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(54) **Titre : ANTICORPS BISPECIFIQUES CIBLANT CD137 ET LEURS UTILISATIONS DANS L'IMMUNOTHERAPIE
ANTICANCEREUSE**
(54) **Title: BISPECIFIC ANTIBODIES TARGETING CD137 AND USES THEREOF FOR ANTI-CANCER IMMUNOTHERAPY**

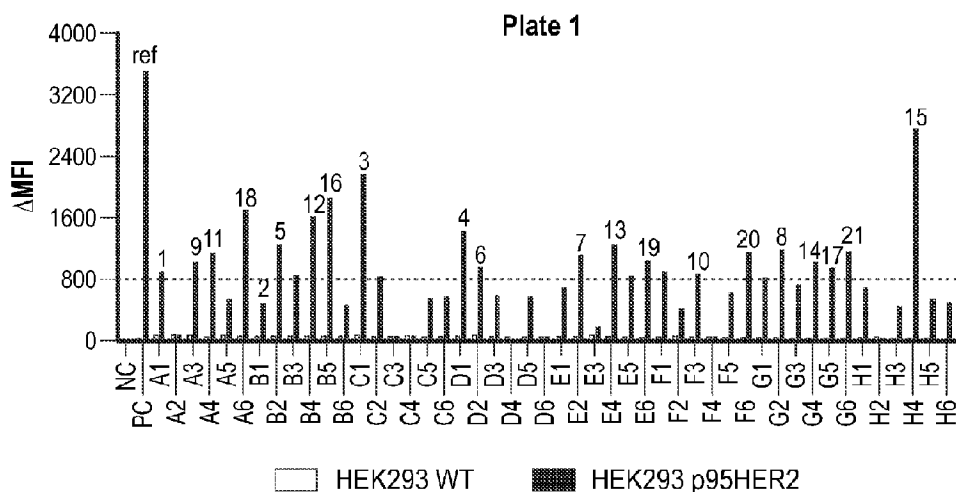


FIGURE 1A

(57) **Abrégé/Abstract:**

Provided herein are antibodies or antigen binding fragments thereof having a binding specificity for p95HER2 or for CD73, bispecific antibodies comprising a first antigen binding region that binds to p95HER2 or CD73 and a second antigen binding region that binds to an immune checkpoint molecule or an immune stimulatory molecule, and antibody-drug conjugates thereof. Also provided herein are pharmaceutical compositions comprising the antibodies, antigen binding fragments thereof, bispecific antibodies or antibody drug conjugates thereof, and methods of use thereof. The methods of use include method of treating cancer.

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Abstract:

Provided herein are antibodies or antigen binding fragments thereof having a binding specificity for p95HER2 or for CD73, bispecific antibodies comprising a first antigen binding region that binds to p95HER2 or CD73 and a second antigen binding region that binds to an immune checkpoint molecule or an immune stimulatory molecule, and antibody-drug conjugates thereof. Also provided herein are pharmaceutical compositions comprising the antibodies, antigen binding fragments thereof, bispecific antibodies or antibody drug conjugates thereof, and methods of use thereof. The methods of use include method of treating cancer.

BISPECIFIC ANTIBODIES TARGETING CD137 AND USES THEREOF FOR ANTI-CANCER IMMUNOTHERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority under 35 U.S.C. § 119(e) of U.S. Provisional Applications Nos. 63/281,347, filed November 19, 2021, and 63/392,474, filed July 26, 2022. The disclosure of the prior applications is considered part of and are herein incorporated by reference in the disclosure of this application in their entirety.

INCORPORATION OF SEQUENCE LISTING

[0002] The material in the accompanying sequence listing is hereby incorporated by reference into this application. The accompanying sequence listing XML file, name AP1110_2WO.xml, was created on November 18, 2022, and is 206 kb.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[0003] The present invention relates generally to antibodies and antigen binding fragments thereof and more specifically to antibodies and antigen binding fragments having cytotoxicity against cancer cells and enhancing T-cell function.

BACKGROUND INFORMATION

[0004] Cancer immunotherapy restores or boosts the natural defense against tumor by the human immune system. Such therapy usually targets specific biological molecules on cancer cells' surface such as tumor-associated antigens (TAAs). Anti-tumor activities are possibly achieved by directing the host immune system toward TAAs, which consequently establishes or induces adaptive immune responses against cancer cells. Over the past few decades, cancer treatment using monoclonal antibodies (mAbs) has achieved great success and many of them have been approved for cancer treatment or clinical trials.

[0005] CD137 (4-1BB/TNFRSF9) was identified in 1989 as an inducible gene that was expressed on antigen-primed T cells but not on resting ones. In addition, it is known to be expressed in dendritic (DCs), natural killer cells (NKs), activated CD4+ and CD8+ T lymphocytes, eosinophils, natural killer T cells (NKTs), and mast cells, but myeloid-derived suppressor cells (MDSCs) were investigated to not express this molecule on their surfaces. The

anti-CD137 antibodies show great potential in anti-cancer activities due to its ability to activate cytotoxic T cells and to increase the production of interferon gamma (IFN- γ).

[0006] Antibody-based strategies for cancer treatment have dramatically advanced in the past 20 years. Since rituximab was approved as the first monoclonal antibody (mAb) for the treatment of cancer in 1997, several mAbs have become standard of care for the treatment of both solid tumors and hematologic malignancies. Most of the approved mAbs (e.g., rituximab, trastuzumab, and cetuximab) target tumor-associated antigens on the surface of cancer cells and inhibit cell growth. Although several effective antibodies have emerged, long-term, durable responses remain elusive, and resistance and relapse remain major problems. Immunomodulatory antibodies have revolutionized cancer immunotherapy and helped garner the breakthrough distinction.

[0007] In addition to checkpoint blockade agents such as ipilimumab and pembrolizumab, agents targeting the tumor necrosis factor (TNF) superfamily of costimulatory receptors have entered development. CD137 is one of the TNF receptor family targets that have advanced into clinical trials. CD137 regulates many immune cells, including CD4⁺ and CD8⁺ T cells, regulatory T cells (Treg), dendritic cells (DC), and natural killer (NK) cells. Recent studies indicate that the addition of anti-CD137 mAbs can augment the antitumor efficacy of immunomodulatory antibodies. However, there is still an unmet need for more specific and more effective antigen binding molecules that target CD137.

SUMMARY OF THE INVENTION

[0008] The present innovation is based on the discovery of anti-p95HER2 antibodies that show a superior activity to bind p95HER2-expressing cancer cells and of anti-CD73 antibodies that show a superior activity to neutralize the enzymatic activity of CD73 in the cell membrane-bound or soluble form. The present invention is further based on the discovery that bispecific antibodies targeting p95HER2 and CD137 possess dual functions, antibody antibody-dependent cell-mediated cytotoxicity (ADCC) and p95HER2-dependent T-cell activation, against p95HER2-positive cancer cells, and that bispecific antibodies targeting CD73 and CD137 possess extraordinary activity to boost T-cell effector function and inhibit tumor growth in vivo better than mono-treatment or combination treatment with each antibody.

[0009] In one embodiment, the invention provides four antibodies or antigen binding fragments thereof: anti-p95HER2 antibody clone R4-3 (p95HER2 #R4-3), anti-p95HER2 antibody clone R4-5 (p95HER2 #R4-5), anti-p95HER2 antibody clone R4-11 (p95HER2 #R4-

11), anti- p95HER2 antibody clone R4-15 (p95HER2 #R4-15), anti- p95HER2 antibody clone R4-38 (p95HER2 #R4-38). In one aspect, the anti-p95HER2 antibodies provided herein include a heavy chain variable (VH) region including an amino acid sequence with at least 80% sequence identity to a sequence selected from SEQ ID NO:1, SEQ ID NO:9, SEQ ID NO:17, SEQ ID NO:25 and SEQ ID NO:33; and a light chain variable (VL) region including an amino acid sequence with at least 80% sequence identity to a sequence selected from SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:18, SEQ ID NO:26 and SEQ ID NO:34.

[0010] In one aspect, the antibody or antigen binding fragment has a VH region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:1 and a VL region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:2 includes (a) VH CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:3, wherein CDR-H2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:4, and wherein CDR-H3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:5; and (b) VL CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:6, wherein CDR-L2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:7, and wherein CDR-L3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:8.

[0011] In another aspect, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:9 and a VL region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:10 includes (a) VH CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:11, wherein CDR-H2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:12, and wherein CDR-H3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:13; and (b) VL CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:14, wherein CDR-L2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:15, and wherein CDR-L3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:16.

[0012] In one aspect, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:17 and a VL region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:18

includes (a) VH CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:19, wherein CDR-H2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:20, and wherein CDR-H3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:21; and (b) VL CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:22, wherein CDR-L2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:23, and wherein CDR-L3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:24.

[0013] In another aspect, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:25 and a VL region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:26 includes (a) VH CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:27, wherein CDR-H2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:28, and wherein CDR-H3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:29; and (b) VL CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:30, wherein CDR-L2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:31, and wherein CDR-L3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:32.

[0014] In yet another aspect, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:33 and a VL region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:34 includes (a) VH CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:35, wherein CDR-H2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:36, and wherein CDR-H3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:37; and (b) VL CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:38, wherein CDR-L2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:39, and wherein CDR-L3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:40.

[0015] In one aspect, the antibody includes an Fc domain. In some aspects, the Fc domain is an IgG, IgE, IgM, IgD, IgA, or IgY domain. In various aspects, the IgG domain is an IgG1,

IgG2, IgG3, or IgG4 domain. In other aspects, the IgG1 domain includes an amino acid sequence of SEQ ID NO:41. In various aspects, the IgG1 domain includes point mutations compared to a wild-type IgG1 domain that modify or enhance antibody-dependent cellular cytotoxicity (ADCC) and/ or complement-dependent cytotoxicity (CDC). In many aspects, point mutations include S241D/A332L/I334E mutations.

[0016] In another embodiment, the invention provides a bispecific antibody including a first antigen binding region and a second antigen binding region, wherein the first antigen binding region binds to p95HER2.

[0017] In one aspect, the bispecific antibody includes a V_H region including complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:19, CDR-H2 is set forth in SEQ ID NO:20, and CDR-H3 is set forth in SEQ ID NO:21 or sequences having 90% identity to SEQ ID NO:19, 20 or 21 and the antigen binding specificity thereof; and a V_L region including CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:22, CDR-L2 is set forth in SEQ ID NO:23, and CDR-L3 is set forth in SEQ ID NO:24 or sequences having 90% identity to SEQ ID NO:19, 20 or 21 and the antigen binding specificity thereof. In another aspect, the V_H region has an amino acid sequence having at least 80% identity to SEQ ID NO:17 and an antigen binding specificity of SEQ ID NOs:19-21 and the V_L region has an amino acid sequence having at least 80% identity to SEQ ID NO:18 and an antigen binding specificity of SEQ ID NOs:22-24. In one aspect, the second antigen binding region binds to an immune checkpoint molecule, or an immune stimulatory molecule. In another aspect, the antibody includes a heavy chain sequence having the amino acid sequence of SEQ ID NO:46 or 47 or a sequence having 90% identity thereto and the binding specificity thereof. In one aspect, the bispecific antibody further includes a light chain having the amino acid sequence of SEQ ID NO:18. In another aspect, the bispecific antibody includes an Fc domain. In some aspects, the Fc domain is an IgG domain, an IgE domain, an IgM domain, and IgD domain, an IgA domain, or an IgY domain. In various aspects, the Fc domain is an IgG domain. In many aspects, the IgG domain is an IgG1 domain, an IgG2 domain, an IgG3 domain, or an IgG4 domain. In one aspect, a scFv is linked to the C-terminus of the Fc domain. In another aspect, the bispecific antibody includes a linker between the Fab domain and the scFv domain. In some aspects, the Fab fragment is linked to the N-terminus of the Fc domain. In various aspects, the Fab includes a p95HER2 binding site, and the scFv includes a CD137 binding site.

