount of multi-specific binding protein and additional therapeutic agent and the relative timing of administration may be selected in order to achieve a desired combined therapeutic effect. For example, when administering a combination therapy to a patient in need of such administration, the therapeutic agents in the combination, or a pharmaceutical composition or compositions comprising the therapeutic agents, may be administered in any order such as, for example, sequentially, concurrently, together, simultaneously and the like. Further, for example, a multi-specific binding protein may be administered during a time when the additional therapeutic agent(s) exerts its prophylactic or therapeutic effect, or *vice versa*.

V. PHARMACEUTICAL COMPOSITIONS

[0321] The present disclosure also features pharmaceutical compositions that contain a therapeutically effective amount of a protein described herein. The composition can be formulated for use in a variety of drug delivery systems. One or more physiologically acceptable excipients or carriers can also be included in the composition for proper formulation. Suitable formulations for use in the present disclosure are found in *Remington's*

Pharmaceutical Sciences, 17th Ed. Mack Publishing Company, Easton, PA(1985). For a brief review of methods for drug delivery, see, e.g., Langer R. (1990) *Science*; 249(4976):1527-1533.

[0322] The intravenous drug delivery formulation of the present disclosure may be contained in a bag, a pen, or a syringe. In certain embodiments, the bag may be connected to a channel comprising a tube and/or a needle. In certain embodiments, the formulation may be a lyophilized formulation or a liquid formulation. In certain embodiments, the formulation may be freeze-dried (lyophilized) and contained in about 12-60 vials. In certain embodiments, the formulation may be freeze-dried and 45 mg of the freeze-dried formulation may be contained in one vial. In certain embodiments, the about 40 mg to about 100 mg of freeze-dried formulation may be contained in one vial. In certain embodiments, freeze dried formulation from 12, 27, or 45 vials are combined to obtain a therapeutic dose of the protein in the intravenous drug formulation. In certain embodiments, the formulation may be a liquid formulation and stored as about 250 mg/vial to about 1000 mg/vial. In certain embodiments, the formulation may be a liquid formulation and stored as about 600 mg/vial. In certain embodiments, the formulation may be a liquid formulation and stored as about 250 mg/vial.

[0323] This present disclosure could exist in a liquid aqueous pharmaceutical formulation including a therapeutically effective amount of the multi-specific binding protein in a buffered solution.

[0324] The compositions disclosed herein may be sterilized by conventional sterilization techniques, or may be filter-sterilized. The resulting aqueous solutions may be packaged for use as-is, or lyophilized, wherein the lyophilized preparation is combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably between 5 and 9 or between 6 and 8, and most preferably between 7 and 8, such as 7 to 7.5. The resulting compositions in solid form may be packaged in multiple single dose units, each containing a fixed amount of the above-mentioned agent or agents. The composition in solid form can also be packaged in a container for a flexible quantity.

[0325] In certain embodiments, the present disclosure provides a formulation with an extended shelf life including the multi-specific binding protein of the present disclosure, in combination with mannitol, citric acid monohydrate, sodium citrate, disodium phosphate

dihydrate, sodium dihydrogen phosphate dihydrate, sodium chloride, polysorbate 80, water, and sodium hydroxide.

[0326] In certain embodiments, an aqueous formulation is prepared including the multi-specific binding protein of the present disclosure in a pH-buffered solution. The buffer of this invention may have a pH ranging from about 4 to about 8, *e.g.*, from about 4.5 to about 6.0, or from about 4.8 to about 5.5, or may have a pH of about 5.0 to about 5.2. Ranges intermediate to the above recited pH's are also intended to be part of this disclosure. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. Examples of buffers that will control the pH within this range include acetate (*e.g.*, sodium acetate), succinate (*e.g.*, sodium succinate), gluconate, histidine, citrate and other organic acid buffers.

[0327] In certain embodiments, the formulation includes a buffer system which contains citrate and phosphate to maintain the pH in a range of about 4 to about 8. In certain embodiments the pH range may be from about 4.5 to about 6.0, or from about pH 4.8 to about 5.5, or in a pH range of about 5.0 to about 5.2. In certain embodiments, the buffer system includes citric acid monohydrate, sodium citrate, disodium phosphate dihydrate, and/or sodium dihydrogen phosphate dihydrate. In certain embodiments, the buffer system includes about 1.3 mg/mL of citric acid (*e.g.*, 1.305 mg/mL), about 0.3 mg/ml of sodium citrate (*e.g.*, 0.305 mg/mL), about 1.5 mg/mL of disodium phosphate dihydrate (*e.g.*, 1.53 mg/mL), about 0.9 mg/mL of sodium dihydrogen phosphate dihydrate (*e.g.*, 0.86 mg/mL), and about 6.2 mg/mL of sodium chloride (*e.g.*, 6.165 mg/mL). In certain embodiments, the buffer system includes 1-1.5 mg/mL of citric acid, 0.25 to 0.5 mg/mL of sodium citrate, 1.25 to 1.75 mg/mL of disodium phosphate dihydrate, 0.7 to 1.1 mg/mL of sodium dihydrogen phosphate dihydrate, and 6.0 to 6.4 mg/mL of sodium chloride. In certain embodiments, the pH of the formulation is adjusted with sodium hydroxide.

[0328] A polyol, which acts as a tonicifier and may stabilize an antibody, may also be included in the formulations described herein. The polyol is added to a formulation in an amount which may vary with respect to the desired isotonicity of the formulation. In certain embodiments, the aqueous formulation may be isotonic. The amount of polyol added may also be altered with respect to the molecular weight of the polyol. For example, a lower amount of a monosaccharide (*e.g.*, mannitol) may be added, compared to a disaccharide (*e.g.*, trehalose). In certain embodiments, the polyol which may be used in the formulation as a tonicity agent is mannitol. In certain embodiments, the mannitol concentration may be about

5 to about 20 mg/mL. In certain embodiments, the concentration of mannitol may be about 7.5 to 15 mg/mL. In certain embodiments, the concentration of mannitol may be about 10-14 mg/mL. In certain embodiments, the concentration of mannitol may be about 12 mg/mL. In certain embodiments, the polyol sorbitol may be included in the formulation.

5 **[0329]** A detergent or surfactant may also be added to the formulations of the present invention. Exemplary detergents include nonionic detergents such as polysorbates (*e.g.*, polysorbates 20, 80 etc.) or poloxamers (*e.g.*, poloxamer 188). The amount of detergent added is such that it reduces aggregation of the formulated antibody and/or minimizes the formation of particulates in the formulation and/or reduces adsorption. In certain
10 embodiments, the formulation may include a surfactant which is a polysorbate. In certain embodiments, the formulation may contain the detergent polysorbate 80 or Tween 80. Tween 80 is a term used to describe polyoxyethylene (20) sorbitanmonooleate (*see, e.g.*, Fiedler H.P., Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und andere Bereiche, 4th Ed., Editio Cantor, Aulendorf, Germany(1996). In certain embodiments, the formulation
15 may contain between about 0.1 mg/mL and about 10 mg/mL of polysorbate 80, or between about 0.5 mg/mL and about 5 mg/mL. In certain embodiments, about 0.1% polysorbate 80 may be added in the formulation.

[0330] In certain embodiments, the multi-specific binding protein product of the present disclosure is formulated as a liquid formulation. The liquid formulation may be present at a
20 10 mg/mL concentration in either a USP / Ph Eur type I 50R vial closed with a rubber stopper and sealed with an aluminum crimp seal closure. The stopper may be made of elastomer complying with USP and Ph Eur. In certain embodiments vials may be filled with 61.2 mL of the multi-specific binding protein product solution in order to allow an extractable volume of 60 mL. In certain embodiments, the liquid formulation may be diluted with 0.9% saline
25 solution.

[0331] In certain embodiments, the liquid formulation of the disclosure may be prepared as a 10 mg/mL concentration solution in combination with a sugar at stabilizing levels. In certain embodiments the liquid formulation may be prepared in an aqueous carrier. In certain embodiments, a stabilizer may be added in an amount no greater than that which may result
30 in a viscosity undesirable or unsuitable for intravenous administration. In certain embodiments, the sugar may be a disaccharide, *e.g.*, sucrose. In certain embodiments, the liquid formulation may also include one or more of a buffering agent, a surfactant, and a preservative.

[0332] In certain embodiments, the pH of the liquid formulation may be set by addition of a pharmaceutically acceptable acid and/or base. In certain embodiments, the pharmaceutically acceptable acid may be hydrochloric acid. In certain embodiments, the base may be sodium hydroxide.

5 [0333] In addition to aggregation, deamidation is a common product variation of peptides and proteins that may occur during fermentation, harvest/cell clarification, purification, drug substance/drug product storage, and sample analysis. Under physiological conditions, deamidation is the loss of ammonia (NH₃) from an asparagine residue of a protein, resulting in a 17 dalton decrease in mass and formation of a succinimide intermediate. Subsequent
10 hydrolysis of succinimide results in an 18 dalton mass increase and formation of aspartic acid or isoaspartic acid. The parameters affecting the rate of deamidation include pH, temperature, solvent dielectric constant, ionic strength, primary sequence, local polypeptide conformation and tertiary structure. The amino acid residues adjacent to Asn in the peptide chain may also affect deamidation rates, *e.g.*, Gly and Ser residues following an Asn residue results in a
15 higher susceptibility to deamidation.

[0334] In certain embodiments, the liquid formulation of the present disclosure may be preserved under conditions of pH and humidity to prevent deamidation of the protein product.

[0335] The aqueous carrier of interest herein is one which is pharmaceutically acceptable (*i.e.*, safe and non-toxic for administration to a human) and is useful for the preparation of a
20 liquid formulation. Illustrative carriers include sterile water for injection (SWFI), bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.*, phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

[0336] A preservative may be optionally added to the formulations herein to reduce bacterial action. The addition of a preservative may, for example, facilitate the production of
25 a multi-use (multiple-dose) formulation.

[0337] Intravenous (IV) formulations may be the preferred administration route in particular instances, such as when a patient is in the hospital after transplantation receiving all drugs via the IV route. In certain embodiments, the liquid formulation is diluted with 0.9% sodium chloride solution before administration. In certain embodiments, the diluted drug
30 product for injection is isotonic and suitable for administration by intravenous infusion.

[0338] In certain embodiments, salt or buffer components may be added in amounts of about 10 mM to about 200 mM. The salts and/or buffers are pharmaceutically acceptable and

are derived from various known acids (inorganic and organic) with “base forming” metals or amines. In certain embodiments, the buffer may be phosphate buffer. In certain embodiments, the buffer may be glycinate, carbonate, citrate buffers, in which case, sodium, potassium or ammonium ions can serve as counterions.

5 **[0339]** A preservative may be optionally added to the formulations herein to reduce bacterial action. The addition of a preservative may, for example, facilitate the production of a multi-use (*i.e.*, multiple-dose) formulation.

10 **[0340]** The aqueous carrier of interest herein is one which is pharmaceutically acceptable (*i.e.*, safe and non-toxic for administration to a human) and is useful for the preparation of a liquid formulation. Illustrative carriers include SWFI, BWFI, a pH buffered solution (*e.g.*, phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

15 **[0341]** This present disclosure could exist in a lyophilized formulation including the proteins and a lyoprotectant. The lyoprotectant may be a sugar, *e.g.*, a disaccharide. In certain embodiments, the lyoprotectant may be sucrose or maltose. The lyophilized formulation may also include one or more of a buffering agent, a surfactant, a bulking agent, and/or a preservative.

20 **[0342]** The amount of sucrose or maltose useful for stabilization of the lyophilized drug product may be in a weight ratio of at least 1:2 protein to sucrose or maltose. In certain embodiments, the protein to sucrose or maltose weight ratio may be from 1:2 to 1:5.

25 **[0343]** In certain embodiments, the pH of the lyophilized formulation, prior to lyophilization, may be set by addition of a pharmaceutically acceptable acid and/or base. In certain embodiments the pharmaceutically acceptable acid may be hydrochloric acid. In certain embodiments, the pharmaceutically acceptable base may be sodium hydroxide.

30 **[0344]** Before lyophilization, the pH of the solution containing the protein of the present disclosure may be adjusted between 6 to 8. In certain embodiments, the pH range for the lyophilized drug product may be from 7 to 8.

35 **[0345]** In certain embodiments of the lyophilized formulation, salt or buffer components may be added in an amount of 10 mM - 200 mM. The salts and/or buffers are pharmaceutically acceptable and are derived from various known acids (inorganic and organic) with “base forming” metals or amines. In certain embodiments, the buffer may be phosphate buffer. In certain embodiments, the buffer may be glycinate, carbonate, citrate buffers, in which case, sodium, potassium or ammonium ions can serve as counterion.

[0346] In certain embodiments, a “bulking agent” may be added to the lyophilized formulation. A “bulking agent” is a compound which adds mass to a lyophilized mixture and contributes to the physical structure of the lyophilized cake (*e.g.*, facilitates the production of an essentially uniform lyophilized cake which maintains an open pore structure). Illustrative bulking agents include mannitol, glycine, polyethylene glycol and sorbitol. The lyophilized formulations of the present invention may contain such bulking agents.

[0347] A preservative may be optionally added to the lyophilized formulations herein to reduce bacterial action. The addition of a preservative may, for example, facilitate the production of a multi-use (*i.e.*, multiple-dose) formulation.

[0348] In certain embodiments, the lyophilized drug product may be reconstituted with an aqueous diluent. The aqueous diluent of interest herein is one which is pharmaceutically acceptable (*e.g.*, safe and non-toxic for administration to a human) and is useful for the preparation of a reconstituted liquid formulation, after lyophilization. Illustrative diluents include SWFI, BWFI, a pH buffered solution (*e.g.*, phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

[0349] In certain embodiments, the lyophilized drug product of the current disclosure is reconstituted with either SWFI, USP or 0.9% sodium chloride for injection, USP. During reconstitution, the lyophilized powder dissolves into a solution.

[0350] In certain embodiments, the lyophilized protein product of the instant disclosure is reconstituted to about 4.5 mL in SWFI and diluted with 0.9% saline solution (sodium chloride solution).

[0351] Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

[0352] The specific dose can be a uniform dose for each patient, for example, 50-5000 mg of protein. Alternatively, a patient's dose can be tailored to the approximate body weight or surface area of the patient. Other factors in determining the appropriate dosage can include the disease or condition to be treated or prevented, the severity of the disease, the route of administration, and the age, sex and medical condition of the patient. Further refinement of the calculations necessary to determine the appropriate dosage for treatment is routinely made by those skilled in the art, especially in light of the dosage information and assays disclosed

herein. The dosage can also be determined through the use of known assays for determining dosages used in conjunction with appropriate dose-response data. An individual patient's dosage can be adjusted as the progress of the disease is monitored. Blood levels of the targetable construct or complex in a patient can be measured to see if the dosage needs to be adjusted to reach or maintain an effective concentration. Pharmacogenomics may be used to determine which targetable constructs and/or complexes, and dosages thereof, are most likely to be effective for a given individual (*see, e.g., Schmitz et al. (2001) Clinica Chimica Acta*; 308: 43-53; Steimer *et al. (2001) Clinica Chimica Acta*; 308: 33-41.).

[0353] In general, dosages based on body weight are from about 0.01 µg to about 100 mg per kg of body weight, such as about 0.01 µg to about 100 mg/kg of body weight, about 0.01 µg to about 50 mg/kg of body weight, about 0.01 µg to about 10 mg/kg of body weight, about 0.01 µg to about 1 mg/kg of body weight, about 0.01 µg to about 100 µg/kg of body weight, about 0.01 µg to about 50 µg/kg of body weight, about 0.01 µg to about 10 µg/kg of body weight, about 0.01 µg to about 1 µg/kg of body weight, about 0.01 µg to about 0.1 µg/kg of body weight, about 0.1 µg to about 100 mg/kg of body weight, about 0.1 µg to about 50 mg/kg of body weight, about 0.1 µg to about 10 mg/kg of body weight, about 0.1 µg to about 1 mg/kg of body weight, about 0.1 µg to about 100 µg/kg of body weight, about 0.1 µg to about 50 µg/kg of body weight, about 0.1 µg to about 10 µg/kg of body weight, about 0.1 µg to about 1 µg/kg of body weight, about 1 µg to about 100 mg/kg of body weight, about 1 µg to about 50 mg/kg of body weight, about 1 µg to about 10 mg/kg of body weight, about 1 µg to about 1 mg/kg of body weight, about 1 µg to about 100 µg/kg of body weight, about 1 µg to about 50 µg/kg of body weight, about 1 µg to about 10 µg/kg of body weight, about 10 µg to about 100 mg/kg of body weight, about 10 µg to about 50 mg/kg of body weight, about 10 µg to about 10 mg/kg of body weight, about 10 µg to about 1 mg/kg of body weight, about 10 µg to about 100 µg/kg of body weight, about 10 µg to about 50 µg/kg of body weight, about 50 µg to about 100 mg/kg of body weight, about 50 µg to about 50 mg/kg of body weight, about 50 µg to about 10 mg/kg of body weight, about 50 µg to about 1 mg/kg of body weight, about 50 µg to about 100 µg/kg of body weight, about 100 µg to about 100 mg/kg of body weight, about 100 µg to about 50 mg/kg of body weight, about 100 µg to about 10 mg/kg of body weight, about 100 µg to about 1 mg/kg of body weight, about 1 mg to about 100 mg/kg of body weight, about 1 mg to about 50 mg/kg of body weight, about 1 mg to about 10 mg/kg of body weight, about 10 mg to about 100 mg/kg of body weight, about 10 mg to about 50 mg/kg of body weight, or about 50 mg to about 100 mg/kg of body weight.

[0354] Doses may be given once or more times daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the targetable construct or complex in bodily fluids or tissues. Administration of the present invention can be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, intracavitary, by perfusion through a catheter or by direct intralesional injection. This may be administered once or more times daily, once or more times weekly, once or more times monthly, or once or more times annually.

[0355] The description above describes multiple aspects and embodiments of the invention. The patent application specifically contemplates all combinations and permutations of the aspects and embodiments.

EXAMPLES

[0356] The invention now being generally described, will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

Example 1 – NKG2D-binding domains bind to NKG2D

NKG2D-binding domains bind to purified recombinant NKG2D

[0357] The nucleic acid sequences of human, mouse or cynomolgus NKG2D ectodomains were fused with nucleic acid sequences encoding human IgG1 Fc domains and introduced into mammalian cells to be expressed. After purification, NKG2D-Fc fusion proteins were adsorbed to wells of microplates. After blocking the wells with bovine serum albumin to prevent non-specific binding, NKG2D-binding domains were titrated and added to the wells pre-adsorbed with NKG2D-Fc fusion proteins. Primary antibody binding was detected using a secondary antibody which was conjugated to horseradish peroxidase and specifically recognizes a human kappa light chain to avoid Fc cross-reactivity. 3,3',5,5'-Tetramethylbenzidine (TMB), a substrate for horseradish peroxidase, was added to the wells to visualize the binding signal, whose absorbance was measured at 450 nM and corrected at 540 nM. An NKG2D-binding domain clone, an isotype control or a positive control (comprising heavy chain and light chain variable domains selected from SEQ ID NOs:101-104, or anti-mouse NKG2D clones MI-6 and CX-5, eBioscience, San Diego, CA) was added to each well.

[0358] The isotype control showed minimal binding to recombinant NKG2D-Fc proteins, while the positive control bound strongest to the recombinant antigens. NKG2D-binding domains produced by all clones demonstrated binding across human, mouse, and cynomolgus recombinant NKG2D-Fc proteins, although with varying affinities from clone to clone.

5 Generally, each anti-NKG2D clone bound to human (FIG. 3) and cynomolgus (FIG. 4) recombinant NKG2D-Fc with similar affinity, but with lower affinity to mouse (FIG. 5) recombinant NKG2D-Fc.

NKG2D-binding domains bind to cells expressing NKG2D

[0359] EL4 mouse lymphoma cell lines were engineered to express human or mouse
10 NKG2D-CD3 zeta signaling domain chimeric antigen receptors. An NKG2D-binding clone, an isotype control, or a positive control was used at a 100 nM concentration to stain extracellular NKG2D expressed on the EL4 cells. The antibody binding was detected using fluorophore-conjugated anti-human IgG secondary antibodies. Cells were analyzed by flow cytometry, and fold-over-background (FOB) was calculated using the mean fluorescence
15 intensity (MFI) of NKG2D-expressing cells compared to parental EL4 cells.

[0360] NKG2D-binding domains produced by all clones bound to EL4 cells expressing human and mouse NKG2D. Positive control antibodies (comprising heavy chain and light chain variable domains selected from SEQ ID NOs:101-104, or anti-mouse NKG2D clones MI-6 and CX-5, eBioscience, San Diego, CA) gave the best FOB binding signal. The
20 NKG2D-binding affinity for each clone was similar between cells expressing human NKG2D (FIG. 6) and mouse (FIG. 7) NKG2D.

Example 2 – NKG2D-binding domains block natural ligand binding to NKG2D

Competition With ULBP-6

[0361] Recombinant human NKG2D-Fc proteins were adsorbed to wells of a microplate,
25 and the wells were blocked with bovine serum albumin to reduce non-specific binding. A saturating concentration of ULBP-6-His-biotin was added to the wells, followed by addition of the NKG2D-binding domain clones. After a 2-hour incubation, wells were washed and ULBP-6-His-biotin that remained bound to the NKG2D-Fc coated wells was detected by streptavidin-conjugated to horseradish peroxidase and TMB substrate. Absorbance was
30 measured at 450 nM and corrected at 540 nM. After subtracting background, specific binding of NKG2D-binding domains to the NKG2D-Fc proteins was calculated from the percentage of ULBP-6-His-biotin that was blocked from binding to the NKG2D-Fc proteins in wells.

The positive control antibody (comprising heavy chain and light chain variable domains selected from SEQ ID NOs:101-104) and various NKG2D-binding domains blocked ULBP-6 binding to NKG2D, while isotype control showed little competition with ULBP-6 (FIG. 8).

[0362] ULBP-6 sequence is represented by SEQ ID NO:632.

5 MAAAAIPALLLCLPLLFLFGWSRARRDDPHSLCYDITVIPKFRPGPRWCAVQGQVD
 EKTFLHYDCGNKTVTPVSPLGKKLNVTMAWKAQNPVLREVVDILTEQLLDIQLNENY
 TPKEPLTLQARMSCEQKAEGHSSGSWQFSIDGQTFLFDSEKRMWTTVHPGARKMK
 EKWENDKDVAMSFHYISMGDCIGWLEDFLMGMDSTLEPSAGAPLAMSSGTTQLRA
 TATTLILCCLLILPCFILPGI (SEQ ID NO:632)

10 Competition With MICA

[0363] Recombinant human MICA-Fc proteins were adsorbed to wells of a microplate, and the wells were blocked with bovine serum albumin to reduce non-specific binding.