[0018] In one embodiment, the invention provides an antibody-drug conjugate including: a therapeutic agent, and any one of the antibodies or antigen binding fragments thereof described herein, or any one of the bispecific antibodies described herein.

[0019] In one aspect, the therapeutic agent is covalently linked to the antibody, antigen binding fragment or bispecific antibody via a linker.

[0020] In another embodiment, the invention provides a pharmaceutical composition including any one of the antibodies or antigen binding fragments thereof described herein, any one of the bispecific antibodies described herein or any one of the antibody-drug conjugates described herein, and at least one pharmaceutical acceptable carrier.

[0021] In one aspect, the pharmaceutically acceptable carrier is conjugated to the C-terminus of the C-terminus of the amino acid sequence of the antibody, antigen binding fragment, bispecific antibody, or antibody-drug conjugate.

[0022] In one embodiment, the invention provides a method of treating cancer including administering to a subject in need thereof an effective amount of any one of the antibodies or antigen binding fragments thereof described herein, any one of the bispecific antibodies described herein, or any one of the antibody-drug conjugates described herein, thereby treating cancer. In one aspect, the cancer is selected from prostate cancer, lung cancer, Non-Small Cell Lung Cancer (NSCLC), melanoma, lymphoma, breast cancer, head and neck cancer, renal cell carcinoma (RCC), ovarian cancer, kidney cancer, urinary bladder cancer, uterine cancer, cervical cancer, ovarian cancer, liver cancer, stomach cancer, colon cancer, rectal cancer, oral cavity cancer, pharynx cancer, pancreatic cancer, thyroid cancer, skin cancer, brain cancer, bone cancer, hematopoietic cancer, or leukemia.

[0023] In another embodiment, the invention provides an isolated amino acid sequence as set forth in SEQ ID NOs:1-42 or 46-47.

[0024] In one embodiment, the invention provides an isolated nucleic acid sequence encoding any one of the antibodies or antigen-binding fragments thereof, or the bispecific antibodies described herein. In another embodiment, the invention provides an isolated nucleic acid encoding any one of SEQ ID NOs:1-42 or 46-47.

[0025] In a further embodiment, the invention provides four antibodies or antigen binding fragments thereof: anti-CD73 antibody clone 3-4 (CD73 #3-4), anti-CD73 antibody clone 3-16 (CD73 #3-16), anti-CD73 antibody clone 3-1-28 (CD73 #3-1-28), and anti-CD73 antibody clone 3-1-37 (CD73 #3-1-37); and three affinity-maturated antibodies derived from anti-CD73

clone 3-1-28: anti-CD73 #3-1-28 (S51A Q54E), anti-CD73 #3-1-28 (S107D S109H), anti-CD73 #3-1-28 (S107D S109T A114S).

[0026] In one aspect, the anti-CD73 antibodies provided herein include a heavy chain variable (VH) region including an amino acid sequence with at least 80% sequence identity to a sequence selected from SEQ ID NO:49, SEQ ID NO:57, SEQ ID NO:65, and SEQ ID NO:73; and a light chain variable (VL) region including an amino acid sequence with at least 80% sequence identity to a sequence selected from SEQ ID NO:50, SEQ ID NO:58, SEQ ID NO:66 and SEQ ID NO:74, SEQ ID NO:81, SEQ ID NO:83 and SEQ ID NO:85.

[0027] In one aspect, the antibody or antigen binding fragment has a VH region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:49 and a VL region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:50 includes (a) VH CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:51, wherein CDR-H2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:52, and wherein CDR-H3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:53; and (b) VL CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:54, wherein CDR-L2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:55, and wherein CDR-L3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:56.

[0028] In another aspect, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:57 and a VL region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:58 includes (a) VH CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:59, wherein CDR-H2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:60, and wherein CDR-H3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:61; and (b) VL CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:62, wherein CDR-L2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:63, and wherein CDR-L3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:64.

[0029] In one aspect, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:65 and a VL

region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:66 includes (a) VH CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:67, wherein CDR-H2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:68, and wherein CDR-H3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:69; and (b) VL CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:70, wherein CDR-L2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:71, and wherein CDR-L3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:72.

[0030] In another aspect, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:73 and a VL region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:74 includes (a) VH CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:75, wherein CDR-H2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:76, and wherein CDR-H3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:77; and (b) VL CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:78, wherein CDR-L2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:79, and wherein CDR-L3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:80.

[0031] In an additional embodiment, the present invention provides three affinity-matured antibodies derived from anti-CD73 clone 3-1-28: anti-CD73 #3-1-28 (S51A Q54E), anti-CD73 #3-1-28 (S107D S109H), anti-CD73 #3-1-28 (S107D S109T A114S).

[0032] In one aspect, the anti-CD73 #3-1-28 (S51A Q54E) antibody or antigen binding fragment thereof having a VL region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:81 includes VL CDR-L2 region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:82. In another aspect, the anti-CD73 #3-1-28 (S107D S109H) antibody or antigen binding fragment thereof having a VH region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:83 includes VH CDR-H3 region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:84. In yet another aspect, the anti-CD73 #3-1-28 (S107D S109T A114S) antibody or antigen binding fragment thereof having a VH region that includes an amino acid sequence having at least 80%

identity to SEQ ID NO:85 includes VH CDR-H3 region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:86.

[0033] In one aspect, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:65 and a VL region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:81 includes (a) VH CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:67, wherein CDR-H2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:68, and wherein CDR-H3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:69; and (b) VL CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:70, wherein CDR-L2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:82, and wherein CDR-L3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:72.

[0034] In another aspect, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:65 and a VL region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:83 includes (a) VH CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:67, wherein CDR-H2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:68, and wherein CDR-H3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:69; and (b) VL CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:70, wherein CDR-L2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:84, and wherein CDR-L3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:72.

[0035] In yet another aspect, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:65 and a VL region that includes an amino acid sequence having at least 80% identity to SEQ ID NO:85 includes (a) VH CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 includes an amino acid sequence having at least 80% identity to SEQ ID NO:67, wherein CDR-H2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:68, and wherein CDR-H3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:69; and (b) VL CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 includes an amino acid sequence having

at least 80% identity to SEQ ID NO:70, wherein CDR-L2 includes an amino acid sequence having at least 80% identity to SEQ ID NO:86, and wherein CDR-L3 includes an amino acid sequence having at least 80% identity to SEQ ID NO:72.

[0036] In one aspect, antibodies or antigen binding fragments thereof provided herein include an Fc domain. In some aspects, the Fc domain is an IgG, IgE, IgM, IgD, IgA, or IgY domain. In various aspects, the IgG domain is an IgG1, IgG2, IgG3, or IgG4 domain. In other aspects, the IgG1 domain includes an amino acid sequence of SEQ ID NO:87. In various aspects, IgG1 includes point mutations compared to wild-type IgG1 that modify or reduce antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC). In many aspects, point mutations include K297A and K322A mutations.

[0037] In another embodiment, the invention provides a bispecific antibody including a first antigen binding region and a second antigen binding region, wherein the first antigen binding region binds to CD73.

[0038] In one aspect, the bispecific antibody includes a VH region including complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof; and a VL region including CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:71, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 71 or 72 and the antigen binding specificity thereof. In another aspect, the bispecific antibody includes a VH region including complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof; and a VL region including CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:82, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 82 or 72 and the antigen binding specificity thereof. In one aspect, the bispecific antibody includes a VH region including complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof;

and a VL region including CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:84, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 84 or 72 and the antigen binding specificity thereof. In another aspect, the bispecific antibody includes a VH region including complementarity-determining region (CDR)-III1, CDR-II2, and CDR-II3, wherein CDR-III1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof; and a VL region including CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:86, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 86 or 72 and the antigen binding specificity thereof. In one aspect, the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:66 and an antigen binding specificity of SEQ ID NOs:70-72. In another aspect, the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:81 and an antigen binding specificity of SEQ ID NOs:70, 82 and 72. In one aspect, the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:82 and an antigen binding specificity of SEQ ID NOs:70, 84 and 72. In another aspect, the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:85 and an antigen binding specificity of SEQ ID NOs:70, 86 and 72. In one aspect the second antigen binding region binds to an immune checkpoint molecule, or an immune stimulatory molecule. In one aspect, the antibody includes a heavy chain sequence having the amino acid sequence of SEQ ID NO:88, 89, 90 or 91 or a sequence having 90% identity thereto and the binding specificity thereof. In another aspect, the bispecific antibody further includes a light chain having the amino acid sequence of SEQ ID NO:66.

[0039] In one embodiment, the invention provides an antibody-drug conjugate including: a therapeutic agent, and any one of the antibodies or antigen binding fragments thereof

described herein, or any one of the bispecific antibodies described herein.

[0040] In another embodiment, the invention provides a pharmaceutical composition including any one of the antibodies or antigen binding fragments thereof described herein, any one of the bispecific antibodies described herein, or any one of the antibody-drug conjugates described herein, and at least one pharmaceutically acceptable carrier.

[0041] In one embodiment, the invention provides a method of treating cancer including administering to a subject in need thereof an effective amount of any one of the antibodies or antigen binding fragments thereof described herein, any one of the bispecific antibodies described herein, or any one of the antibody-drug conjugates described herein, thereby treating cancer.

[0042] In another embodiment, the invention provides an isolated amino acid sequence as set forth in SEQ ID NOs:49-86 or 88-91.

[0043] In one embodiment, the invention provides an isolated nucleic acid sequence encoding any one of the antibodies or antigen-binding fragments thereof, or the bispecific antibodies described herein. In another embodiment, the invention provides an isolated nucleic acid encoding any one of SEQ ID NOs:49-86 or 88-91.

BRIEF DESCRIPTION OF THE DRAWINGS

[0044] **FIGURES 1A-1C** show the screening for phage clones targeted to p95HER2 by flow cytometer. The phage clones that recognize p95HER2 were enriched after four rounds of panning. Positive phage binders were selected by staining p95HER2-HEK293 cells with phage-containing supernatant. **FIGURE 1A** illustrates phage clones with specific binding to p95HER2-HEK293 cells were determined by flow cytometer. **FIGURE 1B** illustrates clones (as numbered 1 to 40) specifically bound to p95HER2-HEK293 cells with Δ MF1 higher than 800 were selected for sequencing. Negative control (NC), stained with secondary antibody alone. Positive control (PC), stained with both primary anti-p95HER2 ref antibody and secondary antibody. **FIGURE 1C** illustrates phage clones with distinct sequences (marked in bold with grey background) were selected for conversion to monoclonal antibodies. Clones were named as R4-X, where X is the number in **FIGURE 1A**.

[0045] **FIGURE 2** shows the purity of Protein A-purified anti- p95HER2 antibody leads by SDS- PAGE. The purity and integrity of one-step Protein A-purified anti- p95HER2 antibody leads was analyzed by SDS-PAGE under non-reducing and reducing conditions.

[0046] **FIGURES 3A-3B** show the binding activity of purified anti-p95HER2 antibody leads by flow cytometer. **FIGURE 3A** is a graph illustrating that P95HER2-expressing HEK293 cells were surface stained with anti-p95HER2 antibody (30, 3, 0.3 nM) on ice for 1hr then incubated with A488-conjugated anti-human IgG secondary antibody on ice for additional 1hr. Cells were analyzed by flow cytometer, and A488 fluorescence intensity was shown by number. **FIGURE 3B** is a graph illustrating that P95HER2-expressing NCI-H292 stable cells were surface stained with selected anti-p95HER2 antibodies and the analyzed by flow cytometer. Binding curve and EC50 were shown.

[0047] **FIGURES 4A-4B** show the binding epitope mapping of anti-p95HER2 antibody leads. **FIGURE 4A** illustrates that anti-p95HER2 #R4-3, R4-5, R4-11, R4-15 and R4-38 antibodies showed widely identical responses against peptides with the consensus motif KFPDE at moderate to high spot intensities and high signal-to-noise ratios. Even though the intensity levels did not vary remarkably, antibody R4-3 showed a slightly weaker and antibody R4-38 the strongest response of these antibodies. **FIGURE 4B** is a heat map showing anti-p95HER2 antibodies binding to linear 5, 10 and 15 amino acid peptides with peptide-peptide overlaps. Binding intensity was shown for each peptide, corrected with its own background value.

[0048] **FIGURE 5** shows the symmetric format of an anti- p95HER2-CD137 bispecific antibody (bsAb).

[0049] **FIGURES 6A-6B** show the protein aggregation and integrity of anti-p95HER2-CD137 bsAb by SEC-HPLC and μ CE-SDS-PAGE, respectively. **FIGURE 6A** shows the analysis of protein aggregation by SEC-HPLC. **FIGURE 6B** shows the analysis of protein integrity by μ CE-SDS-PAGE under non-reducing and reducing conditions, and the data were summarized in the table below.

[0050] **FIGURES 7A-7B** show the binding affinity of anti-p95HER2-CD137 bsAb against p95HER2 and CD137 antigen by biolayer interferometry. **FIGURE 7A** illustrates binding kinetics against human p95HER2 antigen. **FIGURE 7B** illustrates binding kinetics against human CD137 antigen.

[0051] **FIGURE 8** shows the simultaneous binding to p95HER2 and CD137 antigens by anti-p95HER2-CD137 bsAb.

[0052] **FIGURES 9A-9B** show that anti-p95HER2-CD137 bsAb induces IFN- γ production of T-cells upon coculturing with p95HER2-expressing NCI-H292 cells. **FIGURE**

9A illustrates the expression level of p95HER2 on p95HER2-expressing NCI-H292 cells. **FIGURE 9B** illustrates IFN- γ production of T cells upon coculturing with p95HER2-expressing NCI-H292 lung cancer cells.

[0053] **FIGURES 10A-10B** show the antibody-dependent cell-mediated cytotoxicity of anti-p95HER2-CD137 bsAb p95HER2-expressing NCI-H292 cells. **FIGURE 10A** illustrates the expression level of CD137 on CD137-expressing HEK293 cells (left). ADCC of anti-p95HER2-CD137 bsAbs with normal or ADCC-enhancing IgG1 format (right). **FIGURE 10B** illustrates the expression level of p95HER2 on p95HER2-expressing NCI-H292 cells (left). Anti-p95HER2-CD137 bsAb with normal IgG1 format induced a dose-dependent ADCC as well as #R4-11 IgG1 monoclonal antibody (right).

[0054] **FIGURES 11A-11C** show the anti-tumor activity of anti-p95HER2-CD137 bsAb by MDA-MB-231 breast cancer model *in vivo*. **FIGURE 11A** shows p95HER2 expression by MDA-MB-231 cells. **FIGURE 11B** illustrates tumor volume. **FIGURE 11C** illustrates tumor mass.

[0055] **FIGURE 12** shows the pharmacokinetic profiles of anti-p95HER2-CD137 bsAb *in vivo*.

[0056] **FIGURES 13A-13C** shows the screening for phage clones targeted to CD73 by ELISA. **FIGURE 13A** shows ELISA results of phage clones isolated from third-round MDA-MB-231 cell-based panning. **FIGURE 13B** shows ELISA results of phage clones isolated from third-round CD73-overexpressing Expi293 cell-based panning in the third round. **FIGURE 13C** shows a list of clone name numbered in **FIGURE 13A** and **13B**.

[0057] **FIGURE 14** shows the purity of Protein A-purified anti-CD73 antibody leads by SDS-PAGE.

[0058] **FIGURE 15** shows the binding of anti-CD73 antibody to CD73-expressing MDA-MB-231 breast cancer cells.

[0059] **FIGURE 16** shows the blockade of CD73 enzymatic activity by anti-CD73 antibody.

[0060] **FIGURES 17A-17C** show the screening of affinity-matured clones derived from anti-CD73 clone 3-1-28 antibody. **FIGURE 17A** illustrates CD73 binding activities of phage clones. **FIGURE 17B** illustrates binding activities of antibodies converted from phage clones numbered in **FIGURE 17A**. **FIGURE 17C** illustrates binding kinetics of antibodies selected in **FIGURE 17B**.

[0061] **FIGURES 18A-18B** show the blockade of membrane-bound and soluble CD73 enzymatic activity by affinity-maturated anti-CD73#3-1-28 antibodies. **FIGURE 18A** shows membrane-bound CD73 expressed by MDA-MB-231 cells. **FIGURE 18B** shows soluble CD73 secreted by MDA-MB-231 cells.

[0062] **FIGURE 19** shows the symmetric format of an anti-CD73-CD137 bispecific antibody (bsAb).

[0063] **FIGURES 20A-20B** show the protein aggregation and integrity of anti-CD73-CD137 bsAb by SEC-HPLC and μ CE-SDS-PAGE, respectively. **FIGURE 20A** illustrates the analysis of protein aggregation by SEC-HPLC. **FIGURE 20B** illustrates Analysis of protein integrity by μ CE-SDS-PAGE under non-reducing and reducing conditions.

[0064] **FIGURES 21A-21B** show the binding affinity of anti-CD73-CD137 bsAb against CD73 and CD137 antigen, respectively measured by biolayer interferometry. **FIGURE 21A** shows binding kinetics against human CD73 antigen. **FIGURE 21B** shows binding kinetics against human CD137 antigen.

[0065] **FIGURE 22** shows the simultaneous binding of CD73 and CD137 antigens by anti-CD73-CD137 bsAb.

[0066] **FIGURE 23** shows the blockade of CD73 enzymatic activity by anti-CD73-CD137 bsAb. Anti-CD73-CD137 bsAb showed a comparable efficacy to inhibit CD73 enzymatic activity to parental anti-CD73 antibody.

[0067] **FIGURE 24** shows the rescue of AMP-suppressed T-cell activation by anti-CD73-CD137 bsAb.

[0068] **FIGURES 25A-25C** show that anti-CD73-CD137 bsAb induces IFN- γ production of T-cells upon coculturing with CD73-expressing cancer cells. **FIGURE 25A** illustrates the expression level of CD73 on MDA-MB-231 and NCI-H292 cells. **FIGURE 25B** illustrates IFN- γ production of T-cells upon coculturing with CD73-expressing MDA-MB-231, breast cancer cells. **FIGURE 25C** illustrates IFN- γ production of T-cells upon coculturing with CD73-expressing NCI-H292, lung cancer cells.

[0069] **FIGURES 26A-26E** show the anti-tumor activity of anti-CD73-CD137 bsAb by MDA-MB-231 breast cancer model *in vivo*. **FIGURE 26A** illustrates tumor volumes. **FIGURE 26B** illustrates tumor mass. **FIGURE 26C** illustrates percent of alive huCD45+ cells. **FIGURE 26D** illustrates percent of CD8+ T cells among CD3+ cells. **FIGURE 26E** illustrates percent of CD8+ T cells among CD4+ cells.

[0070] **FIGURE 27** shows the pharmacokinetic profiles of anti-CD73-CD137 bsAb *in vivo*.

DETAILED DESCRIPTION OF THE INVENTION

[0071] The present innovation is based on the discovery of anti-p95HER2 antibodies that show a superior activity to bind p95HER2-expressing cancer cells and of anti-CD73 antibodies that show a superior activity to neutralize the enzymatic activity of CD73 in the cell membrane-bound or soluble form. The present invention is further based on the discovery that bispecific antibodies targeting p95HER2 and CD137 possess dual functions, antibody antibody-dependent cell-mediated cytotoxicity (ADCC) and p95HER2-dependent T-cell activation, against p95HER2-positive cancer cells, and that bispecific antibodies targeting CD73 and CD137 possess extraordinary activity to boost T-cell effector function and inhibit tumor growth *in vivo* better than mono-treatment or combination treatment with each antibody.

[0072] Before the present compositions and methods are described, it is to be understood that this invention is not limited to particular compositions, methods, and experimental conditions described, as such compositions, methods, and conditions may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only in the appended claims.

[0073] As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, references to “the method” includes one or more methods, and/or steps of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0074] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

[0075] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, it will be understood that modifications and variations are encompassed within the spirit and scope of the instant

disclosure. The preferred methods and materials are now described.

[0076] p95HER2 targeting peptides

[0077] The receptor tyrosine kinase HER2 (ErbB2) is one of four members (HER1- 4) of epidermal growth factor receptor family and overexpressed in different tumors, including 20~30% of breast and gastric cancers. HER1-4 share a similar structure: an extracellular ligand-binding domain, a short hydrophobic transmembrane region and a cytoplasmic tyrosine kinase domain. Activation HER family through hetero/ homodimerization of receptors, induced by ligand binding or receptor overexpression and result in cell proliferation, differentiation, adhesion, migration and survival.

[0078] A recombinant humanized monoclonal antibody, Trastuzumab, binds to the extracellular domain of HER2 . Trastuzumab improves the relapse-free survival and overall survival in HER2-positive early breast cancer patients. Moreover, combination therapy of Trastuzumab and chemotherapy improves the survival and provides substantial clinical benefits in the HER2-positive advanced breast cancer patients. However, only 20-30% of the HER2-positive breast cancer patients respond to Trastuzumab when given as a monotherapy. The patients that do not response to Trastuzumab is caused by several potential mechanisms which lead to primary or acquired resistance, including activation the signaling of insulin-like growth factor receptor (IGF-1R), inactivation or loss the gene of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and accumulation of p95HER2.

[0079] p95HER2 is the truncated forms of HER2 receptor which lack the extracellular trastuzumab-binding domain and about 30~40% of HER2-positive tumors express p95HER2. In some tumors, p95HER2 are the predominant HER2 forms. p95HER2 was generated by alternative initiation of translation from two methionine residues (611 and 687) or by proteolytic shedding of full-length HER2. The biologic role of p95HER2 has not been fully evaluated and the possible relationship between p95HER2 and full-length HER2 are unknown. p95HER2 possesses tyrosine kinase activity, which is essential for xenograft tumor growth in nude mice. Due to its exclusive expression in cancerous tissues, p95HER2 would be a good target for cancer therapy. A bispecific antibody that engages T cells and p95HER2-expressing cancer cells was developed and showed an effective antitumor activity in orthotopic MCF-7 breast cancer and PDX models. In addition, they also developed p95HER2 CAR T cells with remarkable activities against p95HER2-expressing primary tumor and metastasis in the lung or brain *in vivo*.

[0080] Bispecific antibodies (bsAbs), Blinatumomab (Blincyto®) and Catumaxomab (Removab®), that engage TAA of tumor cells and CD3 of T cells have been demonstrated anticancer efficacy and approved for the treatment of Philadelphia chromosome-negative B-acute lymphoblastic leukemia (B-ALL) and malignant ascites with ovarian cancer. However, the poor efficacy in solid tumors and systemic cytokine release of BiTE limit their usages. Compared to anti-CD3, anti-CD137 agonistic antibody may provide the better therapeutic window and safety profile as the proven efficacy against solid tumors in clinical trials and the crosslinking-dependent agonistic activity. Take advantage of cancer-specific p95HER2 expression and our invented target-dependent T-cell activation platform of crosslinking-dependent anti-CD137 single-chain antibody, it may generate a drug with potent efficacy against p95HER2-positive solid tumors meanwhile with a satisfied safety profile.

[0081] Provided here are novel anti-p95HER2 monoclonal antibodies that were generated and screened for the most potent binding affinity. Also provided herein are novel bispecific antibodies that simultaneously targeting p95HER2 and CD137, to induce a superior T-cell activation in the presence of p95HER2.

[0082] *Anti-p95HER2 antibodies and antigen binding fragments thereof*

[0083] In one embodiment, the invention provides antibodies and antigen binding fragments thereof that bind p95HER2. In one aspect, the invention provides four antibodies or antigen binding fragments thereof: anti-p95HER2 antibody clone R4-3 (p95HER2 #R4-3), anti-p95HER2 antibody clone R4-5 (p95HER2 #R4-5), anti- p95HER2 antibody clone R4-11 (p95HER2 #R4-11), anti- p95HER2 antibody clone R4-15 (p95HER2 #R4-15), anti- p95HER2 antibody clone R4-38 (p95HER2 #R4-38). In one aspect, the anti-p95HER2 antibodies provided herein include a heavy chain variable (VH) region including an amino acid sequence with at least 80% sequence identity to a sequence selected from SEQ ID NO:1, SEQ ID NO:9, SEQ ID NO:17, SEQ ID NO:25 and SEQ ID NO:33; and a light chain variable (VL) region including an amino acid sequence with at least 80% sequence identity to a sequence selected from SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:18, SEQ ID NO:26 and SEQ ID NO:34.