NKG2D-Fc-biotin was added to wells followed by NKG2D-binding domains. After incubation and washing, NKG2D-Fc-biotin that remained bound to MICA-Fc coated wells
 15 was detected using streptavidin-HRP and TMB substrate. Absorbance was measured at 450 nM and corrected at 540 nM. After subtracting background, specific binding of NKG2D-binding domains to the NKG2D-Fc proteins was calculated from the percentage of NKG2D-Fc-biotin that was blocked from binding to the MICA-Fc coated wells. The positive control antibody (comprising heavy chain and light chain variable domains selected from SEQ ID
 20 NOs:101-104) and various NKG2D-binding domains blocked MICA binding to NKG2D, while isotype control showed little competition with MICA (FIG. 9).

Competition With Rae-1 delta

[0364] Recombinant mouse Rae-1 delta-Fc (R&D Systems, Minneapolis, MN) was adsorbed to wells of a microplate, and the wells were blocked with bovine serum albumin to
 25 reduce non-specific binding. Mouse NKG2D-Fc-biotin was added to the wells followed by NKG2D-binding domains. After incubation and washing, NKG2D-Fc-biotin that remained bound to Rae-1delta-Fc coated wells was detected using streptavidin-HRP and TMB substrate. Absorbance was measured at 450 nM and corrected at 540 nM. After subtracting background, specific binding of NKG2D-binding domains to the NKG2D-Fc proteins was
 30 calculated from the percentage of NKG2D-Fc-biotin that was blocked from binding to the Rae-1delta-Fc coated wells. The positive control (comprising heavy chain and light chain variable domains selected from SEQ ID NOs:101-104, or anti-mouse NKG2D clones MI-6

and CX-5, eBioscience, San Diego, CA) and various NKG2D-binding domain clones blocked Rae-1 delta binding to mouse NKG2D, while the isotype control antibody showed little competition with Rae-1 delta (FIG. 10).

Example 3 – NKG2D-binding domain clones activate NKG2D

5 [0365] Nucleic acid sequences of human and mouse NKG2D were fused to nucleic acid sequences encoding a CD3 zeta signaling domain to obtain chimeric antigen receptor (CAR) constructs. The NKG2D-CAR constructs were then cloned into a retrovirus vector using Gibson assembly and transfected into expi293 cells for retrovirus production. EL4 cells were infected with viruses containing NKG2D-CAR together with 8 µg/mL polybrene. 24 hours
10 after infection, the expression levels of NKG2D-CAR in the EL4 cells were analyzed by flow cytometry, and clones which express high levels of the NKG2D-CAR on the cell surface were selected.

[0366] To determine whether NKG2D-binding domains activate NKG2D, they were adsorbed to wells of a microplate, and NKG2D-CAR EL4 cells were cultured on the antibody
15 fragment-coated wells for 4 hours in the presence of brefeldin-A and monensin. Intracellular TNF-alpha production, an indicator for NKG2D activation, was assayed by flow cytometry. The percentage of TNF-alpha positive cells was normalized to the cells treated with the positive control. All NKG2D-binding domains activated both human NKG2D (FIG. 11) and mouse NKG2D (FIG. 12).

Example 4 – NKG2D-binding domains activate NK cells

Primary human NK cells

[0367] Peripheral blood mononuclear cells (PBMCs) were isolated from human peripheral blood buffy coats using density gradient centrifugation. NK cells (CD3⁻ CD56⁺) were isolated using negative selection with magnetic beads from PBMCs, and the purity of
25 the isolated NK cells was typically >95%. Isolated NK cells were then cultured in media containing 100 ng/mL IL-2 for 24-48 hours before they were transferred to the wells of a microplate to which the NKG2D-binding domains were adsorbed, and cultured in media containing fluorophore-conjugated anti-CD107a antibody, brefeldin-A, and monensin. Following culture, NK cells were assayed by flow cytometry using fluorophore-conjugated
30 antibodies against CD3, CD56 and IFNγ. CD107a and IFNγ staining were analyzed in CD3⁻ CD56⁺ cells to assess NK cell activation. The increase in CD107a/IFNγ double-positive cells is indicative of better NK cell activation through engagement of two activating receptors

rather than one receptor. NKG2D-binding domains and the positive control (comprising heavy chain and light chain variable domains selected from SEQ ID NOs:101-104) showed a higher percentage of NK cells becoming CD107a⁺ and IFN γ ⁺ than the isotype control (FIG. 13 and FIG. 14 represent data from two independent experiments, each using a different donor's PBMCs for NK cell preparation).

Primary mouse NK cells

[0368] Spleens were obtained from C57Bl/6 mice and crushed through a 70 μ m cell strainer to obtain a single cell suspension. Cells were pelleted and resuspended in ACK lysis buffer (Thermo Fisher Scientific #A1049201, Carlsbad, CA; 155 mM ammonium chloride, 10mM potassium bicarbonate, 0.01mM EDTA) to remove red blood cells. The remaining cells were cultured with 100 ng/mL hIL-2 for 72 hours before being harvested and prepared for NK cell isolation. NK cells (CD3⁻NK1.1⁺) were then isolated from spleen cells using a negative depletion technique with magnetic beads which typically yields NK cell populations having >90% purity. Purified NK cells were cultured in media containing 100 ng/mL mIL-15 for 48 hours before they were transferred to the wells of a microplate to which the NKG2D-binding domains were adsorbed, and cultured in media containing fluorophore-conjugated anti-CD107a antibody, brefeldin-A, and monensin. Following culture in NKG2D-binding domain-coated wells, NK cells were assayed by flow cytometry using fluorophore-conjugated antibodies against CD3, NK1.1 and IFN γ . CD107a and IFN γ staining were analyzed in CD3⁻NK1.1⁺ cells to assess NK cell activation. The increase in CD107a/IFN γ double-positive cells is indicative of better NK cell activation through engagement of two activating receptors rather than one receptor. NKG2D-binding domains and the positive control (selected from anti-mouse NKG2D clones MI-6 and CX-5, eBioscience, San Diego, CA) showed a higher percentage of NK cells becoming CD107a⁺ and IFN γ ⁺ than the isotype control (FIG. 15 and FIG. 16 represent data from two independent experiments, each using a different mouse for NK cell preparation).

Example 5 – NKG2D-binding domains enhance cytotoxicity against target tumor cells

[0369] Human and mouse primary NK cell activation assays demonstrate increased cytotoxicity markers on NK cells after incubation with NKG2D-binding domains. To address whether this translates into increased tumor cell lysis, a cell-based assay was utilized where each NKG2D-binding domain was developed into a monospecific antibody. The Fc region was used as one targeting arm, while the Fab region (NKG2D-binding domain) acted as

another targeting arm to activate NK cells. THP-1 cells, which are of human origin and express high levels of Fc receptors, were used as a tumor target and a Perkin Elmer DELFIA[®] Cytotoxicity Kit (Waltham, MA) was used. THP-1 cells were labeled with BATDA reagent, and resuspended at 10^5 cells/mL in culture media. Labeled THP-1 cells were then combined with NKG2D antibodies and isolated mouse NK cells in wells of a microtiter plate at 37 °C for 3 hours. After incubation, 20 µl of the culture supernatant was removed, mixed with 200 µl of Europium solution and incubated with shaking for 15 minutes in the dark. Fluorescence was measured over time by a PHERAstar[®] plate reader equipped with a time-resolved fluorescence module (Excitation 337 nm, Emission 620 nm) and specific lysis was calculated according to the kit instructions.

[0370] The positive control, ULBP-6, a natural ligand for NKG2D, showed increased specific lysis of THP-1 target cells by mouse NK cells. NKG2D antibodies also increased specific lysis of THP-1 target cells, while isotype control antibody showed reduced specific lysis. The dotted line indicates specific lysis of THP-1 cells by mouse NK cells without antibody added (FIG. 17).

Example 6 – NKG2D antibodies have high thermostability

[0371] Melting temperatures of NKG2D-binding domains were assayed using differential scanning fluorimetry. The extrapolated apparent melting temperatures of NKG2D-binding domains were high relative to typical IgG1 antibodies (FIG. 18).

Example 7 – Synergistic activation of human NK cells by cross-linking NKG2D and CD16

Primary human NK cell activation assay

[0372] Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral human blood buffy coats using density gradient centrifugation. NK cells were purified from PBMCs using negative selection magnetic beads (StemCell Technologies, Vancouver, Canada; Cat# 17955). NK cells were >90% CD3⁺CD56⁺ as determined by flow cytometry. Cells were then expanded 48 hours in media containing 100 ng/mL hIL-2 (PeproTech, Inc., Rocky Hill, NJ; Cat#200-02) before use in activation assays. Antibodies were coated onto a 96-well flat-bottom plate at a concentration of 2 µg/ml (anti-CD16, BioLegend, San Diego, CA; Cat# 302013) and 5 µg/mL (anti-NKG2D, R&D Systems, Minneapolis, MN; Cat#MAB139) in 100 µl sterile PBS overnight at 4 °C followed by washing the wells thoroughly to remove excess antibody. For the assessment of degranulation, IL-2-activated NK cells were

resuspended at 5×10^5 cells/ml in culture media supplemented with 100 ng/mL hIL2 and 1 μ g/mL APC-conjugated anti-CD107a mAb (BioLegend Cat# 328619). 1×10^5 cells/well were then added onto antibody coated plates. The protein transport inhibitors Brefeldin A (BFA, BioLegend, San Diego, CA; Cat#420601) and Monensin (BioLegend, San Diego, CA; Cat# 420701) were added at a final dilution of 1:1000 and 1:270 respectively. Plated cells were incubated for 4 hours at 37 °C in 5% CO₂. For intracellular staining of IFN γ , NK cells were labeled with anti-CD3 (BioLegend, San Diego, CA; Cat#300452) and anti-CD56 mAb (BioLegend, San Diego, CA; Cat#318328) and subsequently fixed and permeabilized and labeled with anti-IFN γ mAb (BioLegend, San Diego, CA; Cat# 506507). NK cells were analyzed for expression of CD107a and IFN γ by flow cytometry after gating on live CD56⁺CD3⁻cells.

[0373] To investigate the relative potency of receptor combination, crosslinking of NKG2D or CD16 and co-crosslinking of both receptors by plate-bound stimulation was performed. As shown in FIG. 19, expression of CD107a and intracellular IFN γ of IL-2-activated NK cells was analyzed after 4 hours of plate-bound stimulation with anti-CD16, anti-NKG2D, or a combination of both monoclonal antibodies. Combined stimulation of CD16 and NKG2D resulted in percentages of CD107a⁺ cells (FIG. 19A) and IFN γ ⁺ cells (FIG. 19B) that were greater than the additive effect of individual stimulations of CD16 or NKG2D alone (as indicated by the dotted line). Similarly, combined stimulation of CD16 and NKG2D resulted in a greater percentage of CD107a⁺IFN γ ⁺ double-positive cells as compared to the additive effect of individual of each receptor alone (FIG. 19C). Bar graphs show the mean (n=2) $\pm$ SD and are representative of five independent experiments using five different healthy donors.

Example 8 – Binding kinetics of DLL3 antibodies to different variants of DLL3.

[0374] The extracellular domain (ECD) of human DLL3 was (AdipoGen Life Sciences, San Diego, CA) further purified using size exclusion chromatography. Recombinant His-tagged proteins of different domains of human DLL3 (N-terminal, EGF2-6, EGF2-6, EGF4-6, EGF5-6) were expressed in a cell line and purified using size exclusion chromatography.

[0375] The binding kinetics of different anti-DLL3 antibodies to recombinant proteins of different domains of human DLL3 were studied by surface plasmon resonance (SPR) using a Biacore™ 8K instrument. These anti-DLL3 antibodies were produced from mouse hybridomas, and each included a heavy chain variable region and light chain variable region

described herein. Briefly, antibodies recognizing human IgG Fc and antibodies recognizing mouse IgG Fc were immobilized on different channels of a Biacore™ 8K chip to allow simultaneous analysis of human and murine anti-DLL3 antibodies. Murine anti-DLL3 antibodies were captured on the anti-mouse Fc channel of the Biacore chip. The human anti-DLL3 antibody from Stemcentrx (San Francisco, CA) was used as a control and was captured onto anti-human Fc channel of the Biacore™ chip. Different concentrations of DLL3 ECD, DLL3 N-terminal domain, EGF2-6, EGF3-6, EGF4-6, or EGF5-6 domains of DLL3 were injected. Experiments were performed at 37 °C. Biacore™ 8K evaluation software was used for all data analysis. To obtain kinetic rate constants double-referenced data were fit to a 1:1 interaction model using Biacore™ 8K Evaluation software (GE Healthcare, Marlborough, MA). The equilibrium binding constant K_D was determined by the ratio of binding rate constants k_d/k_a .

[0376] FIG. 20 shows binding profiles of murine anti-DLL3 antibodies to the ECD of human DLL3 (AdipoGen Life Sciences, San Diego, CA), obtained by SPR analysis at 37 °C. Table 36 lists the calculated binding kinetics (K_D). The antibodies demonstrate a range of binding affinities to the DLL3 ECD from <0.011 to 8.44 nM. Stemcentrx anti-DLL3 antibody was used as a control.

Table 36

Antibody	Construct	k_a (1/Ms)	k_d (1/s)	K_D (nM)
2F7	hDLL3 ECD	7.21e+04	~1.15e-06	<0.011*
5E7	hDLL3 ECD	3.19e+05	6.47e-05	0.203
8H9	hDLL3 ECD	5.42e+05	4.58e-03	8.44
9E6	hDLL3 ECD	4.08e+05	2.08e-03	5.11
10H5	hDLL3 ECD	4.46e+05	2.74e-03	6.1
Stemcentrx Benchmark Ab	hDLL3 ECD	1.64e+06	3.14e-03	1.92

* Kinetic data for 2F7 are approximate ~ off rate beyond the instrument sensitivity

Mapping of antibody binding epitope on DLL3

[0377] The binding epitope on DLL3 by the anti-DLL3 antibody which included a heavy chain variable region and light chain variable region of clone 5E7 (see Table 2) was identified by SPR analysis at 37 °C. FIG. 21A shows the kinetics of the anti-DLL3 antibody binding to different DLL3 domains (N-terminus, EGF2-6, EGF3-6, EGF4-6, or EGF5-6) illustrated in

FIG. 21B. The antibody binds to constructs incorporating EGF2-6, EGF3-6, and EGF4-6, but shows no binding to the N-terminal domain or EGF5-6 domain of DLL3, indicating that EGF4 is involved in binding to 5E7. Binding kinetics (K_D) of the antibody to different DLL3 domains are listed in Table 37.

5 Table 37

Antibody	Construct	k_a (1/Ms)	k_d (1/s)	K_D (nM)
5E7	hDLL3 ECD	3.19e+05	6.47e-05	0.203
5E7	N-terminal		No binding	
5E7	EGF2-6	5.7e+05	3.82e-04	0.669
5E7	EGF3-6	1.45e+06	2.64e-04	0.184
5E7	EGF4-6	5.46e+05	6.10e-04	1.12
5E7	EGF5-6		No binding	

Example 9 – Epitope binning of anti-DLL3 antibodies

[0378] Binning of different anti-DLL3 antibodies against Stemcentrx antibody was performed by SPR using a Biacore™ 8K instrument. Briefly, murine anti-DLL3 antibodies were captured using an anti-mouse Fc antibody immobilized on a CM5 chip. This was followed by injections of human DLL3 ECD and the Stemcentrx anti-DLL3 antibody (San Francisco, CA) consecutively. Experiments were performed at 25 °C. Biacore™ 8K evaluation software was used for all data analysis. FIGs. 22A-22C show the binning profiles of anti-DLL3 antibodies corresponding to 2F7 (FIG. 22B), 5E7 (FIG. 22C), and 9E6 (FIG. 22A) clones to the ECD of DLL3. The antibody including the 9E6 clone binds to an epitope on DLL3 overlapping with the epitope to which the Stemcentrx anti-DLL3 antibody binds (FIG. 22A). The antibody including the 2F7 or 5E7 clone did not block the binding of the Stemcentrx antibody to the DLL3 ECD, suggesting that the antibodies corresponding to 2F7 (FIG. 22B) and 5E7 (FIG. 22C) bind to epitopes on DLL3 different from the one bound by the Stemcentrx antibody.

Example 10 – Determination of the melting temperatures of anti-DLL3 antibodies

[0379] Determination of the melting temperature of anti-DLL3 antibodies was done by differential scanning fluorimetry analysis using an Applied Biosystems QuantStudio3™ instrument (Thermo Fisher, Waltham, MA) in a temperature range of 15-95 °C. All samples were run in duplicate. Results were analyzed using Applied Biosystems Protein Thermal

Shift™ Software Version 13 (Thermo Fisher, Waltham, MA). As shown in FIG. 23, anti-DLL3 antibodies corresponding to clones 2H7, 8H9, 5E7, 2H6, and 10F5 showed melting temperatures above 70 °C.

Example 11 – Assessment of binding to recombinant human DLL3 and cross-reactivity with DLL1/DLL4

[0380] To investigate the binding of anti-DLL3 antibodies to human DLL3, wells of high binding flat-bottom plates were coated with recombinant human DLL3 diluted to 0.5 µg/ml. To assess cross-reactivity of the antibodies to DLL1/DLL4, which are closely related family members to DLL3, plates were coated with recombinant human DLL1 diluted to 1 µg/ml, or human DLL4 diluted to 0.5 µg/ml. After blocking the plates with PBS containing 1% BSA, a test anti-DLL3 antibody and a positive control antibody for each of DLL3 (R&D Systems, Minneapolis, MN, Cat#MAB4315), DLL1 (BioLegend, San Diego, CA, Cat#MHD1-314) and DLL4 (BioLegend, San Diego, CA, Cat#MHD4-46), respectively, were diluted serially starting from 10 µg/ml and added to the wells. Binding was detected using anti-mouse IgG-HRP and 3,3',5,5'-Tetramethylbenzidine (TMB) substrate. Signals were normalized to the corresponding positive control antibody.

[0381] As shown in FIG. 24, robust binding of anti-DLL3 antibodies to DLL3 in a dose-dependent manner was observed. Table 38 lists the calculated EC₅₀ based on the binding data from FIG. 24. Asterisks indicate anti-DLL3 antibodies that did not reach saturation of binding to DLL3; the corresponding EC₅₀ values are therefore estimates.

Table 38

DLL3 clone	ELISA EC ₅₀ (nM)
2F7	0.30
2H6	0.06
4E4	4.93*
5E7	0.07
8H9	4.58*
9E6	0.45
10F5	0.64
15H1	76.1*
Positive**	0.10

*does not reach saturation;
value is estimate

**commercial reagent mAb

[0382] As shown in FIG. 25A, the anti-DLL3 antibodies displayed little cross-reactivity with human DLL1. As shown in FIG. 25B, the anti-DLL3 antibodies corresponding to clones 2F7, 2H6, 4E4, 8H9, 9E6, and 15H1 also displayed little cross-reactivity with human DLL4. Two antibodies corresponding to clones 5E7 and 10F5 showed weak cross-reactive binding to DLL4 at very high concentrations measured by ELISA. Binding signals were normalized to the corresponding positive control anti-DLL3 antibody and the positive control anti-DLL4 antibody.

10 Example 12 – Assessment of antibody binding to cell-expressed human DLL3

[0383] DLL3-expressing human small cell lung cancer line NCI-H82 was used to assess the binding of anti-DLL3 antibodies to DLL3. Antibodies were serially diluted starting from 2 µg/mL and then incubated with the cells. Binding was detected using a fluorophore-conjugated anti-mouse IgG secondary antibody. Cells were analyzed by flow cytometry, and binding was expressed as mean fluorescence intensity (MFI) relative to the signal from secondary antibody-only control.

[0384] As shown in FIG. 26A, the anti-DLL3 antibodies corresponding to clones 2F7, 2H6, 4E4, 5E7, 8H9, 9E6, 10F5, and 15H1 (2 µg/mL) bound to DLL3 expressed on NCI-H82 cells. Binding of anti-DLL3 antibodies corresponding to clones 5E7, 2H6, 2F7, and 8H9 to DLL3 on NCI-H82 cells was dose-dependent, binding of antibodies corresponding to clones 9E6 and 10F5 to DLL3 was dose-dependent above a 5 nM dose, and binding of antibodies

corresponding to clone 15H1 to DLL3 on NCI-H82 cells was independent of dose (FIG. 26B).

Example 13 – Determination of extent of antibody internalization by DLL3⁺ SCLC lines

[0385] DLL3-expressing human small cell lung cancer cell lines SHP-77 and DMS-79 were used to assess internalization of anti-DLL3 antibodies upon binding to DLL3 on the surface of the cells. Antibodies were diluted to 10 µg/mL and incubated with the cells at 37 °C for 1, 2 or 3 hours, or on ice for 20 minutes. The remaining surface-bound antibodies were then detected using a fluorophore-conjugated anti-mouse IgG secondary antibody. Cells were analyzed by flow cytometry, and the antibody internalization was calculated as a percentage loss of mean fluorescence intensity (MFI) in comparison with the corresponding control condition, when the cells were incubated with the antibody on ice. As shown in FIG. 27, significant internalization of the anti-DLL3 antibodies on SHP-77 cells (FIG. 27A) and DMS-79 cells (FIG. 27B) was observed.

Example 14 – Binding of multi-specific binding proteins to DLL3-positive cells

[0386] The human myeloma cell line RPMI-8226 was transduced to express either the full-length (DLL3-D1-6) or a truncated (DLL3-D1-2) form of the extracellular portion of DLL3. An anti-DLL3 multi-specific binding protein and monoclonal antibody were diluted and incubated with DLL3⁺ RPMI-8226 cells. Binding was detected using a fluorophore conjugated anti-human IgG secondary antibody, and cells were analyzed by flow cytometry.

[0387] As shown in FIG. 28A and FIG. 28B, an anti-DLL3 monoclonal antibody and an anti-DLL3 multi-specific binding protein having the same DLL3-binding Fab bind with a similar dose-response to RPMI-8226 cells transduced to express DLL3-D1-6 or DLL3-D1-2, but anti-DLL3 multi-specific binding protein bind to a higher maximum.

Example 15 – Activation of NK cells by multi-specific binding proteins

[0388] Peripheral blood mononuclear cells (PBMCs) were isolated from human peripheral blood buffy coats using density gradient centrifugation. Isolated PBMCs were washed and NK cells were isolated using a negative selection technique with magnetic beads. The purity of isolated CD3⁺CD56⁺ NK cells was typically >90%. Isolated NK cells were cultured overnight in media containing 100 ng/mL IL-2.