[0084] As used herein, the term “antibody” refers to an immunoglobulin molecule that has the ability to specifically bind to an antigen. The term “antibody” includes, but is not limited to, monoclonal antibodies, polyclonal antibodies, human antibodies, humanized antibodies, chimeric antibodies, bispecific antibodies, and anti-idiotypic antibodies, unless context clearly indicates otherwise. In one aspect, antibodies provided herein include monoclonal antibodies.

Antibodies provided herein include any isotype and class (e.g., IgG, IgE, IgM, IgD, IgA and IgY), or subclass (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2). As used herein, “antigen binding fragment” means a fragment or portion of an immunoglobulin molecule or antibody that has the ability to specifically bind to the same antigen as the immunoglobulin molecule or antibody. Exemplary antigen binding fragments include scFv, Fab, or F(ab)₂ fragments. As used herein, “antigen binding region” means the part of an antibody or immunoglobulin molecule that binds to antigens or proteins by contacting the antigen or protein, for example. An antigen binding region generally includes heavy chain variable (VH) regions and light chain variable (VL) regions. An antigen binding region generally includes one or more antigen binding sites or paratopes.

[0085] Antibodies provided herein have a VH region including an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to a sequence of SEQ ID NO:1; SEQ ID NO:9; SEQ ID NO:17; SEQ ID NO:25 or SEQ ID NO:33. Antibodies provided herein also include an VL region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to a sequence of SEQ ID NO:2; SEQ ID NO:10; SEQ ID NO:18; SEQ ID NO:26 SEQ ID NO:34.

[0086] In general, “sequence identity” or “sequence homology,” which can be used interchangeably, refer to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Typically, techniques for determining sequence identity include determining the nucleotide sequence of a polynucleotide and/or determining the amino acid sequence encoded thereby or the amino acid sequence of a polypeptide and comparing these sequences to a second nucleotide or amino acid sequence. As used herein, the term “percent (%) sequence identity” or “percent (%) identity,” also including “homology,” refers to the percentage of amino acid residues or nucleotides in a

sequence that are identical with the amino acid residues or nucleotides in a reference sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Thus, two or more sequences (polynucleotide or amino acid) can be compared by determining their “percent identity,” also referred to as “percent homology.” The percent identity to a reference sequence (e.g., nucleic acid or amino acid sequences), which may be a sequence within a longer molecule (e.g., polynucleotide or polypeptide), may be calculated as the number of exact matches between two optimally aligned sequences divided by the length of the reference sequence and multiplied by 100. Percent identity may also be determined, for example, by comparing sequence information using the advanced BLAST computer program, including version 2.2.9, available from the National Institutes of Health. The BLAST program is based on the alignment method of Karlin and Altschul, *Proc. Natl. Acad. Sci. USA* 87:2264-2268 (1990) and as discussed in Altschul, et al., *J. Mol. Biol.* 215:403-410 (1990); Karlin and Altschul, *Proc. Natl. Acad. Sci. USA* 90:5873-5877 (1993); and Altschul et al., *Nucleic Acids Res.* 25:3389-3402 (1997). Briefly, the BLAST program defines identity as the number of identical aligned symbols (i.e., nucleotides or amino acids), divided by the total number of symbols in the shorter of the two sequences. The program may be used to determine percent identity over the entire length of the sequences being compared. Default parameters are provided to optimize searches with short query sequences, for example, with the blastp program. The program also allows use of an SEG filter to mask-off segments of the query sequences as determined by the SEG program of Wootton and Federhen, *Computers and Chemistry* 17: 149-163 (1993). Ranges of desired degrees of sequence identity are approximately 80% to 100% and integer values in between. Percent identities between a reference sequence and a claimed sequence can be at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, or at least 99.9%. In general, an exact match indicates 100% identity over the length of the reference sequence. Additional programs and methods for comparing sequences and/or assessing sequence identity include the Needleman-Wunsch algorithm (see, e.g., the EMBOSS Needle aligner available at www.ebi.ac.uk/Tools/psa/emboss_needle/, optionally with default settings), the Smith-Waterman algorithm (see, e.g., the EMBOSS Water aligner available at www.ebi.ac.uk/Tools/psa/emboss_water/, optionally with default settings), the similarity search method of Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci. USA* 85, 2444, or computer

programs which use these algorithms (GAP, BESTFIT, FASTA, BLAST P, BLAST N and TFASTA in Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.). In some aspects, reference to percent sequence identity refers to sequence identity as measured using BLAST (Basic Local Alignment Search Tool). In other aspects, ClustalW is used for multiple sequence alignment. Optimal alignment may be assessed using any suitable parameters of a chosen algorithm, including default parameters.

[0087] In one aspect, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:1 and a VL region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:2. SEQ ID NO:1 provides an amino acid sequence that includes the variable region of anti-p95HER2 antibody clone #4-3 heavy chain. SEQ ID NO: 2 provides an amino acid sequence that includes the variable region of anti- p95HER2 clone #4-3 light chain.

[0088] The antigen binding region of an antibody or antigen binding fragment thereof generally includes complementarity determining regions (CDRs). “Complementarity determining region (CDR)” refers to hypervariable regions of VH and VL. CDRs include the target protein or antigen binding site of an antibody that confers specificity for protein or antigen binding. VH and VL generally include three sequentially numbered CDRs. As used herein, CDR-H1, CDR-H2, and CDR-H3 refer to three consecutively arranged CDRs of the heavy chain variable region (VH), as numbered from the N-terminus of the heavy chain polypeptide. As used herein, CDR-L1, CDR-L2, and CDR-L3 refer to three consecutively arranged CDRs of the light chain variable region (VL), as numbered from the N-terminus of the light chain polypeptide.

[0089] In one aspect, the antigen binding region of the antibody or antigen binding fragment thereof having a VH region that includes an amino acid sequence having at least about 80% identity to SEQ ID NO:1 and a VL region that includes an amino acid sequence having at least about 80% identity to SEQ ID NO:2 has a CDR-H1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:3, a CDR-H2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:4, and a CDR-H3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:5. In another aspect, the antigen binding region of the antibody or antigen binding fragment thereof having a VH region that includes an amino acid sequence having at least about 80% identity to SEQ ID NO:1 and a VL region that includes an amino acid sequence having at least about 80% identity to SEQ ID NO:2 has a CDR-L1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:6, a CDR-L2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at

least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:7, and a CDR-L3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:8.

[0090] In some aspects, the antibody or antigen binding fragment thereof includes a VH region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:9 and a VL region that includes an amino acid sequence having at least about 80%, identity at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:10. SEQ ID NO:9 provides an amino acid sequence that includes the variable region of anti-p95HER2 antibody clone #4-5 heavy chain. SEQ ID NO: 10 provides an amino acid sequence that includes the variable region of anti-p95HER2 clone #4-5 light chain.

[0091] In one aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:9 and a VL region including an amino acid sequence having at least about 80% identity to SEQ ID NO:10 has a CDR-H1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:11,

a CDR-H2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:12, and a CDR-H3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:13. In another aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:9 and a VL region that includes an amino acid sequence having at least about 80% identity to SEQ ID NO:10 has a CDR-L1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:14, a CDR-L2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:15, and a CDR-L3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:16.

[0092] In some aspects, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:17 and a VL region that includes an amino acid sequence having at least about 80%, identity at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:18. SEQ ID NO:17 provides an amino acid sequence that includes the variable region of anti-p95HER2 antibody clone #4-11 heavy chain. SEQ ID NO:18 provides an amino acid sequence that includes the variable region of anti-p95HER2 antibody clone #4-11 light chain.

[0093] In one aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:17 and a VL region including an amino acid sequence having at least about 80% identity to SEQ ID NO:18 has a CDR-H1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:19, a CDR-H2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:20, and a CDR-H3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about

94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:21. In another aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VII region including an amino acid sequence having at least about 80% identity to SEQ ID NO:17 and a VL region including an amino acid sequence having at least about 80%, identity to SEQ ID NO:18 has a CDR-L1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:22, a CDR-L2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:23, and a CDR-L3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:24.

[0094] In some aspects, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:25 and a VL region that includes an amino acid sequence having at least about 80%, identity at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94%

identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:26. SEQ ID NO:25 provides an amino acid sequence that includes the variable region of anti-p95HER2 antibody clone #4-15 heavy chain. SEQ ID NO: 26 provides an amino acid sequence that includes the variable region of anti-p95HER2 antibody clone #4-15 light chain.

[0095] In one aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:25 and a VL region including an amino acid sequence having at least about 80% identity to SEQ ID NO:26 has a CDR-H1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:27, a CDR-H2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:28, and a CDR-H3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:29. In another aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:25 and a VL region including an amino acid sequence having at least about 80%, identity to SEQ ID NO:26 has a CDR-L1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94%

identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:30, a CDR-L2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:31, and a CDR-L3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:32.

[0096] In some aspects, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:33 and a VL region that includes an amino acid sequence having at least about 80%, identity at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:34. SEQ ID NO:33 provides an amino acid sequence that includes the variable region of anti-p95HER2 antibody clone #4-38 heavy chain. SEQ ID NO: 34 provides an amino acid sequence that includes the variable region of anti-p95HER2 antibody clone #4-38 light chain.

[0097] In one aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:33 and a VL region including an amino acid sequence having

at least about 80% identity to SEQ ID NO:34 has a CDR-H1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:35, a CDR-H2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:36, and a CDR-H3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:37. In another aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:33 and a VL region including an amino acid sequence having at least about 80%, identity to SEQ ID NO:34 has a CDR-L1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:38, a CDR-L2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:39, and a CDR-L3 that includes an amino acid sequence having at least about

80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:40.

[0098] Antibodies or antigen binding fragments thereof provided herein further include an Fc domain. As used herein, the term Fc domain refers to an antibody region that includes at least a hinge region, a CH2 domain, and a CH3 domain, unless context clearly indicates otherwise. The terms Fc domain and Fc region may be used interchangeably, unless context clearly indicates otherwise. In certain aspects, the Fc domain is an IgG domain, an IgE domain, an IgM domain, and IgD domain, an IgA domain, or an IgY domain. Fc domains of any sequence and from any species can be used, including human, ape, monkey, mouse, rabbit, goat, sheep, guinea pig, horse, and others. In certain aspects, Fc domains are engineered, i.e., non-naturally occurring or recombinant Fc domains generated using techniques of molecular biology, for example. In some aspects, the IgG domain is an IgG1 domain, an IgG2 domain, an IgG3 domain, or an IgG4 domain.

[0099] *Anti-p95HER2 bispecific antibodies*

[0100] Bispecific molecules, such as bispecific antibodies (bsAbs), provide a means for simultaneously targeting multiple epitopes on the same or different molecular targets with a single therapeutic agent. Without being limited by theory, bispecific molecules as cancer therapeutics have the potential to confer novel or more potent activities, lower the cost of goods, and facilitate the development of new therapeutic regimens as compared to a mixture of two monoclonal antibodies (mAbs), for example.

[0101] Provided herein, in some embodiments, are bispecific antibodies that include a first antigen binding region and a second antigen binding region. Generally, the first antigen binding region and the second antigen binding region specifically bind to different antigens or targets. In some aspects, the first antigen binding region and the second antigen binding region bind to different epitopes in the same antigen or target.