[0389] RPMI-8226 cells transduced to express DLL3 were harvested from culture, pelleted, and re-suspended in culture media to a concentration of 10⁶ cells/mL. 50 µl of cell

suspension was added to individual wells of a 96-well plate. Anti-DLL3 multi-specific binding protein or anti-DLL3 monoclonal antibody was diluted in culture media, and 50 μ l added to duplicate wells containing cell suspension. To enable detection of CD107a and intracellular IFN γ , 50 μ l of activation cocktail [Brefeldin A (BioLegend, San Diego, CA; Cat# 420601), Monensin (BioLegend, San Diego, CA; Cat# 420701), 100 ng/mL IL-2, and fluorophore-conjugated anti-CD107a (BioLegend, San Diego, CA; Cat# 328620)] was added to each well.

[0390] Isolated primary NK cells were harvested from overnight culture, washed, and re-suspended at in culture media to a concentration of 10^6 cells/mL. 50 μ l of isolated primary NK cell suspension was added to RPMI-8226 cell-containing wells and incubated at 37 °C, 5% CO $_2$ for 4 hours.

[0391] Following co-culture, cells were stained for surface markers (CD107a and/or CD69), fixed, permeabilized with permeabilization/wash buffer (BioLegend, San Diego, CA; Cat# 421002), stained for intracellular IFN γ , and analyzed by flow cytometry.

[0392] As shown in FIG.29A and FIG. 29B, anti-DLL3 monoclonal antibody and anti-DLL3 multi-specific binding protein activate purified human NK cells co-cultured with DLL3-expressing target cells. As detected by flow cytometry, expression of CD107a and accumulation of intracellular IFN γ were comparable in NK cells treated with anti-DLL3 multi-specific binding protein and anti-DLL3 monoclonal antibody (FIG. 29A). Following a 20-hour co-culture with DLL3 $^{+}$ target cells, activation marker CD69 was upregulated to a greater extent on NK cells treated with the anti-DLL3 multi-specific binding protein as compared to the anti-DLL3 monoclonal antibody (FIG. 29B).

Example 16 – Enhanced NK cell-mediated lysis of DLL3 $^{+}$ target cells by multi-specific binding proteins

DELFI Cytotoxicity Assay

[0393] RPMI-8226 cells transduced to express DLL3 were harvested from culture, pelleted, and re-suspended in culture media to a concentration of 10^6 cells/mL for labeling with BATDA reagent (Perkin Elmer, Waltham, MA, Cat# AD0116) in accordance with the manufacturer's instructions. After labeling, cells were washed 3x with HEPES buffered saline, re-suspended at a concentration of 5×10^4 cells/mL in culture media, and 100ul of BATDA labeled cells were added to each well of the 96-well plate. Designated wells were

reserved to measure for spontaneous release from target cells, and all other wells were prepared for max lysis of target cells by addition of 1% Triton-X.

[0394] 50 μ l of diluted anti-DLL3 monoclonal antibody or anti-DLL3 multi-specific binding protein was added to designated wells. IL-2 activated, isolated NK cells (prepared as previously described in Example 15) were harvested from culture, washed, and re-suspended at a concentration of 5×10^5 cells/mL. 50 μ l of NK cell suspension was added to designated wells of the 96-well plate to make a total of 200 μ l culture volume and to achieve a final NK cell to RPMI-8226 target cell ratio of 5:1. Plates were incubated at 37 °C, 5% CO₂ for 2-3 hours.

[0395] Following co-culture, cells were pelleted by centrifugation at 200g for 5 minutes. 20 μ L of culture supernatant was transferred to a clean microplate and 200 μ L of room temperature europium solution was added to each well. The microplate was protected from light and incubated on a plate shaker at 250 rpm for 15 minutes. The microplate was read using either Victor3™ (Perkin Elmer, Waltham, MA) or SpectraMax i3X (Molecular Devices, San Jose, CA) instruments. % Specific lysis was calculated as follows:

$$\% \text{ Specific lysis} = [(\text{Experimental release} - \text{Spontaneous release}) / (\text{Maximum release} - \text{Spontaneous release})] \times 100\%$$

[0396] As shown in FIG. 30A, IL-2-activated human PBMC effector cells have limited basal cytotoxic activity against RPMI-8226 cells expressing DLL3-D1-2 after 2 hours co-culture (dotted line). Introduction of anti-DLL3 multi-specific binding protein in co-culture results in a 30% increase in specific lysis as compared to an anti-DLL3 monoclonal antibody having the same Fab arms.

Flow Cytometry Cytotoxicity Assay

[0397] RPMI-8226 cells transduced to express DLL3 were harvested from culture, pelleted, and re-suspended at a concentration of 10^5 cells/mL in culture media and 100 μ l of cell suspension was added to each well of a 96-well plate. Anti-DLL3 multi-specific binding protein or anti-DLL3 monoclonal antibody was diluted in culture media and 50 μ l of each was added to duplicate wells of the 96-well plate. Purified human NK cells (prepared as previously described in Example 15) were harvested from culture, washed, and re-suspended at 2×10^5 cells/mL in culture media. 50 μ l of NK cell suspension was added to wells except for RPMI-8226-only controls. The 96-well plate was incubated at 37 °C, 5% CO₂ for 20 hours. Following co-culture, cells were stained, fixed, and analyzed by flow cytometry.

[0398] As shown in FIG. 30B, IL-2-activated purified human NK cells exhibited basal lysis of RPMI-8226 cells expressing DLL3-D1-6 after 20 hours (dotted line). Co-cultures in the presence of anti-DLL3 monoclonal antibody did not result in a significant increase in cell lysis. However, co-cultures in the presence of anti-DLL3 multi-specific binding protein exhibited enhanced NK cell-mediated killing of RPMI-8226 target cells.

Example 17 – Humanization of murine antibody 5E7

[0399] Humanization of mouse 5E7 was accomplished by grafting mouse CDRs to appropriate human frameworks using molecular operating environment (MOE) protein modelling software. The CDR grafting was based on combination of best sequence match to human frameworks and by homology model. Human germline VH1-3 was selected as the most appropriate acceptor framework for variable heavy chain. For maintaining binding and structural integrity of the VH domain, three residues of the selected human framework were mutated back to the original mouse framework residues. Human germline VK1-39 was selected as the most appropriate acceptor framework for variable light chain. For maintaining binding and structural integrity of the VL domain, three residues of the selected human framework were mutated back to the original mouse framework residues. From this effort the best variant (clone h5E7) was selected.

[0400] The three residues in the VH mutated back to the original mouse framework residues were at Kabat positions 44, 71, and 76, as bolded and underlined in the h5E7 VH sequence below. The heavy chain CDR sequences were also identified and are underlined below.

h5E7 VH sequence

QVQLVQSGAEVKKPGASVKVSCKASGFNIKDDYIHVVRQAPGQGLEWMGWIDSEN
GDTEYASKFQGRVTITADTSANTAYMELSSLRSEDTAVYYCATSSYYSYDLFVYWG
QGTLVTVSS

[SEQ ID NO:668]

[0401] The three residues in the VL mutated back to the original mouse framework residues were at Kabat positions 2, 36, and 42, as bolded and underlined in the h5E7 VL sequence below. The light chain CDR sequences were also identified and are underlined below.

h5E7 VL sequence

DYQMTQSPSSLSASVGDRVITITCKSSQSLLHSNGKTYLNWLQQKPGQAPKLLLYLVS
 KLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCLQTTHLYTFGGGTKLEIK

[SEQ ID NO: 669]

5 **Example 18 – Affinity maturation of h5E7 by CDRH3 mutation**

[0402] A CDRH3 focused library with single, double and triple mutants of h5E7 was displayed as single chain variable fragment (scFv) on the surface of *Saccharomyces cerevisiae*. The starting library diversity was estimated to be around 10^6 . Three rounds of selections were carried out. The first round of selection was performed by magnetic activated cell sorting (MACS) and enriched clones that bound to 100 nM human DLL3 ECD (ECD of DLL3 was purchased from Adipogen (AG-40B-0151) and further purified in house using size exclusion chromatography before use in this experiment). The second and third rounds of selection were carried out on a fluorescence activated cell sorter (FACS). During the second round of selection, biotinylated human DLL3 was titrated down to 1 nM and variants in the library that bound better than parent h5E7 were gated and collected. The third round of selection was focused on enriching binders that have slower off-rate (kd) than the parent h5E7 clone. This was achieved by competing off bound biotinylated human DLL3 from relatively faster kd variants with excess of unbiotinylated hDLL3 or with the murine 5E7 monoclonal antibody (mAb). The clones enriched from the second and third rounds included h5E7-YD-C6, h5E7-YD-F3, h5E7-YD-A6, and h5E7-YD-B5, the sequence of which are shown in Table 2. Consensus sequences of the humanized 5E7 variants are also provided in Table 2.

[0403] The murine 5E7 and all the humanized versions were cloned and expressed as IgG1 mAbs. All heavy chain variable regions (including mouse 5E7) were cloned into the N-terminus of human IgG1 CH1-CH2-CH3 constant region. All light chain variable regions (including mouse 5E7) were cloned into N-terminus of human constant Kappa region. All clones were expressed in the EXPI293 system and purified using protein A MabSelect SuRe resin. When necessary, an additional step of SEC purification was performed.

[0404] The kinetics of binding of different humanized variants and parental murine 5E7 antibody to human DLL3-His was studied by Surface Plasmon Resonance using a Biacore 8K instrument. Briefly, anti-hFc and anti-mFc IgG proteins were covalently immobilized onto different channels of CM5 chip to allow simultaneous analysis of human and murine

antibodies. m5E7 was captured on the anti-mouse Fc channel and humanized variants were captured on the anti-human Fc channel at a desired capture level of ~ 56 RU in HBS-EP+ buffer supplemented with 0.1% BSA. Three buffer blanks and human DLL3-His (analyte) at concentrations 0.411-300 nM (in three fold dilutions) were injected over the surface with captured murine or humanized 5E7 for 300 second association time and let dissociate for 600 second at a flow rate of 30 μ L/min. The surfaces were subjected to regeneration with three 20-second pulses of 10 mM glycine pH 1.70 at a flow rate of 100 μ L/min between every concentration of analyte. The experiment was conducted at 37°C. Data were double referenced and a 1:1 fit model was applied to the sensorgrams in the Biacore Insight Evaluation software.

[0405] As shown in FIG. 46, the murine 5E7 and the humanized variants all bound human DLL3-His. The kinetic parameters of human DLL3-His binding to murine and humanized variants of 5E7 antibody variants were calculated and shown in Table 39.

Table 39

Antibody clone	Antigen	k_a (1/Ms)	k_d (1/s)	K_D (nM)	CDRH3
5E7	hDLL3	1.19×10^6	1.45×10^{-3}	1.22	SSYYSYDLFVY
h5E7	hDLL3	7.99×10^5	6.97×10^{-3}	8.72	SSYYSYDLFVY
h5E7-YD-C6	hDLL3	4.09×10^5	1.37×10^{-3}	3.35	SEYYSYDLFVY
h5E7-YD-F3	hDLL3	3.12×10^5	4.05×10^{-4}	1.29	SSYWSYDLLVY
h5E7-YD-A6	hDLL3	3.96×10^5	4.34×10^{-4}	1.10	SSYWSYDLFVY
h5E7-YD-B5	hDLL3	4.74×10^5	1.30×10^{-4}	0.27	STYWSYDLFVY

[0406] To assess the binding of the mAbs to DLL3 positive cells, human myeloma cell line RPMI-8226 was transduced to express the full-length extracellular portion of DLL3. Anti-DLL3 mAbs were diluted and incubated with DLL3⁺ RPMI-8226 cells. The cells were analyzed by flow cytometry and binding of a mAb was detected using a fluorophore conjugated anti-human IgG secondary antibody.

[0407] As shown in FIG. 47, conversion to a human backbone reduced the binding affinity of 5E7 to DLL3 presented on cells. After introducing mutations in heavy chain CDR3, the C6 and F3 variants of h5E7 showed similar binding affinities to cell surface DLL3

as the murine 5E7. The A6 variant exhibited similar binding affinity as h5E7, and the B5 variant bound with an intermediate affinity.

INCORPORATION BY REFERENCE

[0408] The entire disclosure of each of the patent documents and scientific articles
5 referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[0409] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein.
10 Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A protein comprising:

- (a) a first antigen-binding site that binds NKG2D;
- (b) a second antigen-binding site that binds Delta like canonical Notch

5 ligand 3 (DLL3); and

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

2. The protein according to claim 1, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ
10 ID NO:679, 668, 671, 673, 675, 677, or 130, and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:669 or 131.

3. The protein according to claim 1 or 2, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

15 a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:132;

a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:133; and

20 a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO: 680, 670, 672, 674, 676, 678, or 134.

4. The protein according to claim 3, wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:135;

25 a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:136; and

a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:137.

5. The protein according to claim 1, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:114 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:115.

5 6. The protein according to claim 1 or 5, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:116;

10 a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:117; and

a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:118.

7. The protein according to claim 6, wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

15 a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:119;

a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:120; and

20 a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:121.

8. The protein according to claim 1, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:122 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:123.

25 9. The protein according to claims 1 or 8, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:124;

30 a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:125; and

a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:126.

10. The protein according to claim 9, wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

5 a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:127;

a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:128; and

10 a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:129.

11. The protein according to claim 1, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:138 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:139.

15 12. The protein according to claim 1 or 11, wherein the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:140;

20 a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:141; and

a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:142.

13. The protein according to claim 12, wherein the light chain variable domain of the second antigen-binding site comprises an amino acid sequence including:

25 a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:143;

a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:144; and

30 a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:145.

14. A protein comprising:

- (a) a first antigen-binding site that binds NKG2D;
- (b) a second antigen-binding site that binds mucin 1 (MUC1 or MUC1-C),

5 Plexin-A1, tumor necrosis factor receptor superfamily member 10B (TNFRSF10B), six-transmembrane epithelial antigen of prostate member 1 (STEAP1), CUB domain-containing protein 1 (CDCP1), tyrosine-protein kinase-like 7 (PTK7), AXL receptor tyrosine kinase (AXL), receptor tyrosine-protein kinase ERBB-3 (ERBB-3), endothelin receptor type B (EDNRB), tyrosinase related protein -1 (TYRP1), or oxidized low-density lipoprotein
10 receptor 1 (OLR1); and

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

15. The protein according to claim 14, wherein the second antigen-binding site binds MUC1 (or MUC1-C), the heavy chain variable domain of the second antigen-binding site
15 comprises an amino acid sequence at least 90% identical to SEQ ID NO:203 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:207.

16. The protein according to claim 14 or 15, wherein the second antigen-binding site binds MUC1 (or MUC1-C), the heavy chain variable domain of the second antigen-binding
20 site comprises an amino acid sequence at least 90% identical to SEQ ID NO:211 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:215.

17. The protein according to claim 14, wherein the second antigen-binding site binds Plexin-A1, the heavy chain variable domain of the second antigen-binding site comprises an
25 amino acid sequence at least 90% identical to SEQ ID NO:220 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:224.

18. The protein according to claim 14, wherein the second antigen-binding site binds Plexin-A1, the heavy chain variable domain of the second antigen-binding site comprises an
30 amino acid sequence at least 90% identical to SEQ ID NO:228 and the light chain variable

domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:232.

19. The protein according to claim 14, wherein the second antigen-binding site binds TNFRSF10B, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:237 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:241.

20. The protein according to claim 14, wherein the second antigen-binding site binds TNFRSF10B, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:245 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:249.

21. The protein according to claim 14, wherein the second antigen-binding site binds TNFRSF10B, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:253 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:257.

22. The protein according to claim 14, wherein the second antigen-binding site binds STEAP1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:262 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:266.

23. The protein according to claim 14, wherein the second antigen-binding site binds STEAP1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:270 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:274.

24. The protein according to claim 14, wherein the second antigen-binding site binds CDCP1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:279 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:283, or SEQ ID NO:287.

25. The protein according to claim 14, wherein the second antigen-binding site binds PTK7, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:292 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:296.

26. The protein according to claim 14, wherein the second antigen-binding site binds AXL, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:301 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:305.

27. The protein according to claim 14, wherein the second antigen-binding site binds AXL, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:309 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:313.

28. The protein according to claim 14, wherein the second antigen-binding site binds ERBB-3, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:318 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:322.

29. The protein according to claim 14, wherein the second antigen-binding site binds ERBB-3, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:326 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:330.

30. The protein according to claim 14, wherein the second antigen-binding site binds ERBB-3, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:334 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:338.

31. The protein according to claim 14, wherein the second antigen-binding site binds EDNRB, the heavy chain variable domain of the second antigen-binding site comprises an

amino acid sequence at least 90% identical to SEQ ID NO:343 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:347.

32. The protein according to claim 14, wherein the second antigen-binding site binds EDNRB, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:351 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:355.

33. The protein according to claim 14, wherein the second antigen-binding site binds TYRP1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:360 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:364.

34. The protein according to claim 14, wherein the second antigen-binding site binds TYRP1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:368 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:372.

35. The protein according to claim 14, wherein the second antigen-binding site binds TYRP1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:376 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:380.

36. The protein according to claim 14, wherein the second antigen-binding site binds OLR1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:385 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:389.

37. The protein according to claim 14, wherein the second antigen-binding site binds OLR1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:393 and the light chain variable

domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:397.

38. A protein comprising:

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

(b) a second antigen-binding site that binds disintegrin and metalloproteinase domain-containing protein 12 (ADAM12), urokinase plasminogen activator receptor (PLAUR), C-C motif chemokine receptor 6 (CCR6), or ephrin type-A receptor 4 (EPHA4); and

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

39. The protein according to claim 38, wherein the second antigen-binding site binds PLAUR, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:405 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:409.

40. The protein according to claim 38, wherein the second antigen-binding site binds PLAUR, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:413 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:417.

41. The protein according to claim 38, wherein the second antigen-binding site binds CCR6, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:422 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:426.

42. The protein according to any one of claims 38, wherein the second antigen-binding site binds CCR6, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:430 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:434.

43. The protein according to claim 38, wherein the second antigen-binding site binds EPHA4, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:439 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:443.

44. The protein according to claim 38, wherein the second antigen-binding site binds EPHA4, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:447 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:451.

45. A protein comprising:

- (a) a first antigen-binding site that binds NKG2D;
- (b) a second antigen-binding site that binds CD14, CD163, colony stimulating factor 3 receptor (CSF3R), sialic acid-binding Ig-like lectin 9 (Siglec-9), integrin alpha M (ITGAM), V-type immunoglobulin domain-containing suppressor of T-cell activation (VISTA), V-set domain-containing T-cell activation inhibitor 1(B7-H4), C-C chemokine receptor type 1 (CCR1), leucine rich repeat containing 25 (LRRC25), platelet activating factor receptor (PTAFR), signal regulatory protein beta 1 (SIRPB1), Toll-like receptor 2 (TLR2), Toll-like receptor 4 (TLR4), CD300 molecule like family member b (CD300LB), ATPase Na⁺/K⁺ transporting subunit alpha 3 (ATP1A3), or C-C chemokine receptor type 5 (CCR5); and
- (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.

46. The protein according to claim 45, wherein the second antigen-binding site binds CD14, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:456 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:460.

47. The protein according to claim 45, wherein the second antigen-binding site binds CD14, the heavy chain variable domain of the second antigen-binding site comprises a CDR1 sequence identical to the amino acid sequence of SEQ ID NO:464, a CDR2 sequence identical to the amino acid sequence of SEQ ID NO:465, and a CDR3 sequence identical to

the amino acid sequence of SEQ ID NO:466; and the light chain variable domain of the second antigen-binding site comprises a CDR1 sequence identical to the amino acid sequence of SEQ ID NO:467, a CDR2 sequence identical to the amino acid sequence of SEQ ID NO:468, and a CDR3 sequence identical to the amino acid sequence of SEQ ID NO:469.

- 5 48. The protein according to claim 45, wherein the second antigen-binding site binds CD163, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:471 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:475.
- 10 49. The protein according to claim 45, wherein the second antigen-binding site binds CD163, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:479 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:483.
- 15 50. The protein according to claim 45, wherein the second antigen-binding site binds CSF3R, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:488 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:492.
- 20 51. The protein according to claim 45, wherein the second antigen-binding site binds Siglec-9, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:497 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:501.
- 25 52. The protein according to claim 45, wherein the second antigen-binding site binds Siglec-9, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:505 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:509.
- 30 53. The protein according to claim 45, wherein the second antigen-binding site binds ITGAM, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:514 and the light chain variable

domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:518.

54. The protein according to claim 45, wherein the second antigen-binding site binds CCR1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:523 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:527.

55. The protein according to claim 45, wherein the second antigen-binding site binds CCR1, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:531 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:535.

56. The protein according to claim 45, wherein the second antigen-binding site binds TLR2, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:544 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:548.

57. The protein according to claim 45, wherein the second antigen-binding site binds TLR2, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:552 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:556.

58. The protein according to claim 45, wherein the second antigen-binding site binds TLR4, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:561 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:565.

59. The protein according to claim 45, wherein the second antigen-binding site binds TLR4, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:569 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:573.

60. The protein according to claim 45, wherein the second antigen-binding site binds CCR5, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:581 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:585.

61. The protein according to claim 45, wherein the second antigen-binding site binds CCR5, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:589 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:593.

62. The protein according to claim 45, wherein the second antigen-binding site binds B7-H4, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:598 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:602.

63. The protein according to claim 45, wherein the second antigen-binding site binds B7-H4, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:606 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:610.

64. The protein according to claim 45, wherein the second antigen-binding site binds VISTA, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:615 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:619.

65. The protein according to claim 45, wherein the second antigen-binding site binds VISTA, the heavy chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:623 and the light chain variable domain of the second antigen-binding site comprises an amino acid sequence at least 90% identical to SEQ ID NO:627.

66. The protein according to any one of claims 45 to 65, wherein the protein further comprises a site that binds a tumor-associated antigen.