[0102] In an embodiment, the invention provides bispecific antibodies including a first antigen binding region and a second antigen binding region, wherein the first antigen binding region binds to p95HER2. The first antigen binding region includes a VH region that includes an amino acid sequence having at least 80% identity, at least 85% identity, at least 90% identity,

at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, at least 99.5% identity, at least 99.9% identity, and any number or range in between, to a sequence selected from SEQ ID NO:17 and a VL region that includes an amino acid sequence having at least 80% identity, at least 85% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, at least 99.5% identity, at least 99.9% identity, and any number or range in between, to about 100 to 120 amino acids of an N-terminal sequence of a sequence selected from SEQ ID NO:18; In some aspects, the second antigen binding region of bispecific antibodies provided herein binds to an immune checkpoint molecule, an immune stimulatory molecule, or a tumor antigen.

[0103] In one aspect, the bispecific antibody includes a V_H region including complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:19, CDR-H2 is set forth in SEQ ID NO:20, and CDR-H3 is set forth in SEQ ID NO:21 or sequences having 90% identity to SEQ ID NO:19, 20 or 21 and the antigen binding specificity thereof; and a V_L region including CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:22, CDR-L2 is set forth in SEQ ID NO:23, and CDR-L3 is set forth in SEQ ID NO:24 or sequences having 90% identity to SEQ ID NO:19, 20 or 21 and the antigen binding specificity thereof. In another aspect, the V_H region has an amino acid sequence having at least 80% identity to SEQ ID NO:17 and an antigen binding specificity of SEQ ID NOs:19-21 and the V_L region has an amino acid sequence having at least 80% identity to SEQ ID NO:18 and an antigen binding specificity of SEQ ID NOs:22-24.

[0104] Any combination of first and second antigen binding regions can be included in bispecific antibodies provided herein, including first and second antigen binding regions that bind to any immune checkpoint molecule, any immune stimulatory molecule, or any tumor antigen, for example. Accordingly, in some aspects, the second antigen binding region of bispecific antibodies provided herein binds to any immune checkpoint molecule, any immune stimulatory molecule, or any tumor antigen. In some aspects, the first and second antigen binding regions bind to the same molecule. For example, the first and second antigen binding regions may bind to the same or to a different epitope of the same molecule. In other aspects, the first and second antigen binding regions bind to different molecules.

[0105] In one aspect, bispecific antibodies having a first antigen binding region that binds to p95HER2 and a second antigen binding region that binds to CD137 bind to p95HER2 and CD137 simultaneously (**FIGURE 8**). Without being limited by theory, restricting anti-p95HER2 binding activity to tumor sites that express p95HER2 by designing bispecific antibodies that are able to bind to both p95HER2 and CD137 may reduce the risk of hepatotoxicity and its associated fatality seen in clinical trials with anti-CD137 antibodies such as Urelumab. In addition, it is believed that simultaneous binding to p95HER2 and CD137 may enhance T-cell activation as a result of cross-linking (see also Example 8 below).

[0106] In some aspects, first antigen binding regions and second antigen binding regions include an scFv, an F(ab)₂, an Fab, or any combination thereof. In one aspect, the first antigen binding region includes an scFv and the second antigen binding region includes an Fab. In another aspect, an scFv included in bispecific antibodies provided herein binds to an immune checkpoint molecule, an immune stimulatory molecule, or a tumor antigen. In yet another aspect, an scFv included in bispecific antibodies provided herein binds to a CD137. In some aspects, an Fab included in bispecific antibodies provided herein binds to an immune checkpoint molecule, an immune stimulatory molecule, or a tumor antigen. In one aspect, an Fab included in bispecific antibodies provided herein binds to p95HER2. In another aspect, the scFv of bispecific antibodies provided herein includes an amino acid sequence of SEQ ID NO: 48.

[0107] In some aspects, bispecific antibodies provided herein further include a linker between the VH region and the VL region of the scFv. Any linker can be used. For example, linkers can include any amino acid sequence. Linkers can be of any length, such as one amino acid, two amino acids, three amino acids, four amino acids, five amino acids, six amino acids, seven amino acids, eight amino acids, nine amino acids, ten amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14 amino acids, 15 amino acids, 16 amino acids, 17 amino acids, 18 amino acids, 19 amino acids, 20 amino acids, or more amino acids. Linkers can also include multiples of an amino acid sequence. Any number of multiples of an amino acid sequence can be included in a linker. Exemplary linker sequences are provided in SEQ ID NO:44, SEQ ID NO:45 and SEQ ID NO:160.

[0108] In some aspects, bispecific antibodies provided herein include a heavy chain sequence of SEQ ID NO:46. In another aspect, bispecific antibodies provided herein further include a light chain sequence of SEQ ID NO:18. Heavy chain sequences such as SEQ ID

NO:46, and others, can include one or more G linkers (SEQ ID NO:44), one or more G4S linkers (SEQ ID NO:45 or SEQ ID NO:160), or any multiple of a G linker or G4S linker, although any other suitable linker can be used.

[0109] CD73 targeting peptides

[0110] Immunoregulation of the adaptive immune system has been an attractive field in cancer immunotherapy because of fewer side effects and long-term protection from cancer relapse. Despite the success of anti-PD-1/PD-L1 therapies in advanced cancer, a considerable proportion of patients remain unresponsive to these treatments. This represents that other immunosuppressive mechanisms coexist in the tumor microenvironment, and the adenosinergic pathway has emerged as a key player in it.

[0111] Tumor hypoxia, chronic inflammation, and metabolic changes give rise to extracellular ATP accumulation that promote anti-tumor activity through inductions of inflammasome assembly and proinflammatory cytokines secretion. However, owing to the presence of hypoxia, inflammatory cytokines, and TGF- β , higher levels of ectonucleotidases CD39 and CD73 usually observed in solid tumors compared to the corresponding non-malignant tissues. Extracellular accumulated ATP was sequentially hydrolyzed by tumor associated CD39 and CD73 to adenosine, then mediates its regulatory functions upon binding to adenosine receptors expressed by tumor cells, stromal cells, and infiltrating lymphocytes in tumors.

[0112] In tumor cells, adenosine signaling via A2A and A2B receptors promotes proliferation, metastasis, and secretion of vascular endothelial growth factor (VEGF). In cancer-associated fibroblasts (CAFs), adenosine signaling via A2BR induces the expressions of pro-tumor fibroblast growth factor 2 (FGF2) and CXCL12 that create a pro-tumor microenvironment. In the tumor-infiltrating immune cells, A2A signaling inhibits effector T cell activation through dampening proximal TCR signaling and concurrently inducing co-inhibitory molecules PD-1, LAG3, and TIM3 expression. In addition, A2A signaling promotes differentiation, proliferation, and CTLA-4 expression of murine Treg cells. In NK cells, A2A signaling blocks maturation and cytotoxicity. In professional antigen-presenting cells, macrophage and dendritic cells, A2A and A2B signaling downregulate the expressions of antigen presentation and costimulatory molecules, while promotes M2 macrophage differentiation.

[0113] High levels of CD73 were found in the malignant tissues of various cancer types, including pancreatic, gastrointestinal, renal and lung cancers, and corresponded to a poor prognosis. High levels of soluble CD73 were also observed in the circulation of patients with metastatic melanoma. Given the pro-tumor functions of adenosine and relevance of CD73 in cancer prognosis, CD73 has become an attractive therapeutic target for cancers. In the preclinical studies, antitumor activities of small molecule inhibitors and antibodies target CD73 were attributed not only to the inhibition of tumor cell growth and migration, but the involvement of adaptive immunity as well. In addition, combine anti-CD73 antibody enhanced antitumor activities of antibodies against other immune regulatory molecules, including CTLA-4, PD-1, and CD137. The synergized antitumor activity in the combination with anti-PD-1 antibody was attribute to the activation of A2A signaling that enhance PD-1 expression on tumor-specific CD8+ T. Intriguingly, tumor resistance to anti-CD137 antibody therapy was referred to the upregulation of CD73 on tumor-infiltrating CD8+ T cells, therefore a more effective antitumor activity was elicited by the combination therapy of anti-CD137 and CD73 antibody.

[0114] In clinical studies, several anti-CD73 antibodies including, MEDI9447 (oleclumab), CPI-006, BMS-986179, and NZV930, are undergoing Phase I/II in the monotherapy or combinational therapies. The partial response was observed in 4.8% (1/21) patients with colon rectal cancer, and in 10% (2/20) patients with pancreatic cancer by treating with oleclumab and durvalumab (anti-PD-L1 antibody). The partial response was observed in 13.5% (7/52) patients with head and neck, pancreatic, prostate, anal, and renal cancer by the combination of BMS-986179 and nivolumab (anti-PD-1 antibody).

[0115] Provided herein are novel anti-CD73 monoclonal antibodies that were generated and screened for the most potent binding affinity and enzymatic blockade activity. Also provided herein are a novel bispecific antibody that simultaneously targeting CD73 and CD137 or PD-L1, to induce a superior T-cell activation upon coculturing with CD73+ tumor cells and antitumor activity *in vivo*.

[0116] *Anti-CD73 antibodies and antigen binding fragments thereof*

[0117] In one embodiment, the invention provides antibodies and antigen binding fragments thereof that bind CD73. In one aspect, the invention provides four antibodies or antigen binding fragments thereof: anti-CD73 antibody clone 3-4 (CD73 #3-4), anti-CD73 antibody clone 3-16 (CD73 #3-16), anti-CD73 antibody clone 3-1-28 (CD73 #3-1-28), and

anti-CD73 antibody clone 3-1-37 (CD73 #3-1-37). In another aspect, the invention provides three affinity-maturated antibodies derived from anti-CD73 clone 3-1-28: anti-CD73 #3-1-28 (S51A Q54E), anti-CD73 #3-1-28 (S107D S109H), anti-CD73 #3-1-28 (S107D S109T A114S).

[0118] In one aspect, the anti-CD73 antibodies provided herein include a heavy chain variable (VH) region including an amino acid sequence with at least 80% sequence identity to a sequence selected from SEQ ID NO:49, SEQ ID NO:57, SEQ ID NO:65 and SEQ ID NO:73; and a light chain variable (VL) region including an amino acid sequence with at least 80% sequence identity to a sequence selected from SEQ ID NO:50, SEQ ID NO:58, SEQ ID NO:66, SEQ ID NO:74, SEQ ID NO:81, SEQ ID NO:83 and SEQ ID NO:85.

[0119] Antibodies provided herein have a VH region including an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to a sequence of SEQ ID NO:49, SEQ ID NO:57, SEQ ID NO:65, and SEQ ID NO:73. Antibodies provided herein also include an VL region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to a sequence of SEQ ID NO:50, SEQ ID NO:58, SEQ ID NO:66 and SEQ ID NO:74, SEQ ID NO:81, SEQ ID NO:83 and SEQ ID NO:85.

[0120] In one aspect, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:49 and a VL region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about

91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:50. SEQ ID NO:49 provides an amino acid sequence that includes the variable region of anti-CD73 antibody clone #3-4 heavy chain. SEQ ID NO: 50 provides an amino acid sequence that includes the variable region of anti-CD73 clone #3-4 light chain.

[0121] In one aspect, the antigen binding region of the antibody or antigen binding fragment thereof having a VH region that includes an amino acid sequence having at least about 80% identity to SEQ ID NO:49 and a VL region that includes an amino acid sequence having at least about 80% identity to SEQ ID NO:50 has a CDR-H1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:51, a CDR-H2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:52, and a CDR-H3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:53. In another aspect, the antigen binding region of the antibody or antigen binding fragment thereof having a VH region that includes an amino acid sequence having at least about 80% identity to SEQ ID NO:49 and a VL region that includes an amino acid sequence having at least about 80% identity to SEQ ID NO:50 has a CDR-L1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about

91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:54, a CDR-L2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:55, and a CDR-L3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:56.

[0122] In some aspects, the antibody or antigen binding fragment thereof includes a VH region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:57 and a VL region that includes an amino acid sequence having at least about 80%, identity at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:58. SEQ ID NO:57 provides an amino acid sequence that includes the variable region of anti-CD73 antibody clone #3-16 heavy chain. SEQ ID NO:58 provides an amino acid sequence that includes the variable region of anti-CD73 clone #3-16 light chain.