67. The protein according to claim 66, wherein the tumor-associated antigen is selected from the group consisting of human epidermal growth factor receptor 2 (HER2), CD20, CD33, B-cell maturation antigen (BCMA), prostate-specific membrane (PSMA), DLL3, ganglioside GD2 (GD2), CD123, anoctamin-1 (Ano1), mesothelin, carbonic anhydrase IX (CAIX), tumor-associated calcium signal transducer 2 (TROP2), carcinoembryonic antigen (CEA), claudin-18.2, receptor tyrosine kinase-like orphan receptor 1 (ROR1), trophoblast glycoprotein (5T4), glycoprotein nonmetastatic melanoma protein B (GPNMB), folate receptor-alpha (FR-alpha), pregnancy-associated plasma protein A (PAPP-A), CD37, epithelial cell adhesion molecule (EpCAM), CD2, CD19, CD30, CD38, CD40, CD52, CD70, CD79b, fms-like tyrosine kinase 3 (FLT3), glypican 3 (GPC3), B7 homolog 6 (B7H6), C-C chemokine receptor type 4 (CCR4), C-X-C motif chemokine receptor 4 (CXCR4), receptor tyrosine kinase-like orphan receptor 2 (ROR2), CD133, HLA class I histocompatibility antigen, alpha chain E (HLA-E), epidermal growth factor receptor (EGFR/ERBB-1), insulin-like growth factor 1-receptor (IGF1R), human epidermal growth factor receptor 3 (HER3)/ERBB-3, human epidermal growth factor receptor 4 (HER4)/ERBB-4, MUC1, tyrosine protein kinase MET (cMET), signaling lymphocytic activation molecule F7 (SLAMF7), prostate stem cell antigen (PSCA), MHC class I polypeptide-related sequence A (MICA), MHC class I polypeptide-related sequence B (MICB), TNF-related apoptosis inducing ligand receptor 1 (TRAILR1), TNF-related apoptosis inducing ligand receptor 2 (TRAILR2), melanoma associated antigen 3 (MAGE-A3), B-lymphocyte activation antigen B7.1 (B7.1), B-lymphocyte activation antigen B7.2 (B7.2), cytotoxic T-lymphocyte associated protein 4 (CTLA4), programmed cell death protein 1 (PD1), programmed cell death 1 ligand 1 (PD-L1), and CD25.
68. The protein according to any one of claims 1 to 37 or 45 to 67, wherein the first antigen-binding site binds to NKG2D in humans, non-human primates, and rodents.
69. The protein according to any one of claims 1-67, wherein the first antigen-binding site binds to NKG2D in humans.
70. The protein according to any one of claims 1 to 69, wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.
71. The protein according to claim 70, wherein the heavy chain variable domain and the light chain variable domain are present on the same polypeptide.

72. The protein according to claim 70 or 71, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.
73. The protein according to claim 72, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.
74. The protein according to claim 72 or 73, 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.
75. A protein according to any one of claims 1 to 74, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to an amino acid sequence selected from: SEQ ID NO:1, SEQ ID NO:41, SEQ ID NO:49, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:85, SEQ ID NO:650, SEQ ID NO:653, SEQ ID NO:656, SEQ ID NO:659, SEQ ID NO:662, SEQ ID NO:665, and SEQ ID NO:93.
76. The protein according to any one of claims 1 to 74, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:41 and a light chain variable domain at least 90% identical to SEQ ID NO:42.
77. The protein according to any one of claims 1 to 74, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:49 and a light chain variable domain at least 90% identical to SEQ ID NO:50.
78. The protein according to any one of claims 1 to 74, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:57 and a light chain variable domain at least 90% identical to SEQ ID NO:58.
79. The protein according to any one of claims 1 to 74, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:59 and a light chain variable domain at least 90% identical to SEQ ID NO:60.
80. The protein according to any one of claims 1 to 74, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:61 and a light chain variable domain at least 90% identical to SEQ ID NO:62.

81. The protein according to any one of claims 1 to 74, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:69 and a light chain variable domain at least 90% identical to SEQ ID NO:70.
82. The protein according to any one of claims 1 to 74, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:77 and a light chain variable domain at least 90% identical to SEQ ID NO:78.
83. The protein according to any one of claims 1 to 74, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:85, SEQ ID NO:650, SEQ ID NO:653, SEQ ID NO:656, SEQ ID NO:659, SEQ ID NO:662, or SEQ ID NO:665, and a light chain variable domain at least 90% identical to SEQ ID NO:86.
84. The protein according to any one of claims 1 to 74, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:93 and a light chain variable domain at least 90% identical to SEQ ID NO:94.
85. The protein according to any one of claims 1 to 74, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:101 and a light chain variable domain at least 90% identical to SEQ ID NO:102.
86. The protein according to any one of claims 1 to 74, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:103 and a light chain variable domain at least 90% identical to SEQ ID NO:104.
87. The protein according to any one of claims 1 to 69, wherein the first antigen-binding site is a single-domain antibody.
88. The protein according to claim 87, wherein the single-domain antibody is a V_HH fragment or a V_{NAR} fragment.
89. The protein according to any one of claims 1 to 69, or 87 to 88, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.
90. The protein according to claim 89, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.
91. The protein according to any one of claims 1 to 71 or 75 to 88, wherein the second antigen-binding site is a single-domain antibody.

92. The protein of claim 91, wherein the second antigen-binding site is a V_HH fragment or a V_{NAR} fragment.
93. The protein according to any one claims 1 to 92, wherein the protein comprises a portion of an antibody Fc domain sufficient to bind CD16, wherein the antibody Fc domain
5 comprises hinge and CH2 domains.
94. The protein according to claim 93, wherein the antibody Fc domain comprises hinge and CH2 domains of a human IgG1 antibody.
95. The protein according to claim 93 or 94, wherein the Fc domain comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody.
- 10 96. The protein according to claim 95, wherein the Fc domain comprises amino acid sequence at least 90% identical to the Fc domain of human IgG1 and differs at one or more positions selected from the group consisting of Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, K439.
- 15 97. A formulation comprising a protein according to any one of claims 1 to 96 and a pharmaceutically acceptable carrier.
98. A cell comprising one or more nucleic acids encoding a protein according to any one of claims 1 to 96.
99. A method of directly and/or indirectly enhancing tumor cell death, the method
20 comprising exposing a tumor microenvironment and natural killer cells to a protein according to any one of claims 1 to 96.
100. A method of treating a cancer, wherein the method comprises administering a protein according to any one of claims 1 to 96 or a formulation according to claim 97 to a patient.
101. A method of treating a cancer, wherein the cancer expresses DLL3, the method
25 comprising administration to a patient in need thereof a protein according to any one of claims 1 to 13, or a formulation comprising a protein according to any one of claims 1 to 13 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of small cell lung cancer, large cell neuroendocrine carcinoma, glioblastoma, Ewing sarcoma, and cancers with a neuroendocrine phenotype.
- 30 102. A method of treating a cancer, wherein the cancer expresses MUC1 or MUC1-C, the method comprising administration to a patient in need thereof a protein according to any one

of claims 14 to 16, or a formulation comprising a protein according to any one of claims 14 to 16 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of gastric cancer, colorectal cancer, pancreatic cancer, breast cancer, endometrial cancer, lung cancer, bladder cancer, cervical cancer, head and neck cancer, ovarian cancer, renal cell cancer, and multiple myeloma.

103. A method of treating a cancer, wherein the cancer expresses Plexin-A1, the method comprising administration to a patient in need thereof a protein according to any one of claims 14, 17 or 18, or a formulation comprising a protein according to any one of claims 14, 17 or 18 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of head and neck cancer, gastric cancer, pancreatic cancer, prostate cancer, and glioma.

104. A method of treating a cancer, wherein the cancer expresses TNFRSF10B, the method comprising administration to a patient in need thereof a protein according to any one of claims 14 or 19 to 21, or a formulation comprising a protein according to any one of claims 14 or 19 to 21 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of liver cancer, pancreatic cancer, stomach cancer, renal cancer, breast cancer, ovarian cancer, endometrial cancer, and melanoma.

105. A method of treating a cancer, wherein the cancer expresses STEAP1, the method comprising administration to a patient in need thereof a protein according to any one of claims 14, 22 or 23, or a formulation comprising a protein according to any one of claims 14, 22 or 23 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of prostate cancer, bladder cancer, colon cancer, pancreatic cancer, ovarian cancer, testicular cancer, breast cancer, cervical cancer and Ewing sarcoma.

106. A method of treating a cancer, wherein the cancer expresses CDCP1, the method comprising administration to a patient in need thereof a protein according to claim 14 or 24, or a formulation comprising a protein according to claim 14 or 24 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of colon cancer, lung cancer, gastric cancer, breast cancer, pancreatic carcinomas, head and neck cancer, bladder cancer, ovarian cancer, endometrial cancer, and skin cancer.

107. A method of treating a cancer, wherein the cancer expresses PTK7, the method comprising administration to a patient in need thereof a protein according to claim 14 or 25, or a formulation comprising a protein according to claim 14 or 25 and a pharmaceutically

acceptable carrier, wherein the cancer is selected from the group consisting of lung cancer, head and neck cancer, stomach cancer, prostate cancer, testicular cancer, endometrial cancer, breast cancer, melanoma, skin cancer, and leukemia.

108. A method of treating a cancer, wherein the cancer expresses AXL, the method
5 comprising administration to a patient in need thereof a protein according to any one of claims 14, 26 or 27, or a formulation comprising a protein according to any one of claims 14, 26 or 27 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of breast cancer, lung cancer, colon cancer, prostate cancer, renal cancer, esophageal cancer, liver cancer, pancreatic cancer, Kaposi's sarcoma, acute myeloid
10 leukemia, glioma, and mesothelioma.

109. A method of treating a cancer, wherein the cancer expresses ERBB-3, the method comprising administration to a patient in need thereof a protein according to any one of claims 14 or 28 to 30, or a formulation comprising a protein according to any one of claims 14 or 28 to 30 and a pharmaceutically acceptable carrier, wherein the cancer is selected from
15 the group consisting of prostate cancer, bladder cancer, breast cancer, ovarian cancer, colon cancer, pancreas cancer, stomach cancer, oral cavity cancer, head and neck cancer, lung cancer and melanoma.

110. A method of treating a cancer, wherein the cancer expresses EDNRB, the method comprising administration to a patient in need thereof a protein according to any one of
20 claims 14, 31 or 32, or a formulation comprising a protein according to any one of claims 14, 31 or 32 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of melanoma, uveal melanoma, and glioma.

111. A method of treating a cancer, wherein the cancer expresses TYRP1, the method comprising administration to a patient in need thereof a protein according to any one of
25 claims 14 or 33 to 35, or a formulation comprising a protein according to any one of claims 14 or 33 to 35 and a pharmaceutically acceptable carrier, wherein the cancer is melanoma.

112. A method of treating a cancer, wherein the cancer expresses OLR1, the method comprising administration to a patient in need thereof a protein according to any one of claims 14, 36 or 37, or a formulation comprising a protein according to any one of claims 14,
30 36 or 37 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of gastric cancer, colorectal cancer, pancreatic cancer, prostate cancer, breast cancer, and endometrial cancer.

113. A method of treating a cancer, wherein the cancer expresses ADAM12, the method comprising administration to a patient in need thereof a protein according to claim 38, or a formulation comprising a protein according to claim 38 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of prostate cancer, breast cancer, basal cell carcinoma, squamous cell carcinoma, melanoma, pancreatic cancer, bladder cancer, colorectal cancer, lung cancer, liver cancer, esophageal cancer, Non-Hodgkin's lymphoma, ovarian cancer, and uterine cancer.

114. A method of treating a cancer, wherein the cancer expresses PLAUR, the method comprising administration to a patient in need thereof a protein according to any one of claims 38 to 40, or a formulation comprising a protein according to any one of claims 38 to 40 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of breast cancer, colorectal cancer, non-small cell lung cancer, multiple myeloma, and oral cancer.

115. A method of treating a cancer, wherein the cancer expresses CCR6, the method comprising administration to a patient in need thereof a protein according to any one of claims 38, 41 or 42, or a formulation comprising a protein according to any one of claims 38, 41 or 42 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of colorectal cancer, breast cancer, prostate cancer, cervical cancer, liver cancer, lung cancer, and cutaneous T-cell lymphoma.

116. A method of treating a cancer, wherein the cancer expresses EPHA4, the method comprising administration to a patient in need thereof a protein according to any one of claims 38, 43 or 44, or a formulation comprising a protein according to any one of claims 38, 43 or 44 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of melanoma, glioma, prostate cancer, breast cancer, small cell lung cancer, endometrial cancer, esophageal cancer, gastric cancer, and colorectal cancer.

117. A method of treating a cancer in a patient, wherein a tumor microenvironment in the patient comprises a myeloid-derived suppressor cell (MDSC) or tumor-associated macrophage (TAM) expressing one or more than one antigen selected from the group consisting of CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and CCR5, the method comprising administration to the patient in need thereof a protein according to any one of claims 45 to 67,

or a formulation comprising a protein according to any one of claims 45 to 67 and a pharmaceutically acceptable carrier, wherein the cancer is a myeloid cell malignancy.

118. The method according to claim 117, wherein the cancer is selected from the group consisting of acute myeloid leukemia, myelodysplastic and/or myeloproliferative neoplasms, acute lymphoblastic leukemia, B-cell lymphoma, chronic neutrophilic leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia.

119. A method of treating a cancer in a patient, wherein a tumor microenvironment in the patient comprises a myeloid-derived suppressor cell (MDSC) or tumor-associated macrophage (TAM) expressing one or more than one antigen selected from the group consisting of CD14, CD163, CSF3R, Siglec-9, ITGAM, VISTA, B7-H4, CCR1, LRRC25, PTAFR, SIRPB1, TLR2, TLR4, CD300LB, ATP1A3, and CCR5, the method comprising administration to the patient in need thereof a protein according to any one of claims 45 to 67, or a formulation comprising a protein according to any one of claims 45 to 67 and a pharmaceutically acceptable carrier, wherein the cancer is selected from the group consisting of bladder cancer, colon cancer, prostate cancer, breast cancer, glioblastoma, hepatocellular carcinoma, head and neck cancer, lung cancer, ovarian cancer, pancreatic cancer, kidney cancer and melanoma.

FIG. 1

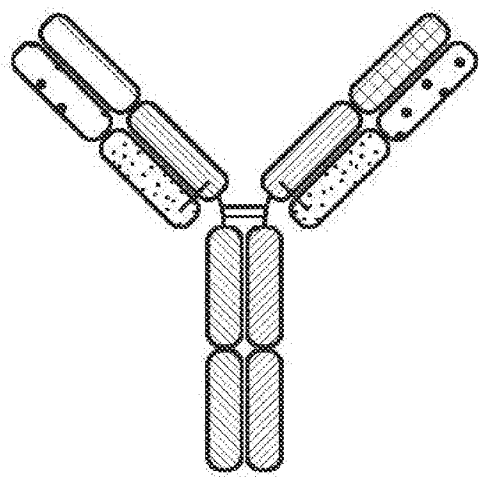


FIG. 2

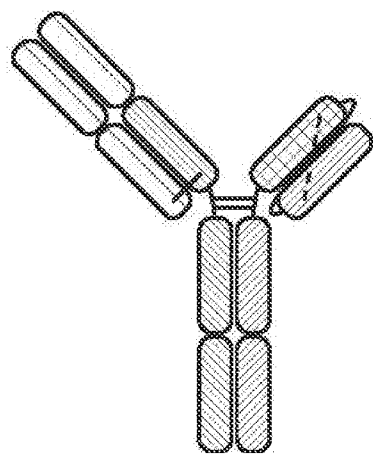


FIG. 3

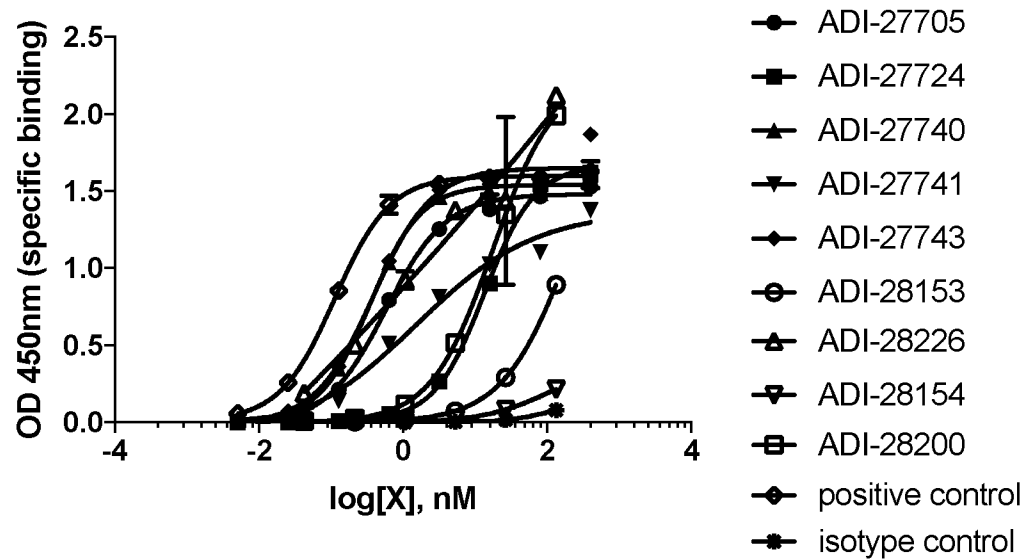


FIG. 4

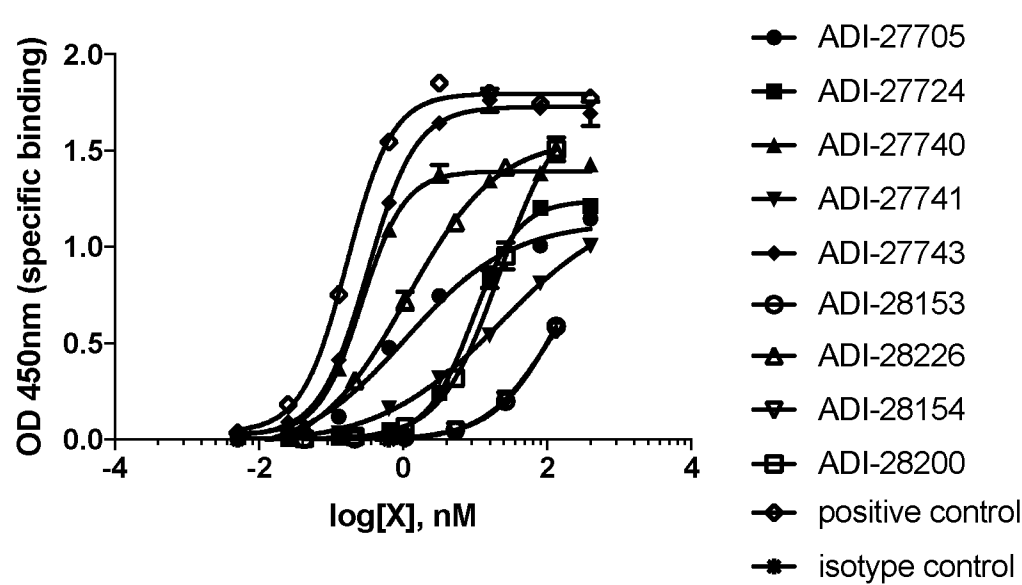


FIG. 5

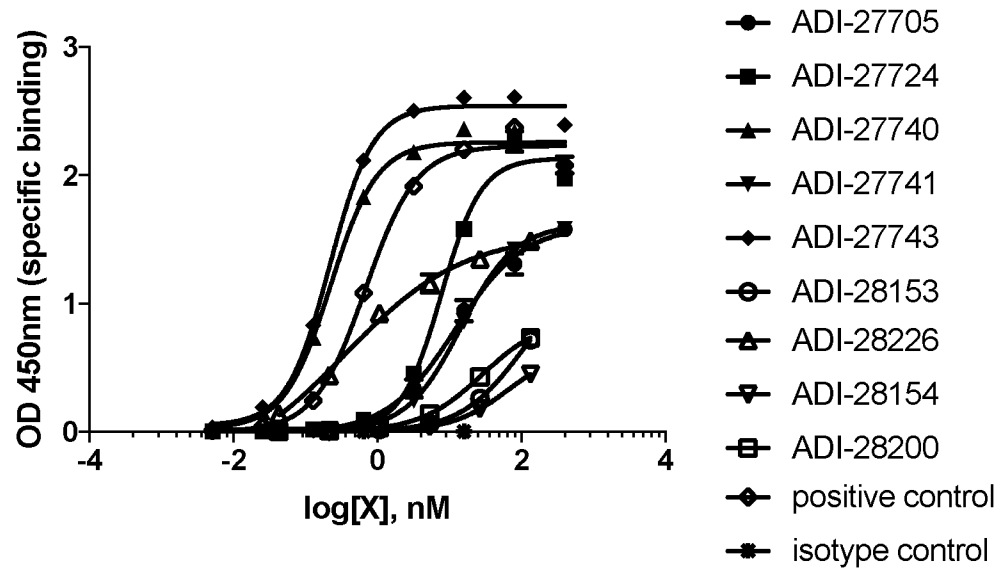


FIG. 6

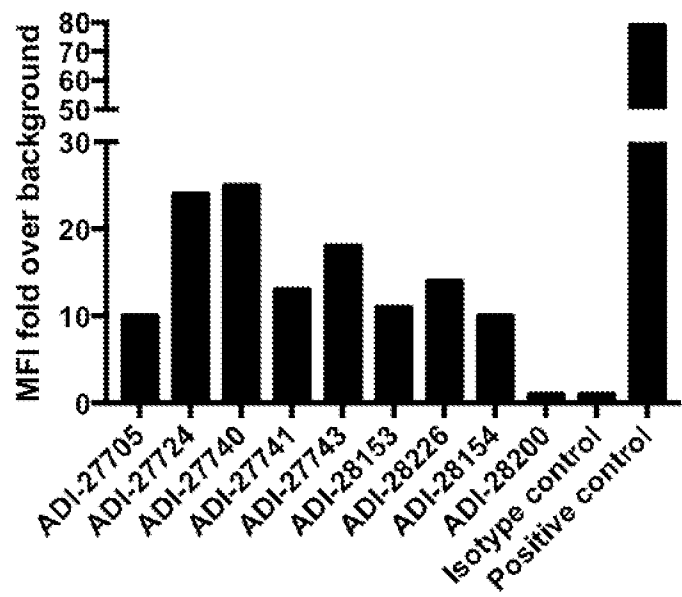


FIG. 7

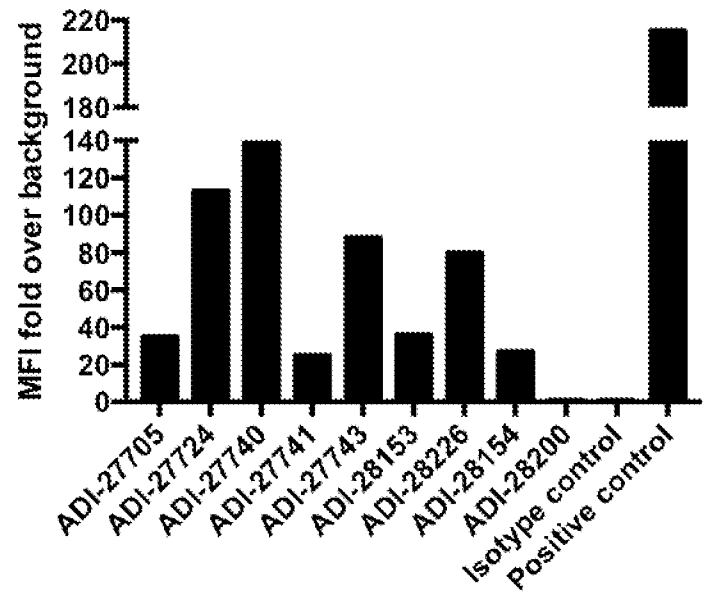


FIG. 8

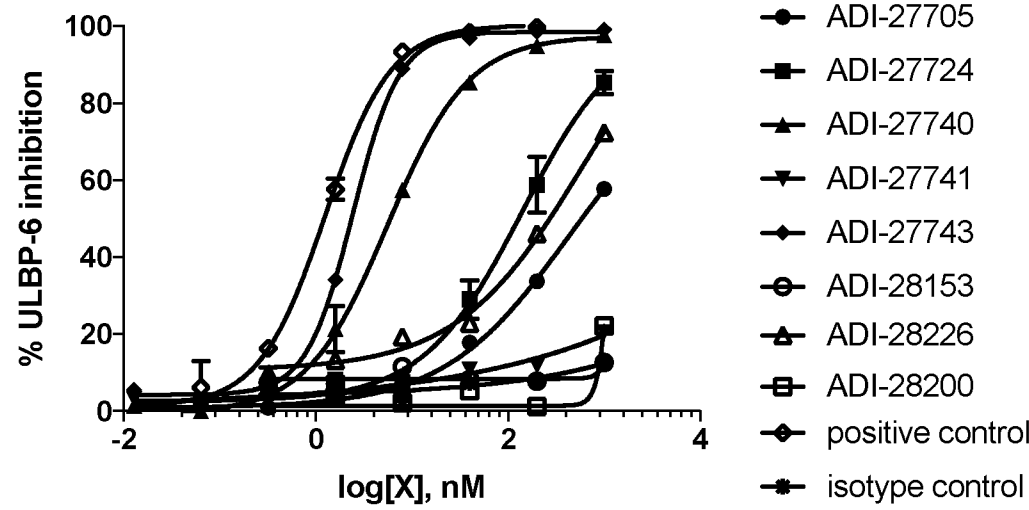


FIG. 9

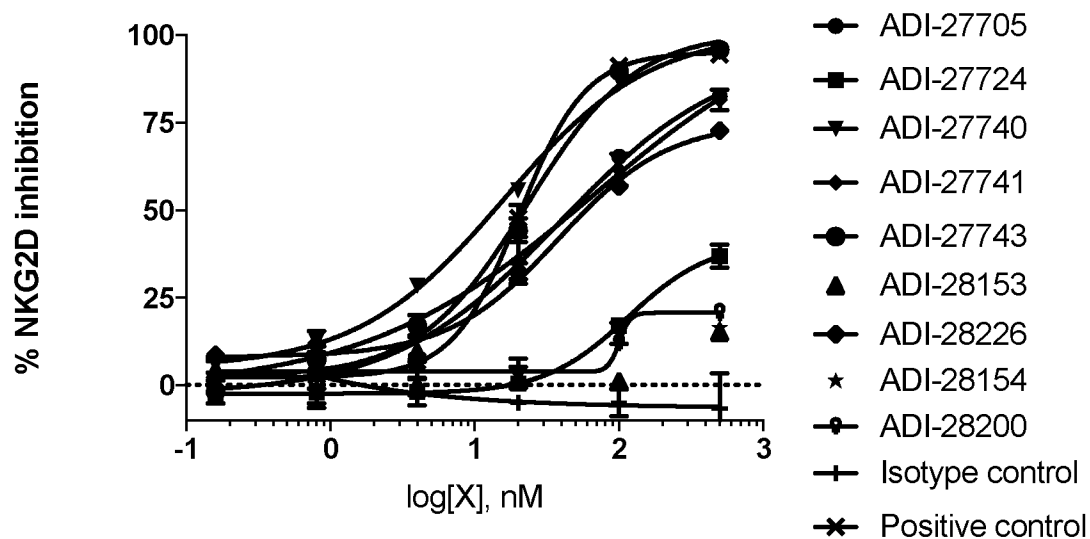


FIG. 10

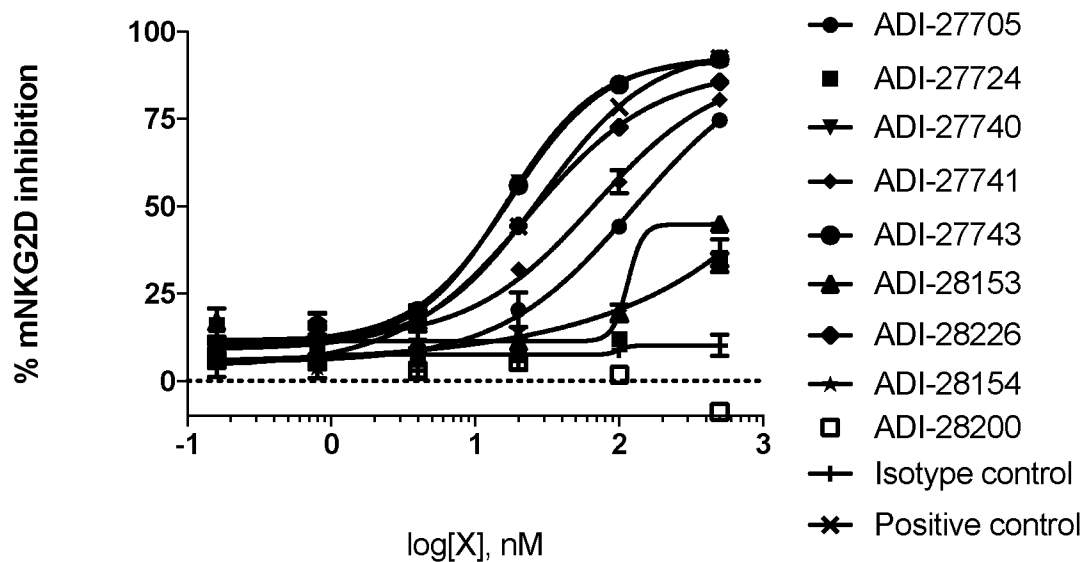


FIG. 11

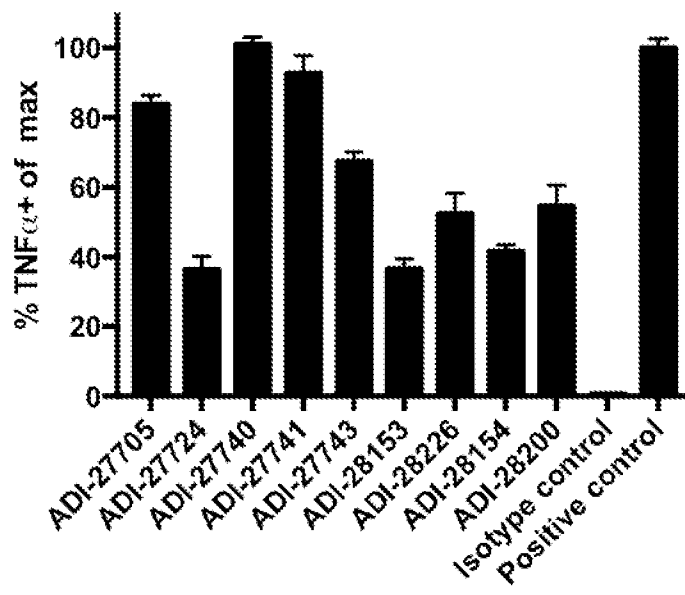


FIG. 12

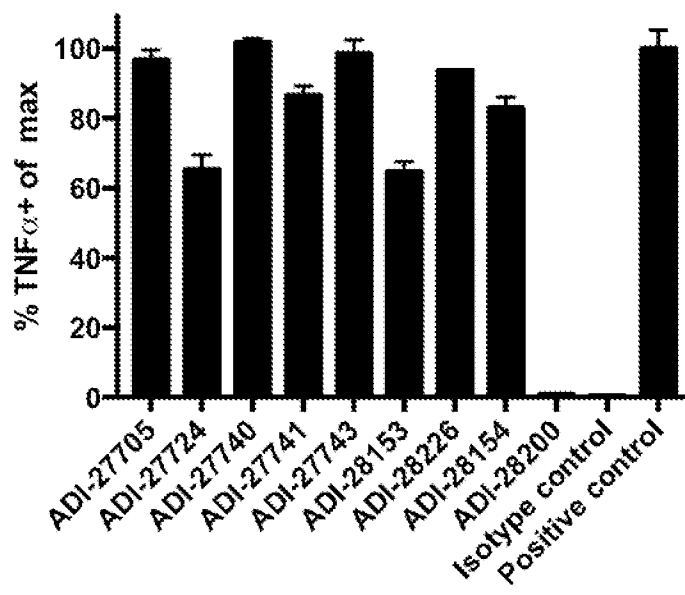


FIG. 13

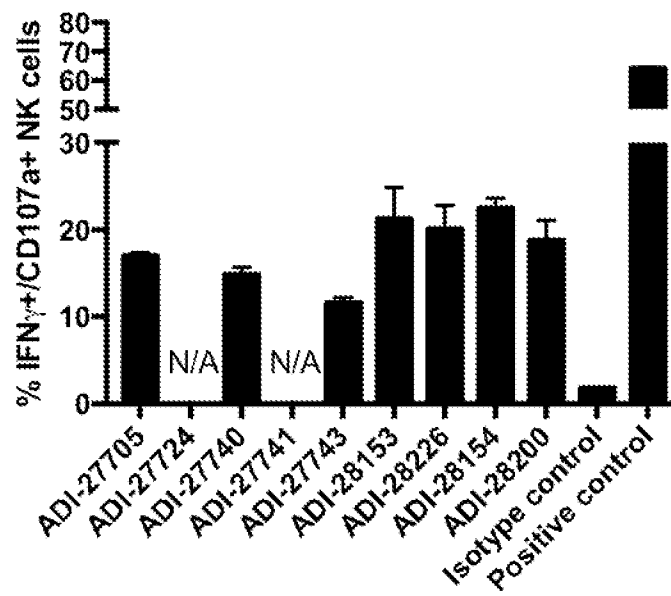


FIG. 14

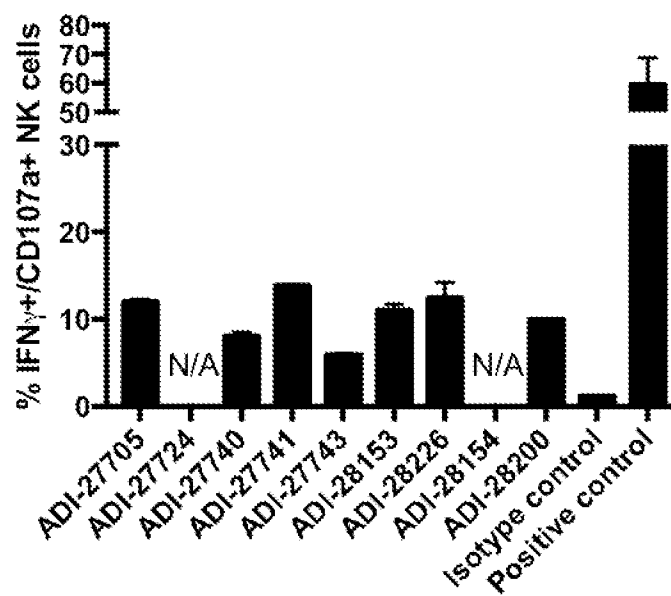


FIG. 15

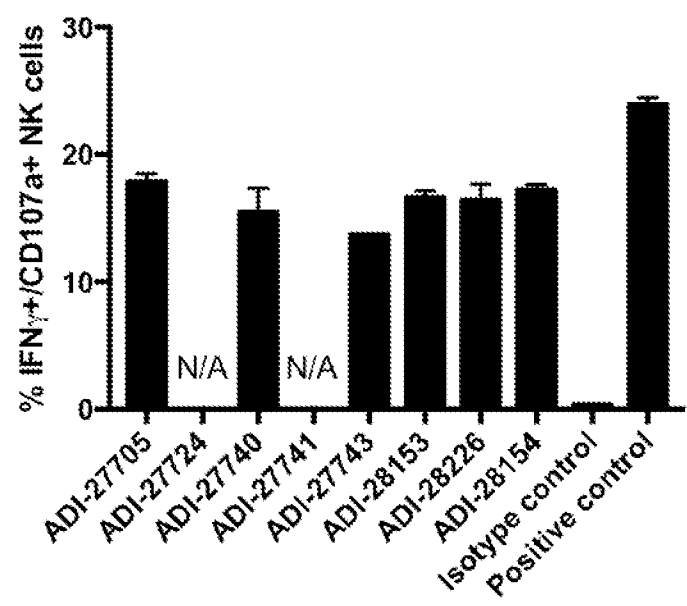


FIG. 16

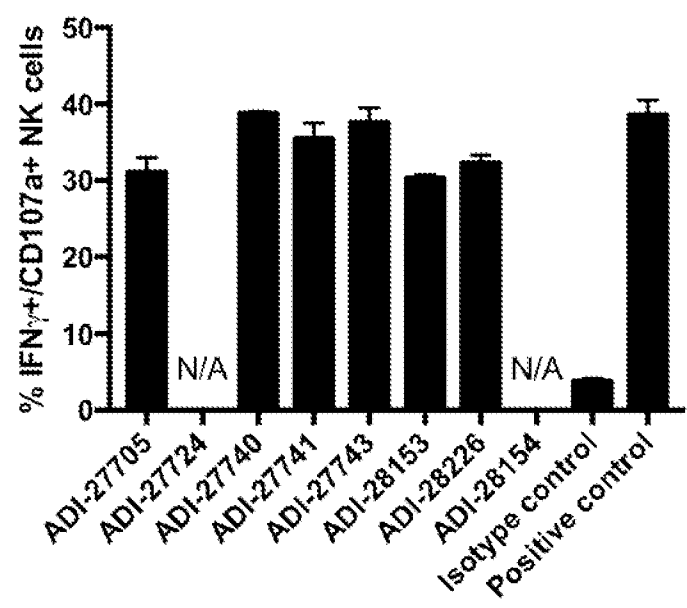


FIG. 17

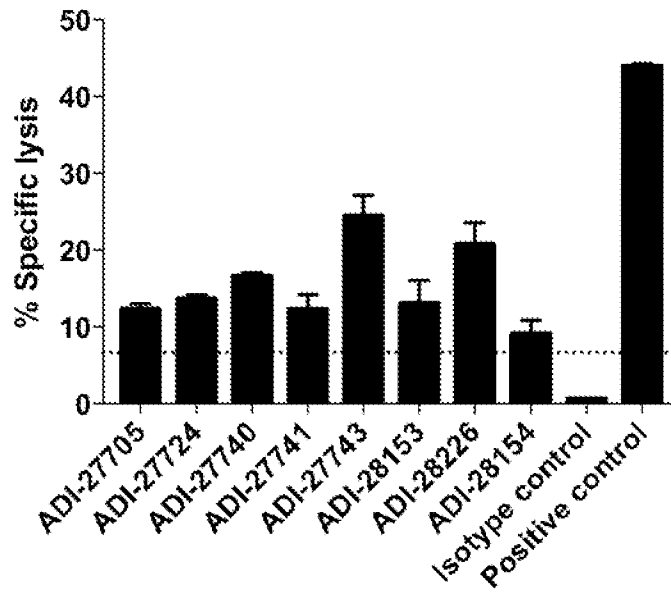


FIG. 18

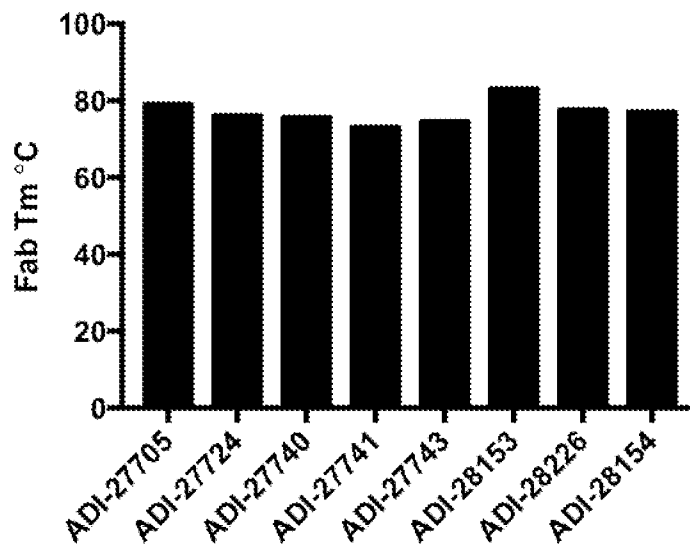