[0123] In one aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least

about 80% identity to SEQ ID NO:57 and a VL region including an amino acid sequence having at least about 80% identity to SEQ ID NO:58 has a CDR-H1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:59, a CDR-H2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:60, and a CDR-H3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:61. In another aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:57 and a VL region that includes an amino acid sequence having at least about 80%, identity to SEQ ID NO:58 has a CDR-L1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:62, a CDR-L2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between,

to SEQ ID NO:63, and a CDR-L3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:64.

[0124] In some aspects, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:65 and a VL region that includes an amino acid sequence having at least about 80%, identity at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:66. SEQ ID NO:65 provides an amino acid sequence that includes the variable region of anti-CD73 antibody clone #3-1-28 heavy chain. SEQ ID NO:66 provides an amino acid sequence that includes the variable region of anti-CD73 clone #3-1-28 light chain.

[0125] In one aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:65 and a VL region including an amino acid sequence having at least about 80% identity to SEQ ID NO:66 has a CDR-H1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:67, a CDR-H2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92%

identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:68, and a CDR-H3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:69. In another aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:65 and a VL region including an amino acid sequence having at least about 80%, identity to SEQ ID NO:66 has a CDR-L1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:70, a CDR-L2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:71, and a CDR-L3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:72.

[0126] In some aspects, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at

least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:73 and a VL region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:74. SEQ ID NO:73 provides an amino acid sequence that includes the variable region of anti-CD73 antibody clone #3-1-37 heavy chain. SEQ ID NO:74 provides an amino acid sequence that includes the variable region of anti-CD73 clone #3-1-37 light chain.

[0127] In one aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:73 and a VL region including an amino acid sequence having at least about 80% identity to SEQ ID NO:74 has a CDR-H1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:75, a CDR-H2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:76, and a CDR-H3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:77. In

another aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:73 and a VL region including an amino acid sequence having at least about 80%, identity to SEQ ID NO:74 has a CDR-L1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:78, a CDR-L2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:79, and a CDR-L3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:80.

[0128] In some aspects, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:65 and a VL region that includes an amino acid sequence having at least about 80%, identity at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:81. SEQ ID NO:65

provides an amino acid sequence that includes the variable region of anti-CD73 antibody clone #3-1-28 heavy chain. SEQ ID NO:81 provides an amino acid sequence that includes the variable region of anti-CD73 clone #3-1-28 (S51A Q54E) light chain.

[0129] In one aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VII region including an amino acid sequence having at least about 80% identity to SEQ ID NO:65 and a VL region including an amino acid sequence having at least about 80% identity to SEQ ID NO:81 has a CDR-H1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:67, a CDR-H2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:68, and a CDR-H3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:69. In another aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:65 and a VL region including an amino acid sequence having at least about 80%, identity to SEQ ID NO:81 has a CDR-L1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:70, a CDR-L2 that

includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:82, and a CDR-L3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:72.

[0130] In some aspects, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:65 and a VL region that includes an amino acid sequence having at least about 80%, identity at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:83. SEQ ID NO:65 provides an amino acid sequence that includes the variable region of anti-CD73 antibody clone #3-1-28 heavy chain. SEQ ID NO:83 provides an amino acid sequence that includes the variable region of anti-CD73 clone #3-1-28 (S107D S109H) light chain.

[0131] In one aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:65 and a VL region including an amino acid sequence having at least about 80% identity to SEQ ID NO:83 has a CDR-H1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at

least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:67, a CDR-H2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:68, and a CDR-H3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:69. In another aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:65 and a VL region including an amino acid sequence having at least about 80%, identity to SEQ ID NO:83 has a CDR-L1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:70, a CDR-L2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:84, and a CDR-L3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about

98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:72.

[0132] In some aspects, the antibody or antigen binding fragment thereof has a VH region that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:65 and a VL region that includes an amino acid sequence having at least about 80%, identity at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:85. SEQ ID NO:65 provides an amino acid sequence that includes the variable region of anti-CD73 antibody clone #3-1-28 heavy chain. SEQ ID NO:85 provides an amino acid sequence that includes the variable region of anti-CD73 clone #3-1-28 (S107D S109T A114S) light chain.

[0133] In one aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:65 and a VL region including an amino acid sequence having at least about 80% identity to SEQ ID NO:85 has a CDR-H1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:67, a CDR-H2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:68, and a CDR-H3 that includes an amino acid sequence

having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:69. In another aspect, the antigen binding region of the antibody or antigen binding fragment thereof that includes a VH region including an amino acid sequence having at least about 80% identity to SEQ ID NO:65 and a VL region including an amino acid sequence having at least about 80%, identity to SEQ ID NO:85 has a CDR-L1 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:70, a CDR-L2 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:86, and a CDR-L3 that includes an amino acid sequence having at least about 80% identity, at least about 85% identity, at least about 90% identity, at least about 91% identity, at least about 92% identity, at least about 93% identity, at least about 94% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, at least about 99.9% identity, and any number or range in between, to SEQ ID NO:72.

[0134] Antibodies or antigen binding fragments thereof provided herein further include an Fc domain. In certain aspects, the Fc domain is an IgG domain, an IgE domain, an IgM domain, and IgD domain, an IgA domain, or an IgY domain. In some aspects, the IgG domain is an IgG1 domain, an IgG2 domain, an IgG3 domain, or an IgG4 domain.

[0135] *Anti-CD73 bispecific antibodies*

[0136] Provided herein are bi-functional proteins, including bispecific antibodies. As used herein, the term “bi-functional protein” refers to a protein that has at least two functions. A

non-limiting example of a bi-functional protein includes a bispecific antibody that is capable of binding to two antigens. Bispecific antibodies provided herein can include isolated, functional scFv fragments that bind to CD137 and that are fused to the C-terminus of the Fc domain of an anti-CD73 antibody, for example. In some aspects, a C-terminally positioned scFv that binds CD137 in fusion constructs provided herein is fused to an Fc domain of an antibody that binds to other immunoregulatory molecules, such as CD40 or CTLA-4, for example. In other aspects, a C-terminally positioned scFv can bind to an immunoregulatory molecule, such as CD40 or CTLA-4, for example.

[0137] In one embodiment, the invention provides a bispecific antibody including a first antigen binding region and a second antigen binding region, wherein the first antigen binding region binds to CD73. The first antigen binding region includes a V_H region that includes an amino acid sequence having at least 80% identity, at least 85% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, at least 99.5% identity, at least 99.9% identity, and any number or range in between, to a sequence selected from SEQ ID NO:65 and a V_L region that includes an amino acid sequence having at least 80% identity, at least 85% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, at least 99.5% identity, at least 99.9% identity, and any number or range in between, to about 100 to 120 amino acids of an N-terminal sequence of a sequence selected from SEQ ID NO:66; In some aspects, the second antigen binding region of bispecific antibodies provided herein binds to an immune checkpoint molecule, an immune stimulatory molecule, or a tumor antigen.

[0138] In one aspect, the bispecific antibody includes a V_H region including complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof; and a V_L region including CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:71, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 71 or 72 and the antigen binding specificity thereof. In another aspect, the V_H region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding

specificity of SEQ ID NOs:67-69 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:66 and an antigen binding specificity of SEQ ID NOs:70-72.

[0139] The first antigen binding region includes a V_H region that includes an amino acid sequence having at least 80% identity, at least 85% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, at least 99.5% identity, at least 99.9% identity, and any number or range in between, to a sequence selected from SEQ ID NO:65 and a V_L region that includes an amino acid sequence having at least 80% identity, at least 85% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, at least 99.5% identity, at least 99.9% identity, and any number or range in between, to about 100 to 120 amino acids of an N-terminal sequence of a sequence selected from SEQ ID NO:83; In some aspects, the second antigen binding region of bispecific antibodies provided herein binds to an immune checkpoint molecule, an immune stimulatory molecule, or a tumor antigen.

[0140] In one aspect, the bispecific antibody includes a V_H region including complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof; and a V_L region including CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:84, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 84 or 72 and the antigen binding specificity thereof. In another aspect, the V_H region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the V_L region has an amino acid sequence having at least 80% identity to SEQ ID NO:83 and an antigen binding specificity of SEQ ID NOs:70, 84 and 72.

[0141] The first antigen binding region includes a V_H region that includes an amino acid sequence having at least 80% identity, at least 85% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, at least 99.5% identity, at least 99.9% identity, and any number or range in between, to a sequence

selected from SEQ ID NO:65 and a VL region that includes an amino acid sequence having at least 80% identity, at least 85% identity, at least 90% identity, at least 91% identity, at least 92% identity, at least 93% identity, at least 94% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, at least 99.5% identity, at least 99.9% identity, and any number or range in between, to about 100 to 120 amino acids of an N-terminal sequence of a sequence selected from SEQ ID NO:85; In some aspects, the second antigen binding region of bispecific antibodies provided herein binds to an immune checkpoint molecule, an immune stimulatory molecule, or a tumor antigen.

[0142] In one aspect, the bispecific antibody includes a V_H region including complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof; and a V_L region including CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:85, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 86 or 72 and the antigen binding specificity thereof. In another aspect, the V_H region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the V_L region has an amino acid sequence having at least 80% identity to SEQ ID NO:85 and an antigen binding specificity of SEQ ID NOs:70, 86 and 72.

[0143] In some aspects, the second antigen binding region of bispecific antibodies provided herein binds to an immune checkpoint molecule, an immune stimulatory molecule, or a tumor antigen.

[0144] Any combination of first and second antigen binding regions can be included in bispecific antibodies provided herein, including first and second antigen binding regions that bind to any immune checkpoint molecule, any immune stimulatory molecule, or any tumor antigen, for example. Accordingly, in some aspects, the second antigen binding region of bispecific antibodies provided herein binds to any immune checkpoint molecule, any immune stimulatory molecule, or any tumor antigen. In some aspects, the first and second antigen binding regions bind to the same molecule. For example, the first and second antigen binding regions may bind to the same or to a different epitope of the same molecule. In other aspects, the first and second antigen binding regions bind to different molecules.

[0145] In one aspect, bispecific antibodies having a first antigen binding region that binds to CD73 and a second antigen binding region that binds to CD137 bind to CD73 and CD137 simultaneously (**FIGURE 22**). Without being limited by theory, restricting anti-CD73 binding activity to tumor sites that express CD73 by designing bispecific antibodies that are able to bind to both CD73 and CD137 may reduce the risk of hepatotoxicity and its associated fatality seen in clinical trials with anti-CD137 antibodies such as Urelumab. In addition, it is believed that simultaneous binding to CD73 and CD137 may enhance T-cell activation as a result of cross-linking (see also Example 21 below).

[0146] In some aspects, first antigen binding regions and second antigen binding regions include an scFv, an F(ab)₂, an Fab, or any combination thereof. In one aspect, the first antigen binding region includes an scFv and the second antigen binding region includes an Fab. In another aspect, an scFv included in bispecific antibodies provided herein binds to an immune checkpoint molecule, an immune stimulatory molecule, or a tumor antigen. In yet another aspect, an scFv included in bispecific antibodies provided herein binds to a CD137. In some aspects, an Fab included in bispecific antibodies provided herein binds to an immune checkpoint molecule, an immune stimulatory molecule, or a tumor antigen. In one aspect, an Fab included in bispecific antibodies provided herein binds to CD73. In one aspect, the antibody includes a heavy chain sequence having the amino acid sequence of SEQ ID NO:88, 89, 90 or 91 or a sequence having 90% identity thereto and the binding specificity thereof. In another aspect, the bispecific antibody further includes a light chain having the amino acid sequence of SEQ ID NO:66. In another aspect, the scFv of bispecific antibodies provided herein includes an amino acid sequence of SEQ ID NO:91.