FIG. 19A

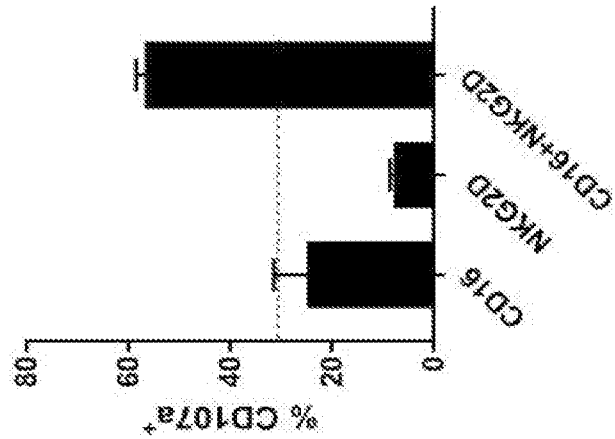


FIG. 19B

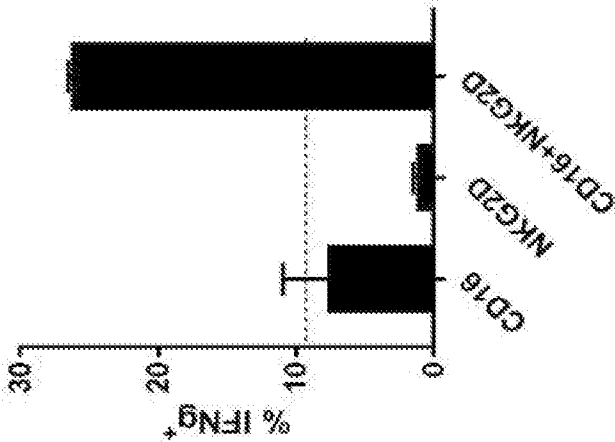


FIG. 19C

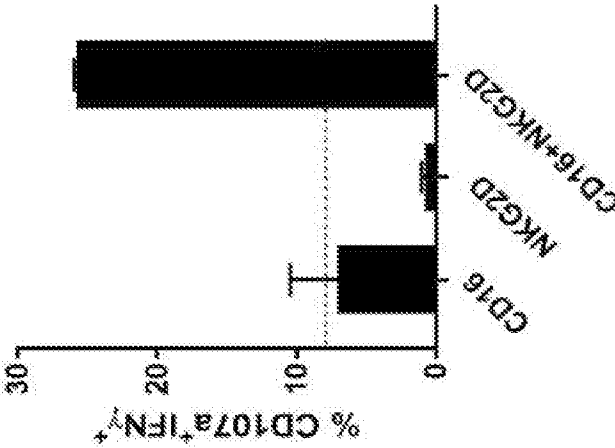


FIG. 20

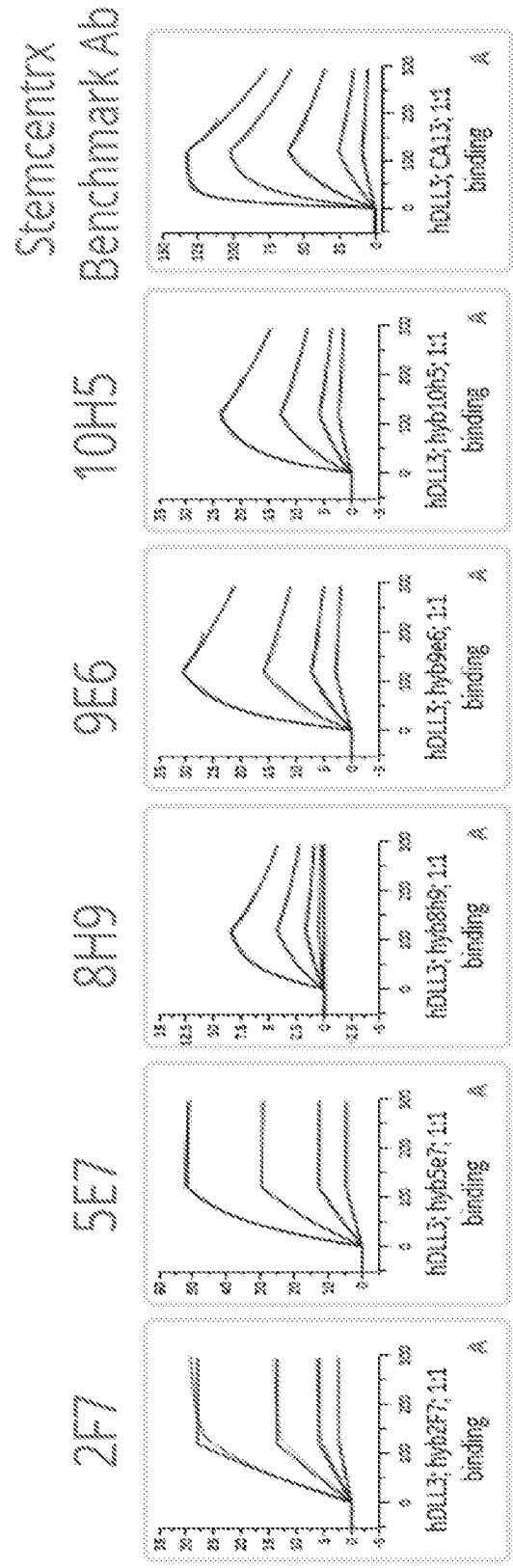


FIG. 21A

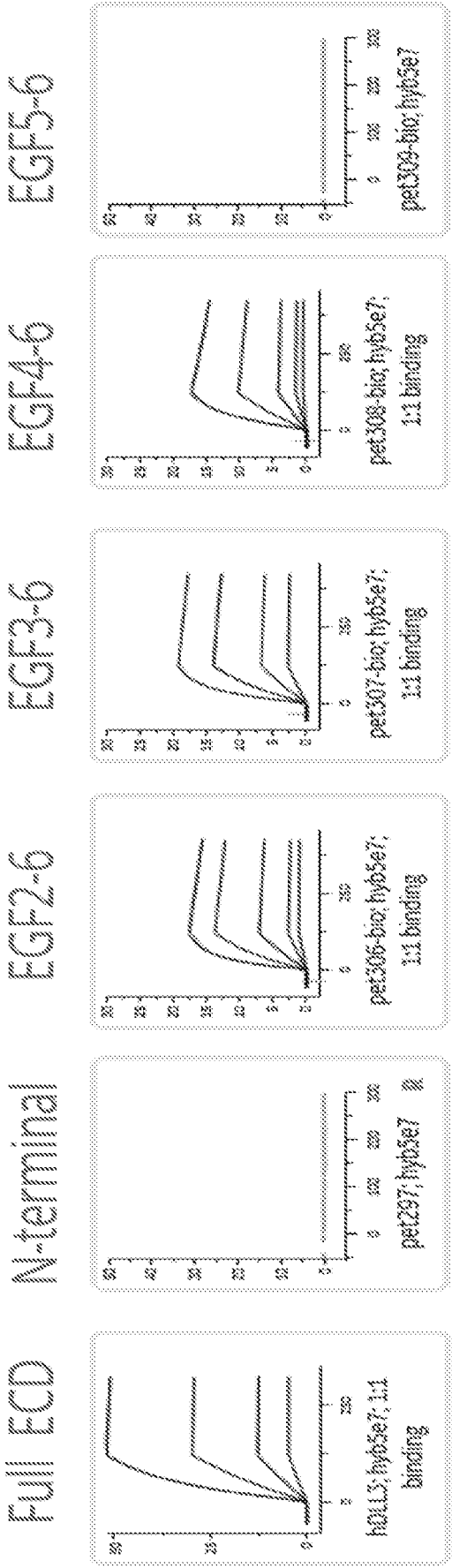


FIG. 21B

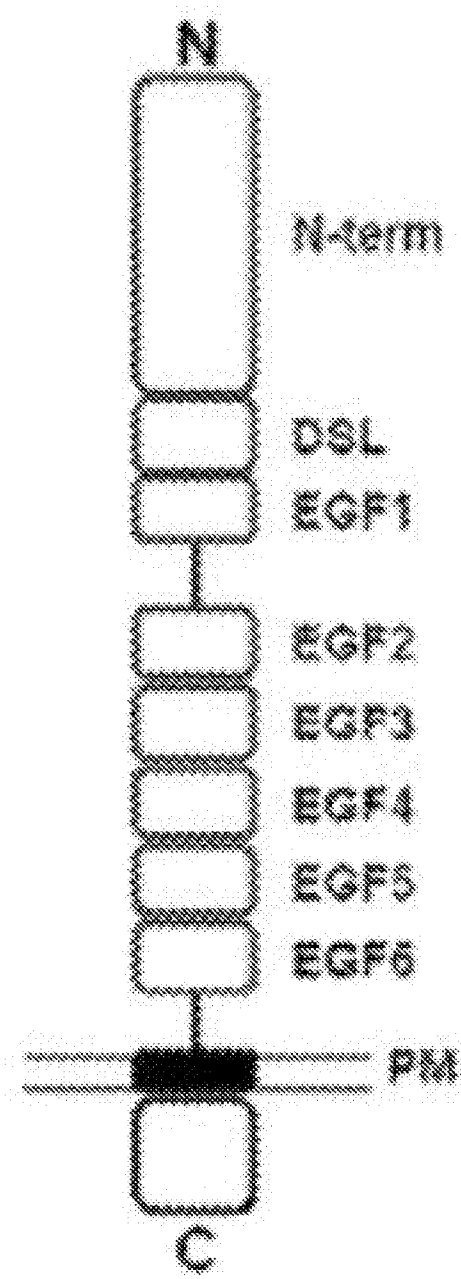


FIG. 22A

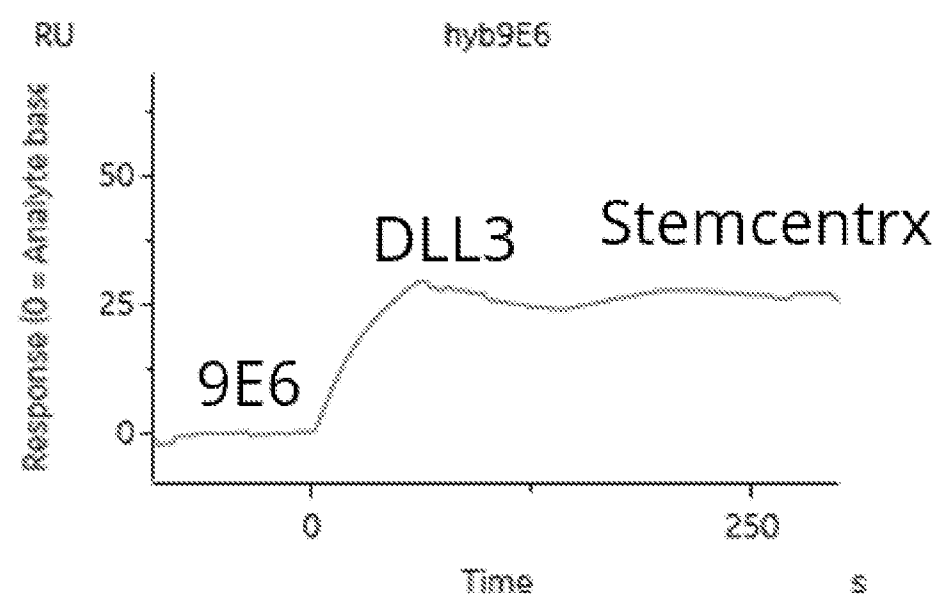


FIG. 22B

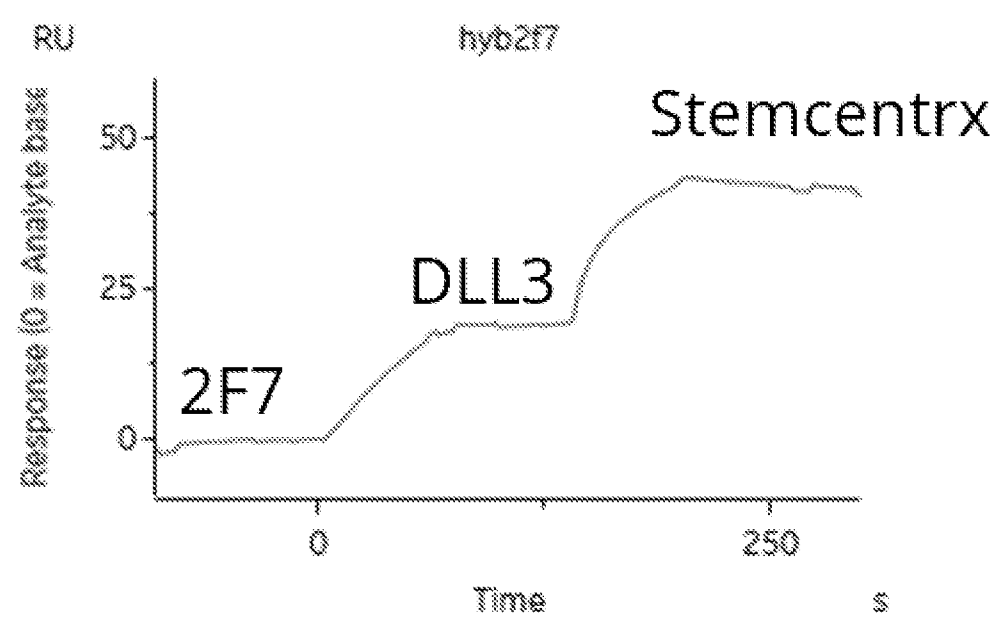


FIG. 22C

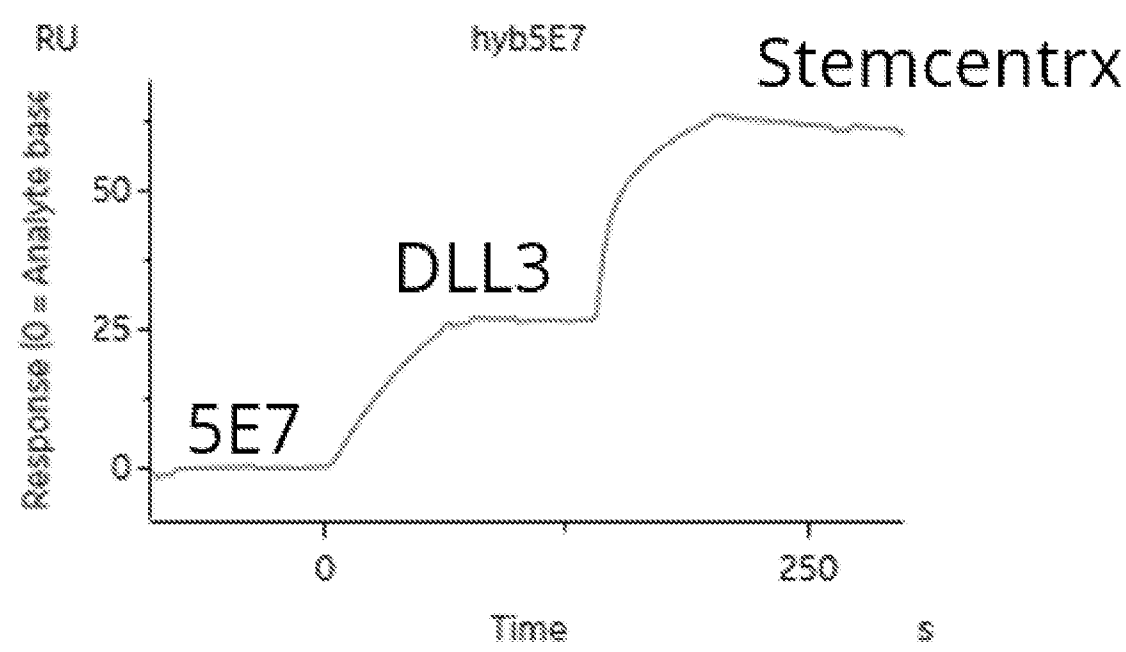


FIG. 23

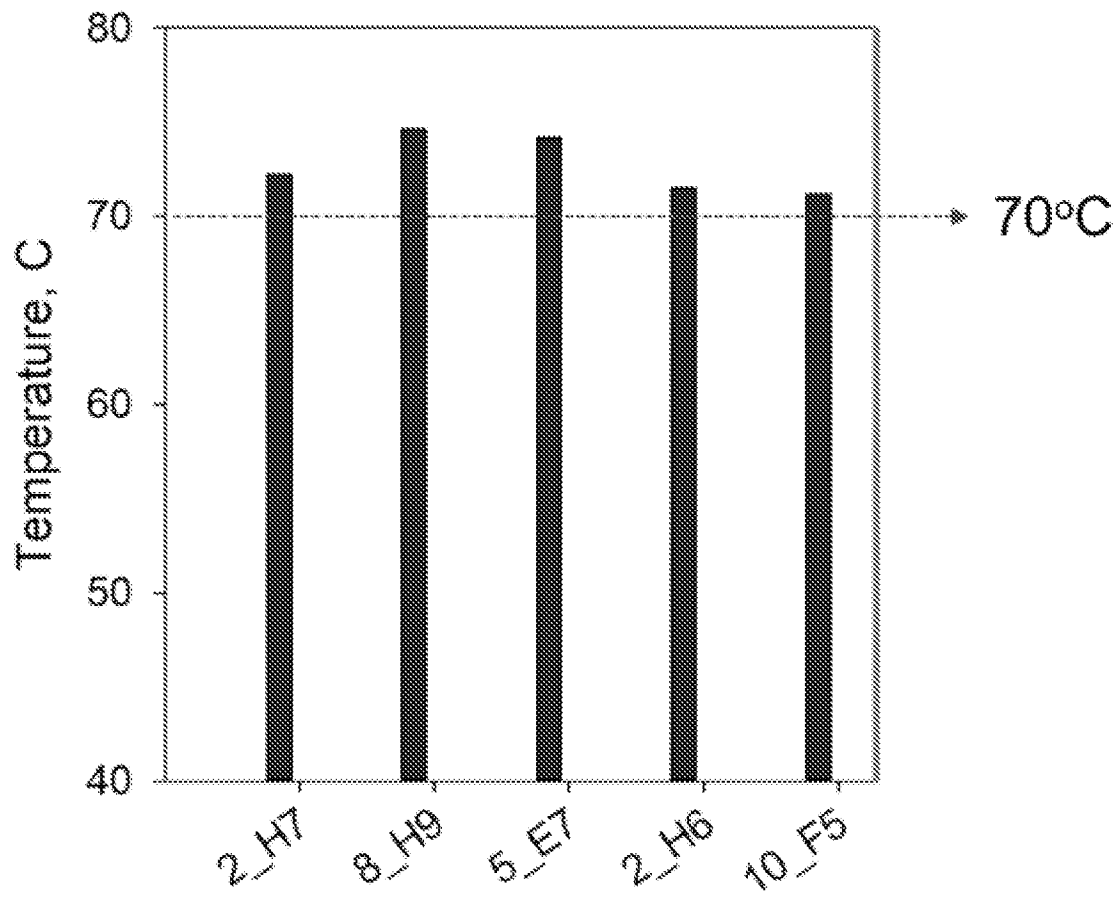


FIG. 24

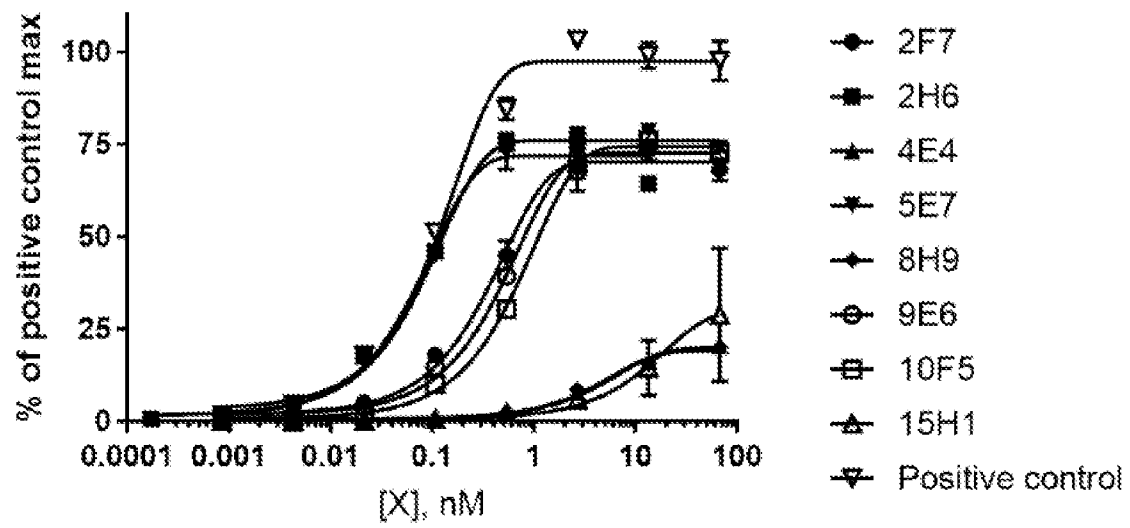


FIG. 25A

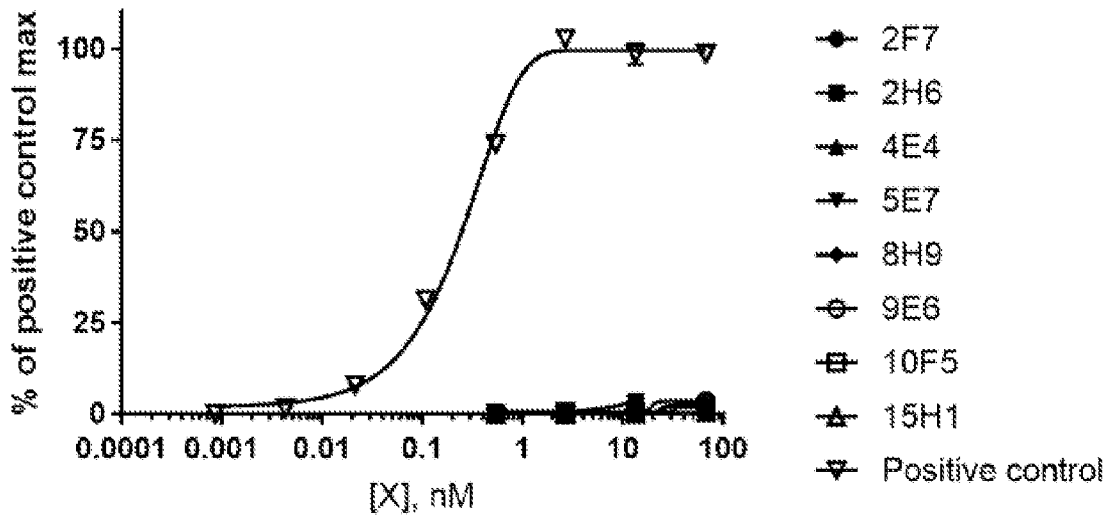


FIG. 25B

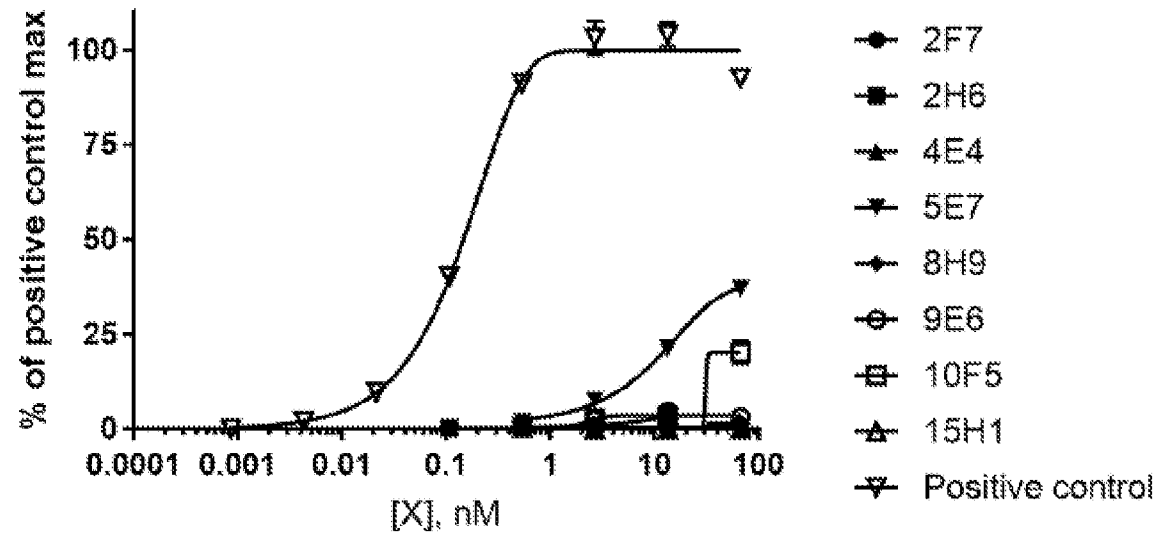


FIG. 26A

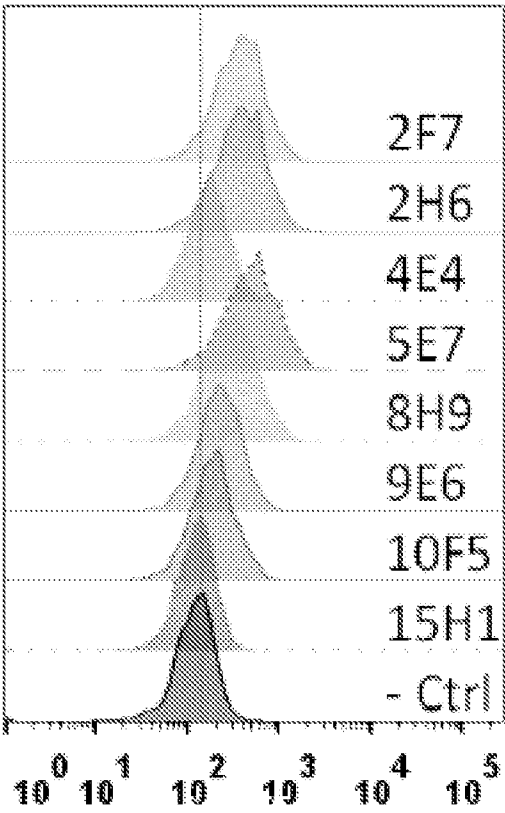


FIG. 26B

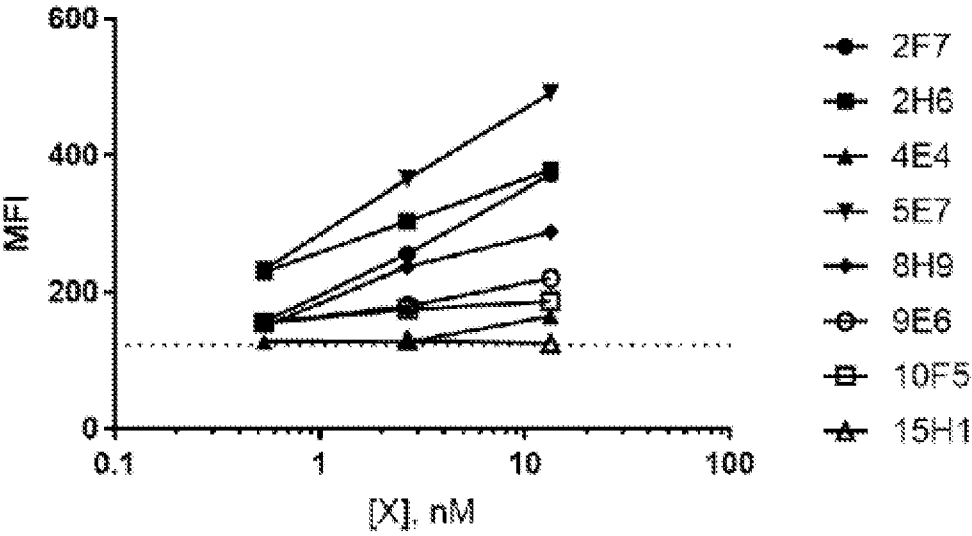


FIG. 27A

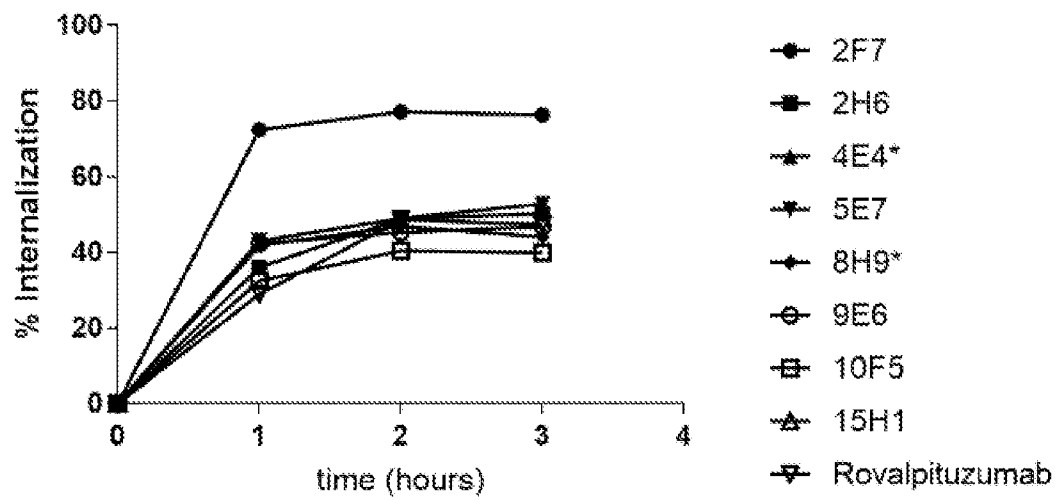
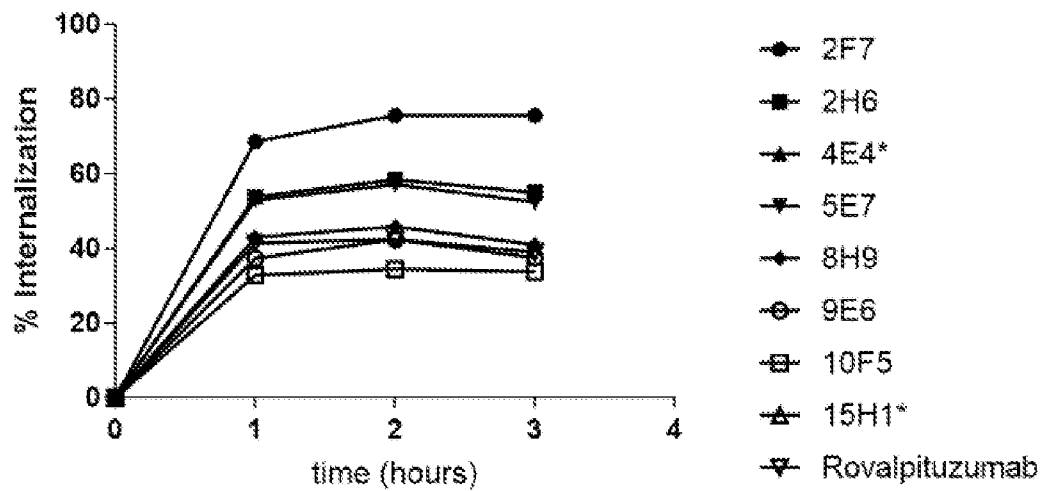


FIG. 27B



*binding insufficient to assess internalization

FIG. 28A

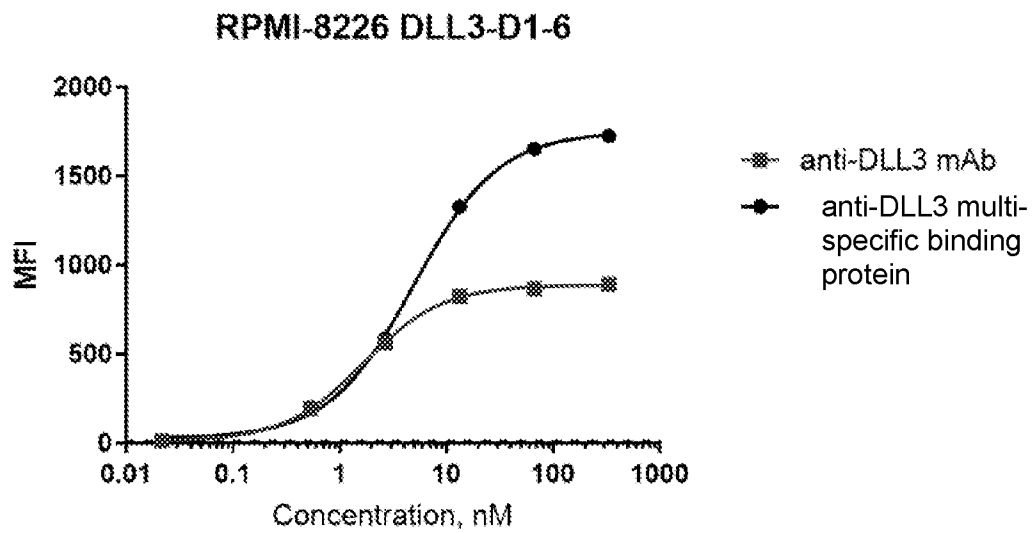


FIG. 28B

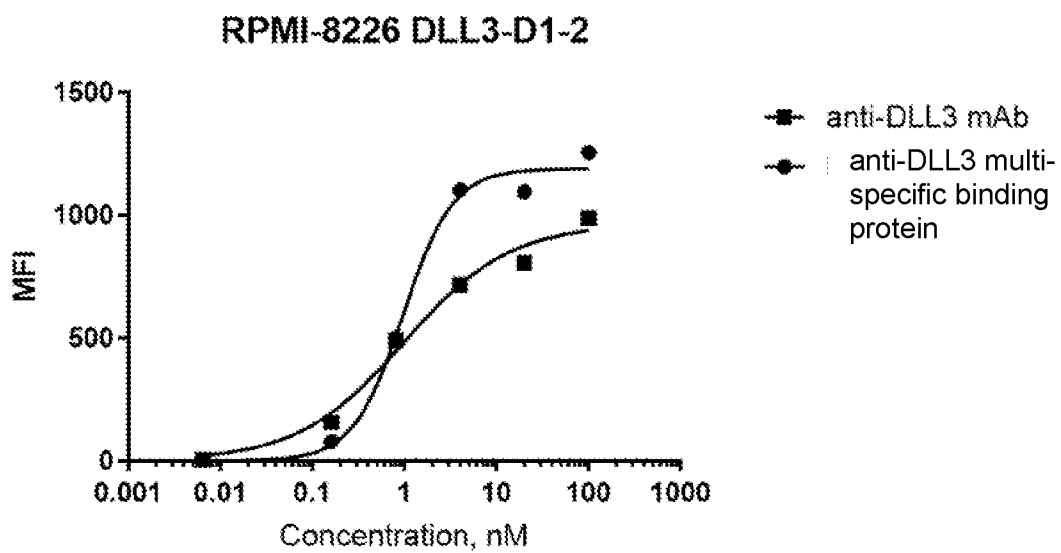


FIG. 29A

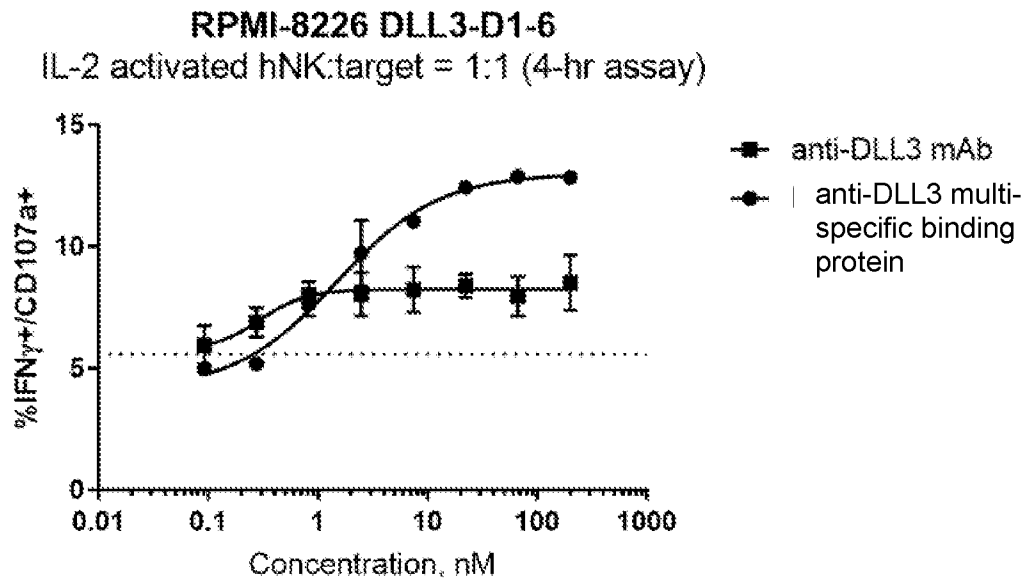


FIG. 29B

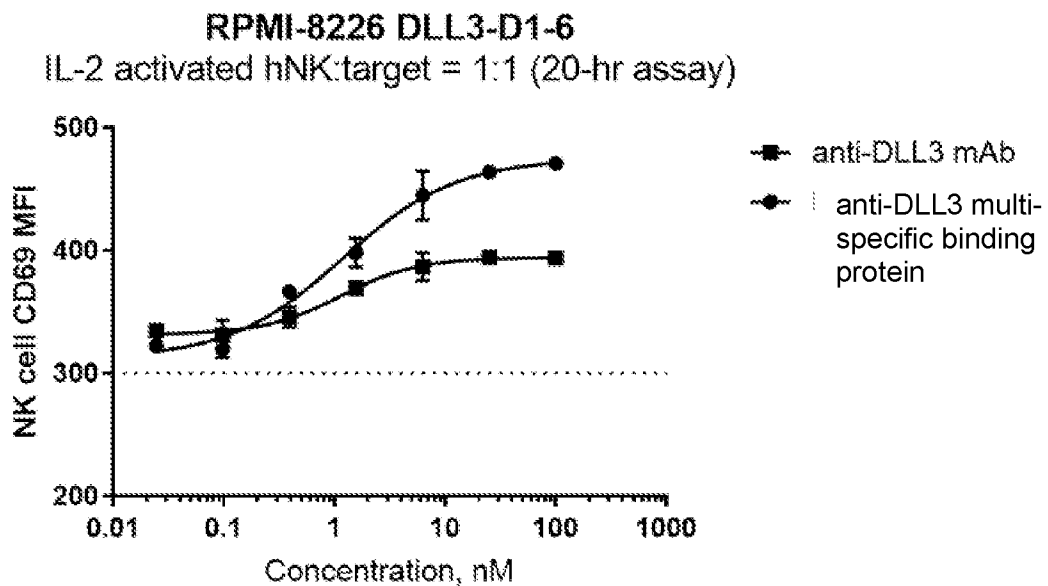


FIG. 30A

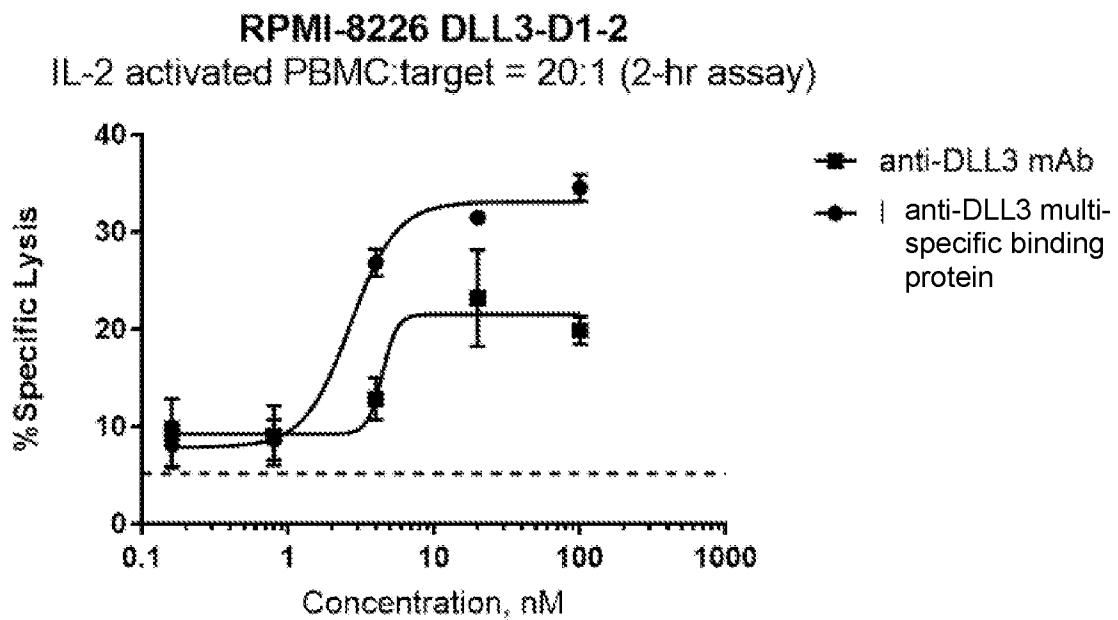


FIG. 30B

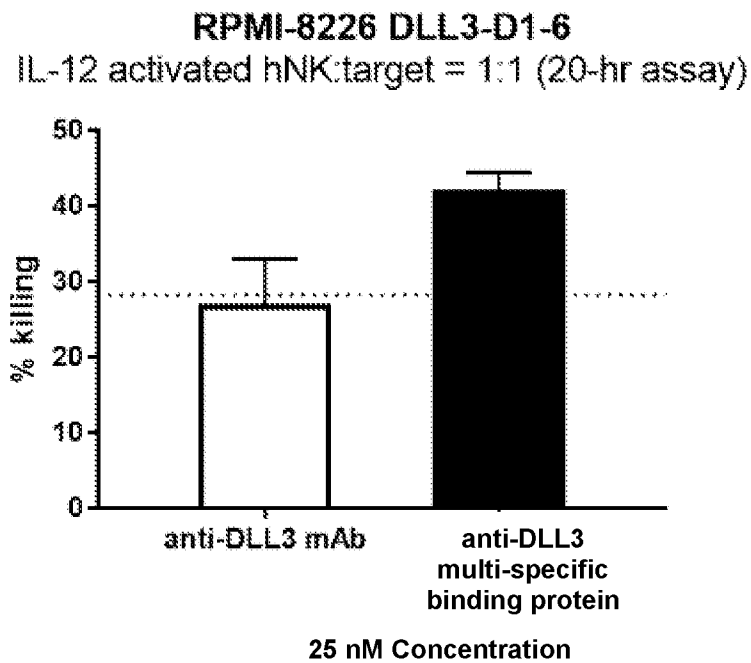


FIG. 31

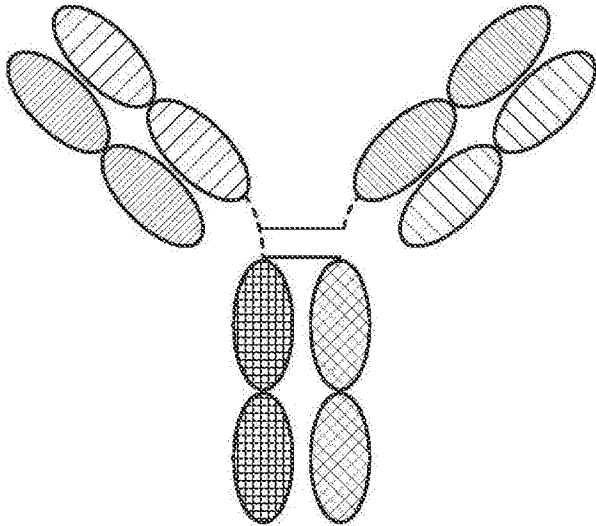


FIG. 32

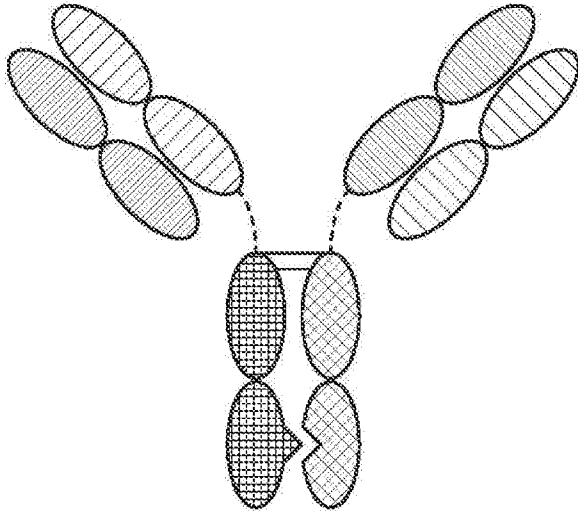


FIG. 33

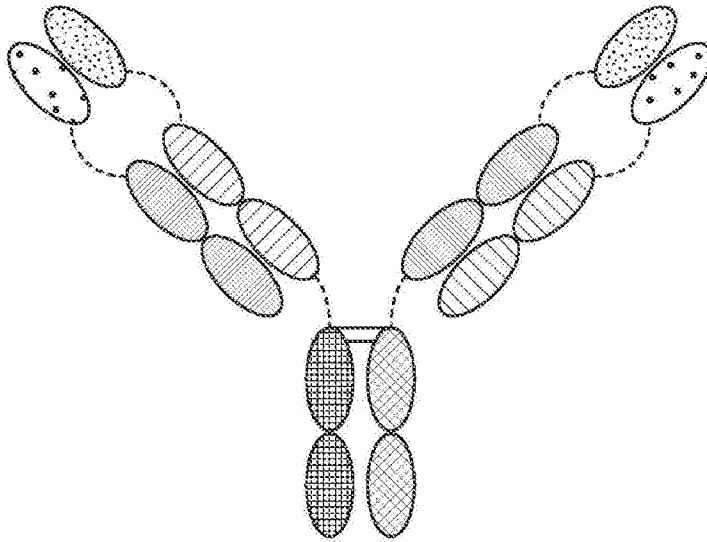


FIG. 34

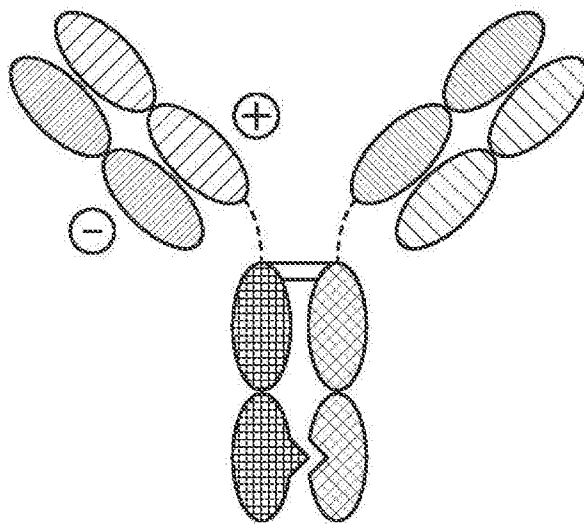


FIG. 35

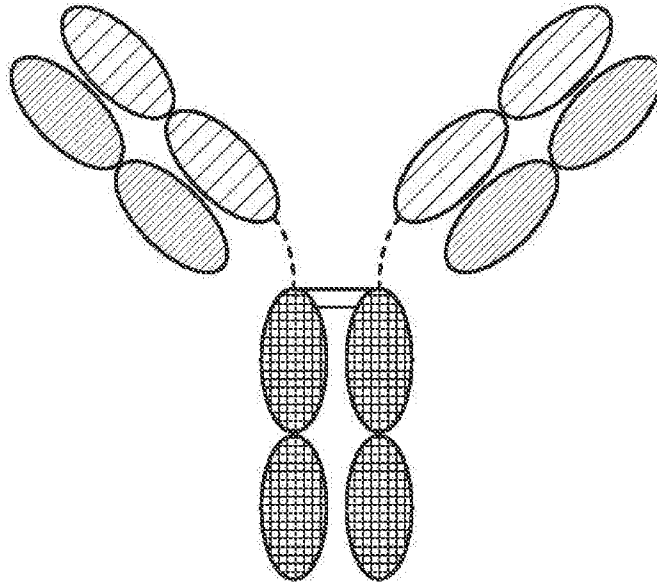


FIG. 36

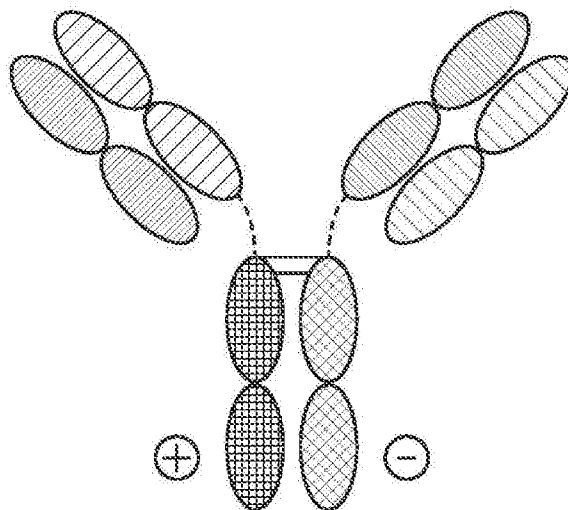


FIG. 37

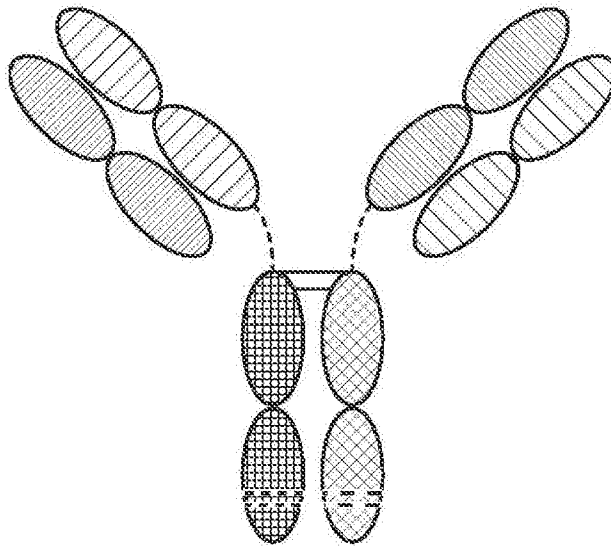


FIG. 38

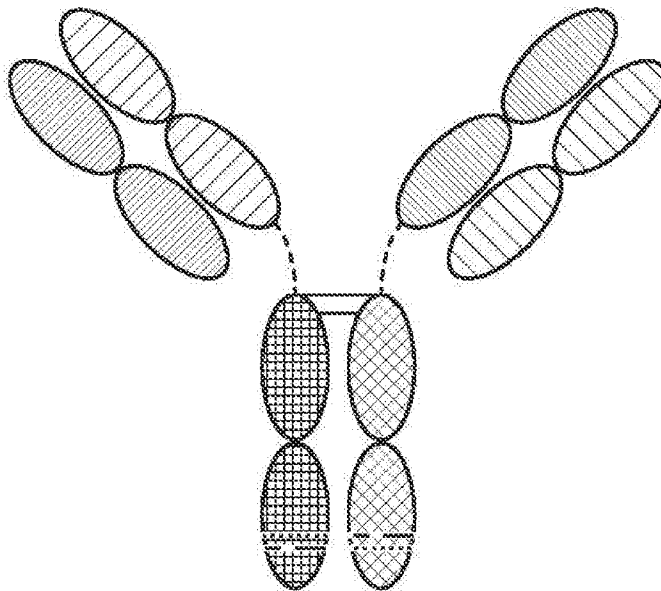


FIG. 39

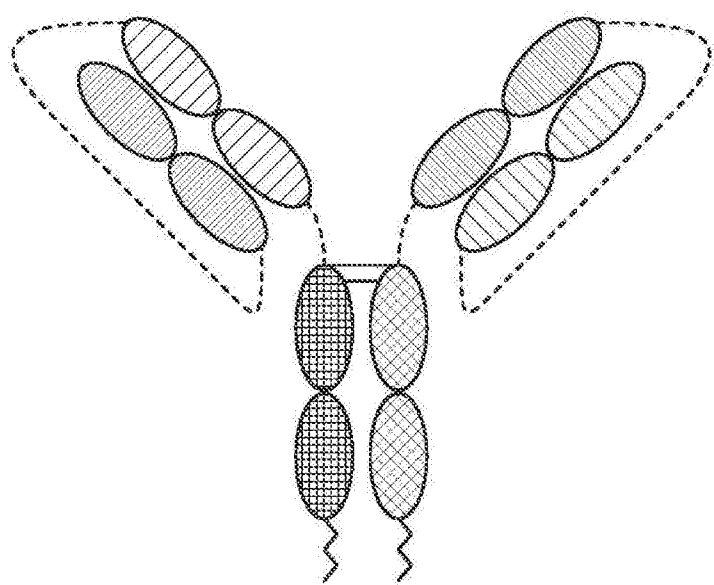


FIG. 40

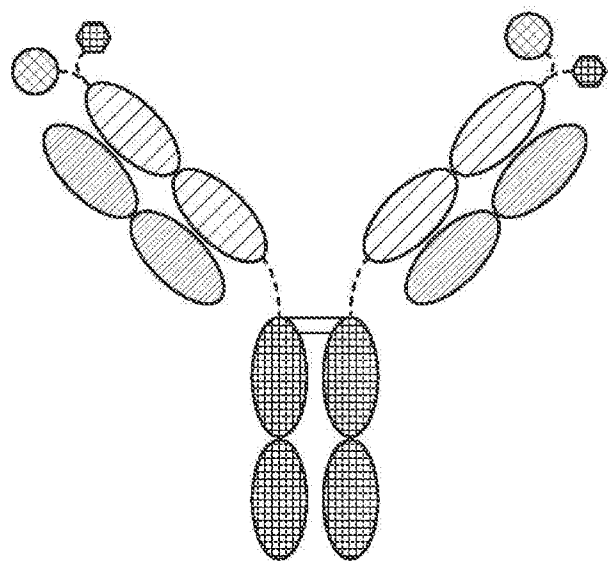


FIG. 41A

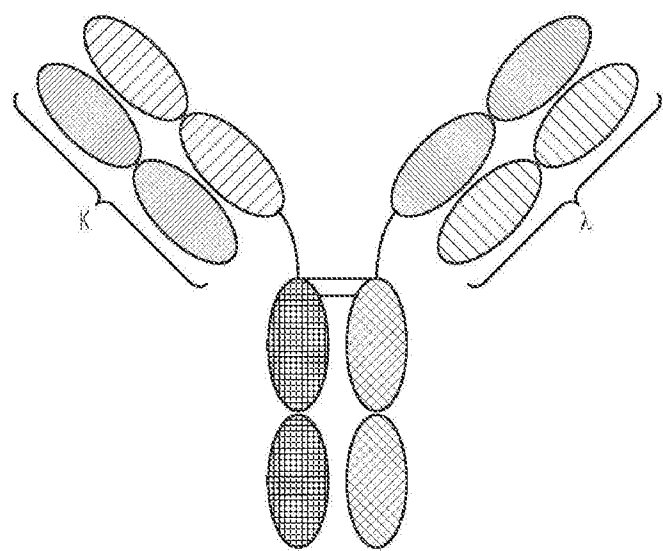


FIG. 41B

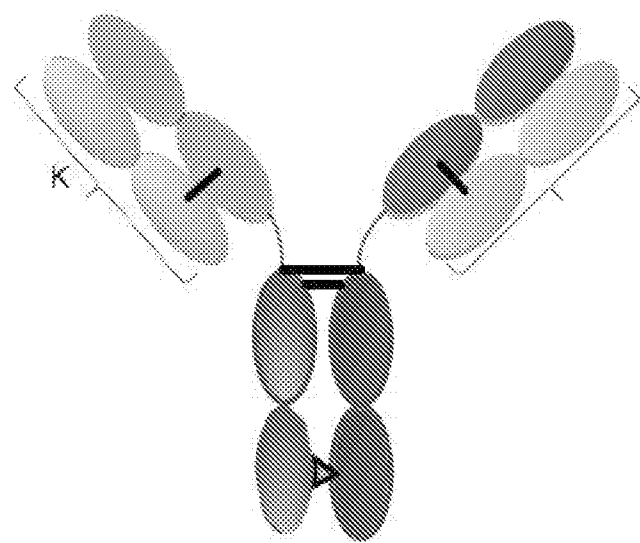


FIG. 42

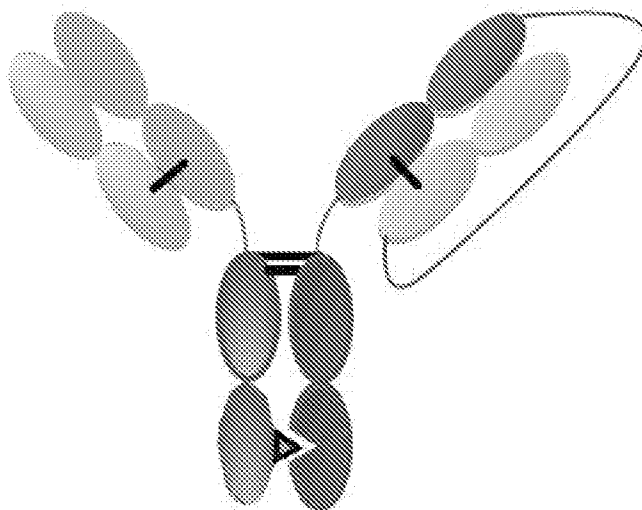


FIG. 43

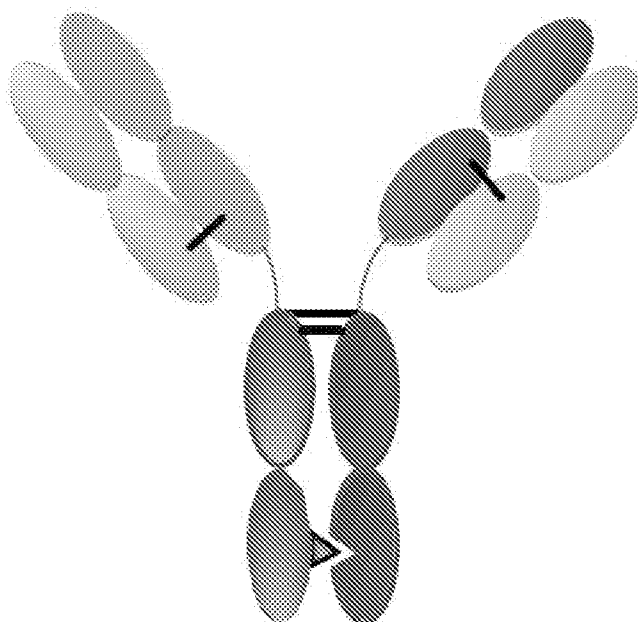


FIG. 44

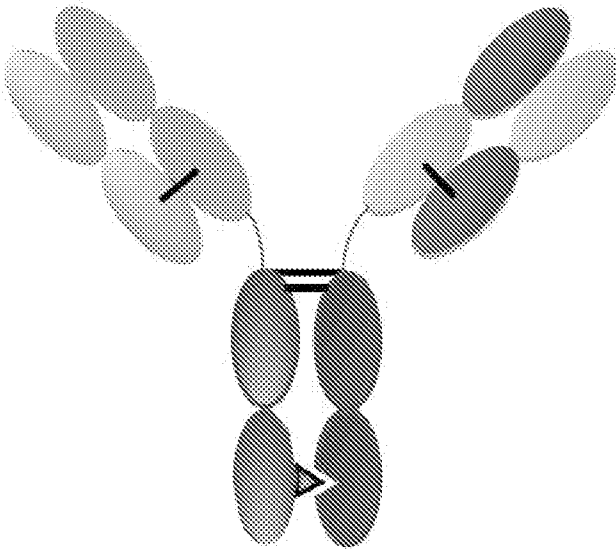


FIG. 45

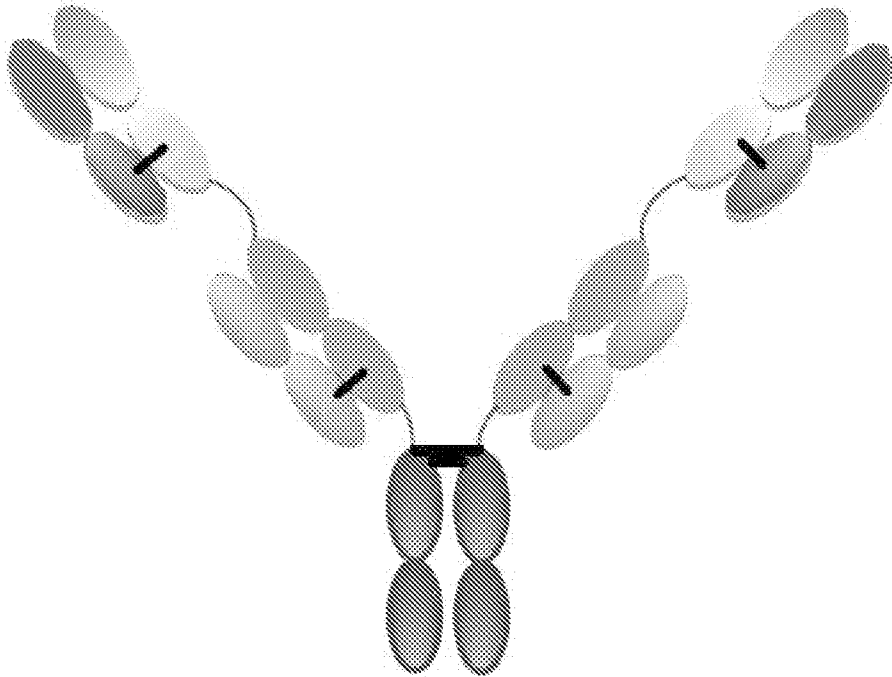


FIG. 46

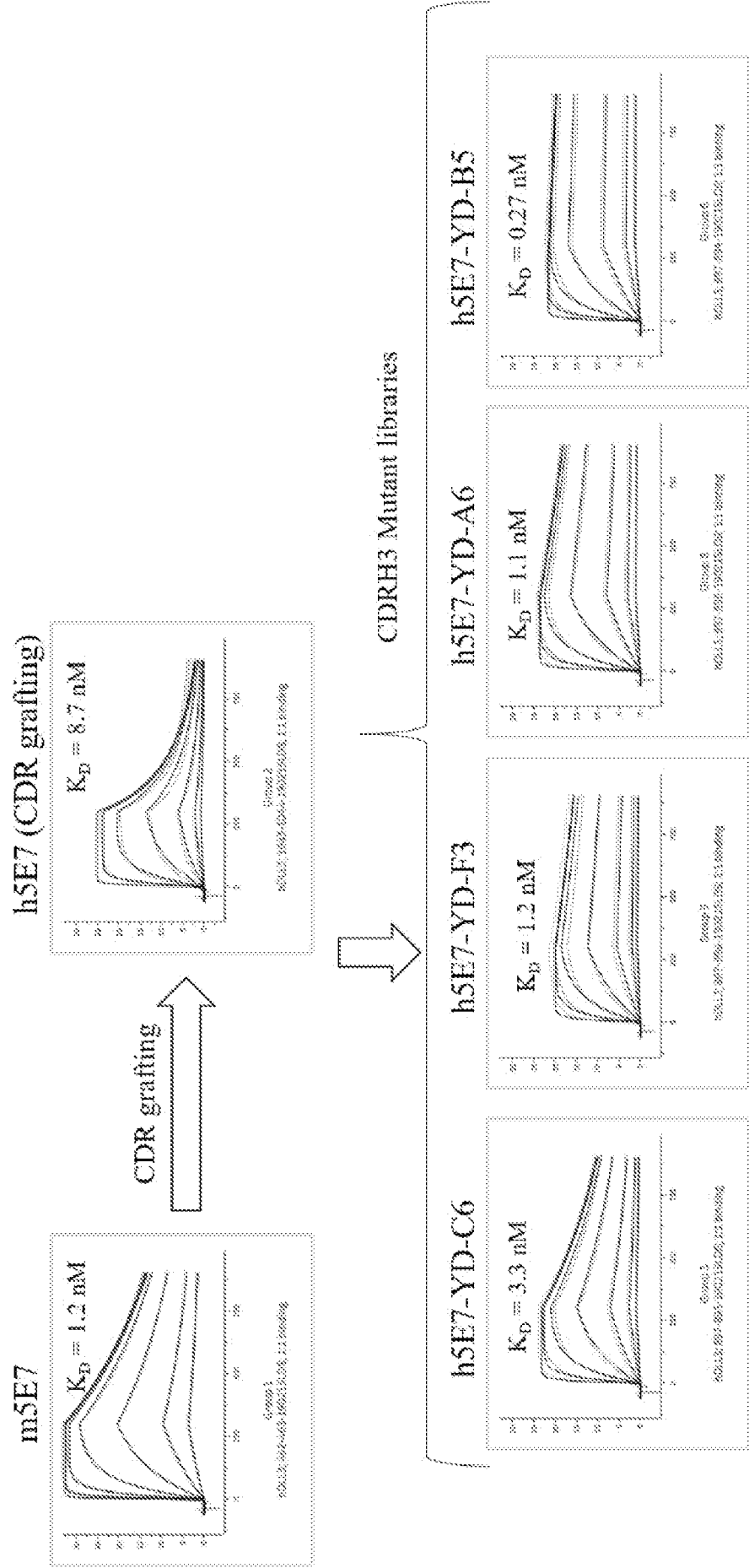
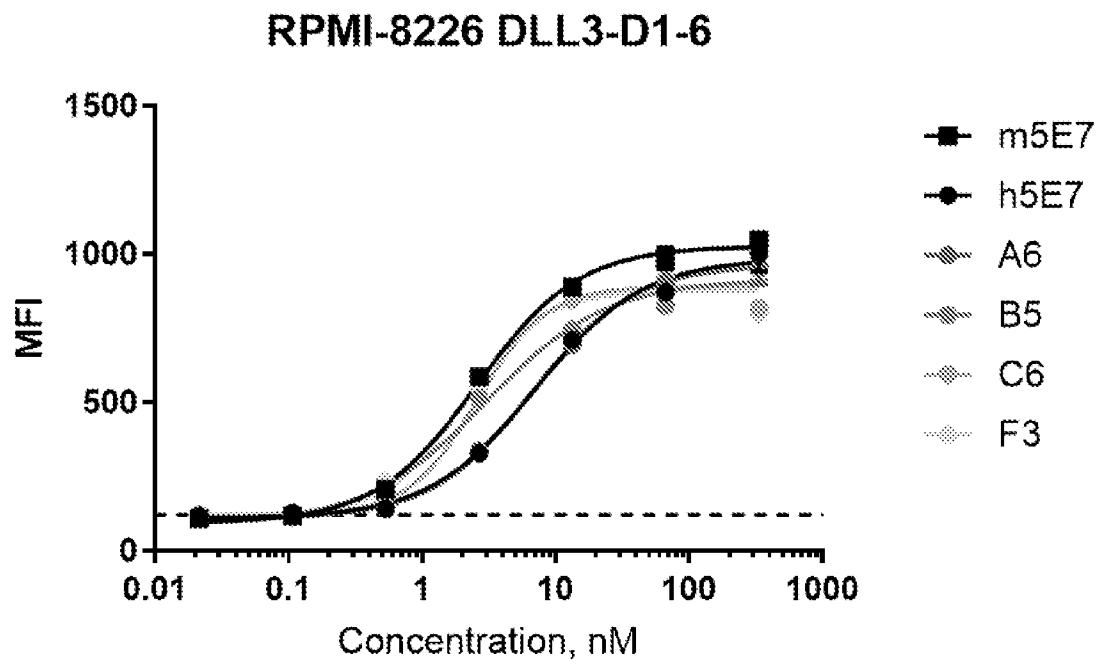


FIG. 47



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/25567

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/17, A61K 39/395, C07K 16/18, C07K 16/28 (2019.01)

CPC - C07K 16/2803, C07K 16/28/51, A61K 2039/505, A61K 2039/507, C07K 2317/31, C07K 2317/76

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y -- A	DE 10,2013,019,352 A1 (ELKE) 17 September 2015 (17.09.2015) abstract; pg 3, para 2	1 --- 2-4
Y -- A	US 2004/0115198 A1 (SPIES et al.) 17 June 2004 (17.06.2004) Claims 1, 2, 6	1 --- 2-4
Y -- A	WO 2017/021349 A1 (AMGEN RESEARCH (MUNICH) GMBH) 09 February 2017 (09.02.2017) abstract, pg 3, para 2, pg 33, para 5, to pg 34, para 1	1 --- 2-4
A	WO 2017/218707 A2 (XENCOR, INC.) 21 December 2017 (21.12.2017) para [0006], SEQ ID NO: 17256	2-4
A	WO2017075432 A1 (ALECTOR, LLC) 04 May 2017 (04.05.2017) abstract, SEQ ID NO: 669	2-4

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

05 August 2019

Date of mailing of the international search report

22 AUG 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/25567

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 68-102, 117-119
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- see extra sheet for Box No. III Observations where unity of invention is lacking -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4 limited to SEQ ID NOs: 679, 669, 132, 133, 680, 135, 136, 137

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/25567

Continuation of:

Box No. III. Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Groups I+: Claims 1-67, 103-116, drawn to a protein comprising:

- (a) a first antigen-binding site that binds NKG2D;
- (b) a second antigen-binding site that binds tumor-associated antigen; and
- (c) a third antigen-binding site that binds CD16, and a method of treating a cancer.

The protein/method will be searched to the extent that the tumor-associated antigen encompasses DLL3 comprising VH of SEQ ID NO: 679, VL of SEQ ID NO: 669, VH CDR1-3 of SEQ ID NOs: 132, 133 and 680, respectively; and VL CDR1-3 of SEQ ID NOs: 135, 136, and 137, respectively. It is believed that claims 1-4 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 679, 669, 132, 133, 680, 135, 136, 137. Additional protein(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected antigen-binding site that binds tumor-associated antigen. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a protein comprising a second antigen-binding site that binds MUC1 comprising VH of SEQ ID NO: 203, VL of SEQ ID NO: 207 (Claims 14-15). [Note, The first invention is limited to the first species of each variable in SEQ ID NO: 680 (SXYXSYDLXVY), corresponding to SEQ ID NO: 680 (SSYYSYDLFVY).]