[0147] In some aspects, bispecific antibodies provided herein further include a linker between the VH region and the VL region of the scFv. Any linker can be used. For example, linkers can include any amino acid sequence. Linkers can be of any length, such as one amino acid, two amino acids, three amino acids, four amino acids, five amino acids, six amino acids, seven amino acids, eight amino acids, nine amino acids, ten amino acids, 11 amino acids, 12 amino acids, 13 amino acids, 14 amino acids, 15 amino acids, 16 amino acids, 17 amino acids, 18 amino acids, 19 amino acids, 20 amino acids, or more amino acids. Linkers can also include multiples of an amino acid sequence. Any number of multiples of an amino acid sequence can be included in a linker. Exemplary linker sequences are provided in SEQ ID NO:44, SEQ ID NO:45 and 160.

[0148] In some aspects, bispecific antibodies provided herein include a heavy chain sequence of SEQ ID NO:88, SEQ ID NO:89, or SEQ ID NO:91. In another aspect, bispecific antibodies provided herein further include a light chain sequence of SEQ ID NO:66 or SEQ ID NO:81. Heavy chain sequences such as SEQ ID NO:88, SEQ IDNO:89, SEQ IDNO:91, and others, can include one or more G linkers (SEQ ID NO:44), one or more G4S linkers (SEQ ID NO:45 and SEQ ID NO:160), or any multiple of a G linker or G4S linker, although any other suitable linker can be used.

[0149] **Antibody-drug conjugates**

[0150] In one embodiment, the invention provides an antibody-drug conjugate including: a therapeutic agent, and any one of the antibodies or antigen binding fragments thereof described herein, or any one of the bispecific antibodies described herein. For example, the antibody, antigen binding fragment thereof or the bispecific antibody include anti-p95HER2 antibody, antigen binding fragment thereof, bispecific antibody targeting p95HER2 and a second target, anti-CD73 antibody, antigen binding fragment thereof, and bispecific antibody targeting CD73 and a second target, such as those described herein.

[0151] The term “antibody drug conjugate” can be used interchangeably with the term “immunoconjugate” or “conjugate” as used herein and refers to a compound or a derivative thereof, such as a drug (i.e., an anti-cancer drug) that is linked to a cell binding agent (i.e., an antibody or fragment thereof, or a bispecific antibody) and is defined by a generic formula: C-L-A, wherein C=cytotoxin, L=linker, A=cell binding agent or antibody. Immunoconjugates can also be defined by the generic formula in reverse order: A-L-C.

[0152] In one aspect, the therapeutic agent is covalently linked to the antibody, antigen binding fragment or bispecific antibody via a linker.

[0153] By “therapeutic agent” it is meant any drug, compound or small molecule, useful for the treatment of cancer, that can be linked to the binding agent described herein. In various aspects, the therapeutic agent is a chemotherapeutic agent. Chemotherapeutic and antineoplastic agents are well known cytotoxic agents, and include but are not limited to: (i) anti-microtubules agents comprising vinca alkaloids (vinblastine, vincristine, vinflunine, vindesine, and vinorelbine), taxanes (cabazitaxel, docetaxel, larotaxel, ortataxel, paclitaxel, and tesetaxel), epothilones (ixabepilone), and podophyllotoxin (etoposide and teniposide); (ii) antimetabolite agents comprising anti-folates (aminopterin, methotrexate, pemetrexed, pralatrexate, and raltitrexed), and deoxynucleoside analogues (azacitidine, capecitabine,

carmofur, cladribine, clofarabine, cytarabine, decitabine, doxifluridine, floxuridine, fludarabine, fluorouracil, gemcitabine, hydroxycarbamide, mercaptopurine, nelarabine, pentostatin, tegafur, and thioguanine); (iii) topoisomerase inhibitors comprising Topoisomerase I inhibitors (belotecan, camptothecin, cositecan, gimatecan, exatecan, irinotecan, lurtotecan, silatecan, topotecan, and rubitecan) and Topoisomerase II inhibitors (aclerubicin, amrubicin, daunorubicin, doxorubicin, epirubicin, etoposide, idarubicin, merbarone, mitoxantrone, novobiocin, pirarubicin, teniposide, valrubicin, and zorubicin); (iv) alkylating agents comprising nitrogen mustards (bendamustine, busulfan, chlorambucil, cyclophosphamide, estramustine phosphate, ifosamide, mechlorethamine, melphalan, prednimustine, trofosfamide, and uramustine), nitrosoureas (carmustine (BCNU), fotemustine, lomustine (CCNU), N-Nitroso-N-methylurea (MNU), nimustine, ranimustine semustine (MeCCNU), and streptozotocin), platinum-based (cisplatin, carboplatin, dicycloplatin, nedaplatin, oxaliplatin and satraplatin), aziridines (carboquone, thiotepa, mytomyacin, diaziquone (AZQ), triaziquone and triethylenemelamine), alkyl sulfonates (busulfan, mannosulfan, and treosulfan), non-classical alkylating agents (hydrazines, procarbazine, triazenes, hexamethylmelamine, altretamine, mitobronitol, and pipobroman), tetrazines (dacarbazine, mitozolomide and temozolomide); (v) anthracyclines agents comprising doxorubicin and daunorubicin. Derivatives of these compounds include epirubicin and idarubicin; pirarubicin, aclerubicin, and mitoxantrone, bleomycins, mitomycin C, mitoxantrone, and actinomycin; (vi) enzyme inhibitors agents comprising FI inhibitor (Tipifarnib), CDK inhibitors (Abemaciclib, Alvociclib, Palbociclib, Ribociclib, and Seliciclib), PrI inhibitor (Bortezomib, Carfilzomib, and Ixazomib), PhI inhibitor (Anagrelide), IMPDI inhibitor (Tiazofurin), LI inhibitor (Masoprocol), PARP inhibitor (Niraparib, Olaparib, Rucaparib), HDAC inhibitor (Belinostat, Panobinostat, Romidepsin, Vorinostat), and PIKI inhibitor (Idelalisib); (vii) receptor antagonist agent comprising ERA receptor antagonist (Atrasentan), Retinoid X receptor antagonist (Bexarotene), Sex steroid receptor antagonist (Testolactone); (viii) ungrouped agent comprising Amsacrine, Trabectedin, Retinoids (Alitretinoin Tretinoin) Arsenic trioxide, Asparagine depleters (Asparaginase/Pegaspargase), Celecoxib, Demecolcine, Elesclomol, Elsamitracin, Etoglucid, Lonidamine, Lucanthone, Mitoguazone, Mitotane, Oblimersen, Omacetaxine mepesuccinate, and Eribulin.

[0154] Pharmaceutical compositions

[0155] In one embodiment, the invention provides a pharmaceutical composition including

any one of the antibodies or antigen binding fragments thereof described herein, any one of the bispecific antibodies described herein or any one of the antibody-drug conjugates described herein, and at least one pharmaceutical acceptable carrier.

[0156] For example, the pharmaceutical composition includes an anti-p95HER2 antibody, an antigen binding fragment thereof, a bispecific antibody targeting p95HER2 and a second target, an antibody-drug conjugate thereof, an anti-CD73 antibody, an antigen binding fragment thereof, a bispecific antibody targeting CD73 and a second target, or an antibody-drug conjugate thereof, such as those described herein.

[0157] As used herein, "pharmaceutical composition" refers to a formulation comprising an active ingredient, and optionally one or more pharmaceutically acceptable carriers, diluents or excipients. The term "active ingredient" can interchangeably refer to an "effective ingredient" and is meant to refer to any agent that is capable of inducing a sought-after effect upon administration. In one embodiment, the active ingredient includes a biologically active molecule. As used herein, the phrase "biologically active molecule" refers to a molecule that has a biological effect in a cell. In certain embodiments the active molecule is an antibody, an antibody fragment, a bispecific antibody or an antibody-drug conjugate, such as those described herein or a morpholino.

[0158] By "pharmaceutically acceptable" it is meant the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof, nor to the activity of the active ingredient of the formulation. Pharmaceutically acceptable carriers, excipients or stabilizers are well known in the art, for example Remington's Pharmaceutical Sciences, 16th edition, Osol, A. Ed. (1980). Pharmaceutically acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and may include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating

agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (for example, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Examples of carrier include, but are not limited to, liposome, nanoparticles, ointment, micelles, microsphere, microparticle, cream, emulsion, and gel. Examples of excipient include, but are not limited to, anti-adherents such as magnesium stearate, binders such as saccharides and their derivatives (sucrose, lactose, starches, cellulose, sugar alcohols and the like) protein like gelatin and synthetic polymers, lubricants such as talc and silica, and preservatives such as antioxidants, vitamin A, vitamin E, vitamin C, retinyl palmitate, selenium, cysteine, methionine, citric acid, sodium sulfate and parabens. Examples of diluent include, but are not limited to, water, alcohol, saline solution, glycol, mineral oil and dimethyl sulfoxide (DMSO).

[0159] In some aspects, the pharmaceutically acceptable carrier is conjugated to the C-terminus of one or more polypeptides of the antibody or antigen binding fragment. Any suitable means of conjugating the pharmaceutically acceptable carrier can be used, including covalent conjugation and use of linkers, for example.

[0160] **Methods of use**

[0161] In one embodiment, the invention provides a method of treating cancer including administering to a subject in need thereof an effective amount of any one of the antibodies or antigen binding fragments thereof described herein, any one of the bispecific antibodies described herein, or any one of the antibody-drug conjugates described herein, thereby treating cancer.

[0162] As used herein, the terms “treat,” “treatment,” “therapy,” “therapeutic,” and the like refer to obtaining a desired pharmacologic and/or physiologic effect, including, but not limited to, alleviating, delaying or slowing the progression, reducing the effects or symptoms, preventing onset, inhibiting, ameliorating the onset of a diseases or disorder, obtaining a beneficial or desired result with respect to a disease, disorder, or medical condition, such as a therapeutic benefit and/or a prophylactic benefit. “Treatment,” as used herein, includes any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject, including a subject which is predisposed to the disease or at risk of acquiring the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and/or (c) relieving the disease, i.e., causing regression of the disease. A therapeutic benefit includes eradication or amelioration of the underlying

disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the subject, notwithstanding that the subject may still be afflicted with the underlying disorder. In some aspects, for prophylactic benefit, treatment or compositions for treatment are administered to a subject at risk of developing a particular disease, or to a subject reporting one or more of the physiological symptoms of a disease, even though a diagnosis of this disease may not have been made. The methods of the present disclosure may be used with any mammal or other animal. In some aspects, treatment results in a decrease or cessation of symptoms. A prophylactic effect includes delaying or eliminating the appearance of a disease or condition, delaying or eliminating the onset of symptoms of a disease or condition, slowing, halting, or reversing the progression of a disease or condition, or any combination thereof.

[0163] As used herein, the term “subject” refers to any individual or patient on which the methods disclosed herein are performed. The term “subject” can be used interchangeably with the term “individual” or “patient.” The subject can be a human, although the subject may be an animal, as will be appreciated by those in the art. Thus, other animals, including mammals such as rodents (including mice, rats, hamsters and guinea pigs), cats, dogs, rabbits, farm animals including cows, horses, goats, sheep, pigs, etc., and primates (including monkeys, chimpanzees, orangutans and gorillas) are included within the definition of subject.

[0164] As used herein, the term “effective amount” or “therapeutically effective amount” refers to that amount of an antibody, an antigen binding fragment thereof, or other composition described herein that is sufficient to induce the intended effect, including but not limited to disease treatment, as defined herein. The therapeutically effective amount may vary depending upon the intended treatment application (e.g., *in vivo*), or the patient and disease condition being treated, e.g., the weight and age of the patient, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The term also applies to a dose that will induce a particular response in a target cell. The specific dose will vary depending on the particular antibody, an antigen binding fragment thereof, or other composition chosen, the dosing regimen to be followed, whether it is administered in combination with other compounds, timing of administration, the tissue to which it is administered, and the physical delivery system in which it is carried.