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

No technical features are shared between the amino acid sequences of antigen-binding sites of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of including: a protein comprising three antigen binding sites, there shared technical features are previously disclosed by DE 10,2013,019,352 A1 (Elke) and US 2004/0115198 A1 to Spies et al. (hereinafter "Spies").

Elke teaches a protein (pg 3, para 2, schematic of a triple fusion protein (Triple Body), the detection and elimination of CD19 + or CD138 + tumor cells by NK cells.; see Fig. 1; abstract, treatment of malignant myeloma and other CD138 positive tumors, which acts through the activation of an anti-tumor immune response after simultaneous stimulation of NKG2D receptor and the NKp30 receptor.), comprising:

- (a) a first ligand that binds NKG2D (pg 3, para 2, the recruitment and activation of NK cells via the ligands of NKG2D; para [0002], the ligands are suitable for tumor therapy. NKG2D- and NKp30 ligand-based recombinant constructs convey promising anti-tumor activity, as demonstrated in various pre-clinical studies.);
- (b) a second antigen-binding site that binds tumor-associated antigen (pg 3, para 2, schematic of a triple fusion protein (Triple Body).....Binding to CD19 + / CD138 + cells via an antibody derivative with specificity for the corresponding antigen (scFv: single chain); and
- (c) a third antigen-binding site that binds CD16 (pg 3, para 2, schematic of a triple fusion protein (Triple Body).....a single chain, which for the activation of CD16 leads to NK cells.). Elke does not specifically teach a first antigenic-binding site that binds NKG2D. However, it is well known to one of ordinary skill in the art that NK cells are activated by antibody to NKG2D, as taught by Spies (Claim 1, A method for expanding a human T-cell population that expresses a natural or engineered NKG2D comprising contacting said population with an NKG2D ligand.; Claim 2, wherein the NKG2D ligand is an anti-NKG2D antibody, or an NKG2D-binding fragment thereof.; Claim 6, wherein said cell population is a T cell population, an NK cell population.). Thus, it would have been obvious to one of ordinary skill in the art to have applied the antigenic-binding site that binds NKG2D of Spies to the protein of Elke.

Elke teaches a method of treating a cancer (abstract, The invention is an agent for treatment of malignant myeloma and other CD138 positive tumors, which acts through the activation of an anti-tumor immune response after simultaneous stimulation of NKG2D receptor and the NKp30 receptor.).

Some inventions of Group I+ further share the technical feature of an antigenic-binding site that binds DLL3, comprising VH, VL, VHCDR1-3 and VLCDR1-3, which is disclosed by WO 2017/021349 A1 to Amgen Research (Munich) GMBH (hereinafter "Amgen") (Claim 1, A bispecific antibody construct comprising a first binding domain which binds to human DLL3 on the surface of a target cell and a second binding domain which binds to human CD3 on the surface of a T cell and at least macaque CD3.; Claim 6, wherein the first binding domain comprises a VH region comprising CDR-H1, CDR-H2 and CDR-H3 and a VL region comprising CDR-L1, CDR-L2 and CDR-L3.).

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.