[0165] In some aspects, antibodies of the invention or antigen binding fragments thereof are used as a monotherapy or combined with other therapeutic agents such as radiotherapy, cytotoxic chemotherapy, immunotherapy including immune checkpoint inhibitors, and other immunoregulatory agents, such as vaccines, interleukins, cytokines, chemokines, and biologics as a combination therapy. Exemplary interleukins for immune therapy include IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-18, IL-21, and IL-23. Exemplary cytokines for immune therapy include interferons, TNF- α , TGF- β , G-CSF, and GM-CSF. Exemplary chemokines for immune therapy include CCL3, CCL26, and CXCL7. Exemplary biologics include CAR T-cell therapy, tumor-infiltrating lymphocyte (TIL) therapy, and monoclonal antibodies, such as alemtuzumab, trastuzumab, ibritumomab tiuxetan, brentuximab vedotin, ado-trastuzumab emtansine, blinatumomab, bevacizumab, and cetuximab. Antibodies also include checkpoint inhibitors, including PD-1 inhibitors, such as pembrolizumab, nivolumab, and cemiplimab, for example, PD-L1 inhibitors, such as atezolizumab, avelumab and durvalumab, for example, CTLA-4 inhibitors, such as ipilimumab, for example, and other checkpoint inhibitors, such as an anti B7-H3 antibody, an anti-KIR antibody and an anti-LAG3 antibody, for example.

[0166] In some embodiments, methods of treating cancer in a subject are provided herein. Methods of treating cancer include administering to a subject an amount of any bispecific antibody or an antigen binding fragment thereof provided herein, effective for treating the cancer.

[0167] Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. In 2015, about 90.5 million people had cancer, about 14.1 million new cases occur a year and it caused about 8.8 million deaths (15.7% of deaths). The most common types of cancer in males are lung cancer, prostate cancer, colorectal cancer and stomach cancer. In females, the most common types are breast cancer, colorectal cancer, lung cancer and cervical cancer. The term “cancer” refers to a group of diseases characterized by abnormal and uncontrolled cell proliferation starting at one site (primary site) with the potential to invade and to spread to others sites (secondary sites, metastases) which differentiate cancer (malignant tumor) from benign tumor. Virtually all the organs can be affected, leading to more than 100 types of cancer that can affect humans. Cancers can result from many causes including genetic predisposition, viral infection, exposure to ionizing radiation, exposure environmental pollutant, tobacco and or alcohol use, obesity, poor diet,

lack of physical activity or any combination thereof. As used herein, “neoplasm” or “tumor” including grammatical variations thereof, means new and abnormal growth of tissue, which may be benign or cancerous. In a related aspect, the neoplasm is indicative of a neoplastic disease or disorder, including but not limited, to various cancers. For example, such cancers can include prostate, pancreatic, biliary, colon, rectal, liver, kidney, lung, testicular, breast, ovarian, pancreatic, brain, and head and neck cancers, melanoma, sarcoma, multiple myeloma, leukemia, lymphoma, and the like.

[0168] In some aspects, the cancer is prostate cancer, lung cancer, non-small cell lung cancer (NSCLC), melanoma, lymphoma, breast cancer, head and neck cancer, renal cell carcinoma (RCC), ovarian cancer, kidney cancer, urinary bladder cancer, uterine cancer, cervical cancer, ovarian cancer, liver cancer, stomach cancer, colon cancer, rectal cancer, oral cavity cancer, pharynx cancer, pancreatic cancer, thyroid cancer, skin cancer, brain cancer, bone cancer, hematopoietic cancer, or leukemia.

[0169] Amino acid and nucleic acid sequences

[0170] In some embodiments, provided herein are isolated amino acid sequences encoding any of the antibodies, antigen binding fragment thereof or bispecific antibodies described herein.

[0171] The terms “peptide”, “polypeptide” and “protein” are used interchangeably herein and refer to any chain of at least two amino acids, linked by a covalent chemical bond. As used herein polypeptide can refer to the complete amino acid sequence coding for an entire protein or to a portion thereof. A “protein coding sequence” or a sequence that “encodes” a particular polypeptide or peptide, is a nucleic acid sequence that is transcribed (in the case of DNA) and is translated (in the case of mRNA) into a polypeptide in vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, cDNA from prokaryotic or eukaryotic mRNA, genomic DNA sequences from prokaryotic or eukaryotic DNA, and even synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the coding sequence.

[0172] In various aspects, the amino acid sequences are as set forth in SEQ ID NOs:1-40 and 46-91.

[0173] Also provided herein are isolated nucleic acid sequences encoding the amino acid sequences provided herein.

[0174] As used herein, the term “nucleic acid” refers to polynucleotides such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Nucleic acids include but are not limited to genomic DNA, cDNA, mRNA, iRNA, miRNA, tRNA, ncRNA, rRNA, and recombinantly produced and chemically synthesized molecules such as aptamers, plasmids, anti-sense DNA strands, shRNA, ribozymes, nucleic acids conjugated and oligonucleotides. According to the invention, a nucleic acid may be present as a single-stranded or double-stranded and linear or covalently circularly closed molecule. A nucleic acid can be isolated. The term “isolated nucleic acid” means, that the nucleic acid (i) was amplified in vitro, for example via polymerase chain reaction (PCR), (ii) was produced recombinantly by cloning, (iii) was purified, for example, by cleavage and separation by gel electrophoresis, or (iv) was synthesized, for example, by chemical synthesis. A nucleic acid can be employed for introduction into, i.e., transfection of, cells, in particular, in the form of RNA which can be prepared by in vitro transcription from a DNA template. The RNA can moreover be modified before application by stabilizing sequences, capping, and polyadenylation.

[0175] In some embodiments, provided herein are isolated nucleic acid sequences encoding any one of the amino acid sequences of SEQ ID NOs:1-40 and 46-91.

[0176] In some embodiments, the invention further provides the expression, purification and characterization of anti-p95HER2 and anti-CD73 antibodies, as detailed in the examples below. A signal sequence can be included in expression constructs for antibodies provided herein. Any suitable signal sequence can be used, such as a sequence of SEQ ID NO:43.

[0177] Presented below are examples discussing anti-p95HER2 and anti-CD73 antibodies, antigen binding fragment thereof and bispecific antibody thereof, contemplated for the discussed applications. The following examples are provided to further illustrate the embodiments of the present invention but are not intended to limit the scope of the invention. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

EXAMPLES

EXAMPLE 1

ANTIBODY GENERATION FROM THE OMNIMAB LIBRARY

[0178] To generate antibodies against p95HER2, panning was carried out with the OmniMab phagemid library built on IgG variable region sequences collected from over a hundred healthy donors' PBMCs by AP Biosciences Inc. (AP Biosciences, Inc.). Phage display was carried out using Hyperphage (M13K07ΔpIII, Progen, Heidelberg, Germany).

[0179] Panning was performed using p95HER2-overexpressing HEK293 cells in the first round, then using recombinant p95HER2 protein produced by Expi-293 cells in the following three rounds selection. Briefly, for first round cell panning, the omniMab phage library was mixed with 1-fold 1%BSA in PBS. The phage/PBS mixture were added to the tube that contented wild type Expi293 cells for subtraction. Then wild type Expi293 cells were collected with centrifugation and washed cells three times with 0.5%BSA in PBS to collect the phage that didn't recognize Expi293 cells. And mixed the collection phage with full length p95HER2 transfected Expi293 with shaking. Washed transfected Expi293 cells with 0.5%BSA in PBS 10 times and eluted the phage that binding to p95HER2 transfected Expi293 cells with TEA solution. The eluted phages were collected to infect the TG1 for phage rescue.

[0180] For second to fourth round solid phase panning, secretory p95HER2 ECD (ERBB2_HUMAN amino acid positions 611 to 652) with signal peptides and FLAG-taq was produced on Expi293 platform. The culture supernatant was collected with centrifugation as target antigen for panning using. Immune tubes were pre-coated with anti-FLAG mouse antibody with PBS and loaded with various FLAG-taq recombinant protein. The immune tubes and phage from previous panning rounds were mixed with 5% NFM or 1%BSA in PBS for blocking, then adding phage mixture to the preloading immune tubes containing FLAG-tag recombinant protein (except p95HER2 ECD-FLAG) for subtraction. Other immune tubes were added to the culture supernatant containing secretory p95HER2 ECD FLAG-tag. After incubation, collected and washed tubes with 1% tween-20 in PBS 3 times to collect the phage that didn't recognize the immune tubes. Then transfer the phage mixture to the immune tube containing p95HER2 ECD with FLAG-taq for antigen binding. After incubation, washed the immune tubes with 1% tween-20 in PBS 10 times and eluted the binding phage with TEA solution. The eluted phage were collected to infect the TG1 for phage rescue. And then, the single clones specific for p95HER2 were isolated and determined by FACS (**FIGURES 1A-**

1C), and selected for sequencing to confirm the diversity of heavy chains and light chains. The reference antibody used in this study was generated from US2018118849A1 SEQ ID NOs: 20 and 21 with human IgG1 backbone and produced on ExpiCHO platform.

[0181] The unique antibody sequences were then sub-cloned and expressed for further analysis.

EXAMPLE 2

SUBCLONING, EXPRESSION, AND PURIFICATION OF P95HER2 SPECIFIC BINDING PROTEINS IN THE FORM OF IGGs

[0182] To quickly screen candidates, the heavy chains and light chains of positive p95HER2 binders identified by FACS were amplified, digested and subcloned into an in-house generated IgG expression vector carrying the IgG1 constant region (SEQ ID NO:41). After sequence validation, plasmids were prepared and transfected into ExpiCHO cells (Invitrogen) for antibody production. After 6 days of culture, antibody was affinity purified from culture supernatant by Protein A chromatography. Purified antibody was concentrated and dialyzed in PBS buffer. Antibody concentration was determined using NanoDrop2000 spectrophotometer. Antibody purity and integrity were determined by NuPAGE (ThermoFisher, Cat. No. NP0321BOX) under non-reducing or reducing conditions and visualized by Coomassie Brilliant Blue G-250 staining solution.

[0183] **FIGURE 2** shows the PAGE results of purified anti-p95HER2 antibody leads. Results indicated that proteins have a molecular weight of about 145 kDa under non-reducing conditions, and heavy chain and light chain have a molecular weight of 55kDa and 25kDa, respectively, under reducing conditions. Most of antibody leads showed greater than 90% purity from one-step Protein A chromatography.

[0184] **FIGURES 3A-3B** show the binding activity of purified anti-p95HER2 antibody leads by flow cytometer.

EXAMPLE 3

BINDING EPITOPE MAPPINGS OF ANTI-P95HER2 MONOCLONAL ANTIBODY

[0185] The binding epitope mappings were conducted by PEPperPRINT Inc. To determine the binding epitopes, GS linker elongated full-length p95HER2 peptide sequence was

converted into linear 5, 10 and 15 amino acid peptides each overlapped with 4, 9 and 14 amino acids.

[0186] The linear peptide microarrays were incubated with the antibody leads at a concentration of 1 µg/ml followed by staining with secondary (Goat anti-human IgG (H+L) DyLight680) and control (Mouse monoclonal anti-IIA (12CA5) DyLight800) antibodies as well as read-out with Odyssey Imaging System (LI-COR, Inc). Quantification of spot intensities and peptide annotation were done with PepSlide® Analyzer (SICASYS Software GmbH). The epitope for p95HER2 antibodies were shown and summarized in the **FIGURE 4A**. The heat map of epitope mapping result showed that both anti-p95HER2 #R4-3, R4-5, R4-11, R4-15 and R4-38 could recognize motif KFPDE (SEQ ID NO:96) strongly. However, the signal from 10 and 15 amino acid peptides containing KFPDE motif disclosed that the structure of p95HER2 were not simple linear. Relatively stronger signal was generated when the motif KFPDE is located at the edge of the amino acid sequence, i.e., amino acid nearby motif KFPDE would affect the binding affinity of anti-p95HER2 antibodies (**FIGURE 4B**).

EXAMPLE 4

EVALUATION OF PROTEIN AGGREGATION OF ANTI- P95HER2-CD137 SCFV BISPECIFIC ANTIBODY BY SEC-HPLC

[0187] Purified and concentrated antibody (> 1 mg/ml) was applied to determine variants and aggregations by SEC-HPLC analysis. SEC-HPLC was performed using a Waters ACQUITY Arc system with Waters 2489 UV/Vis detector. Samples were load into XBridge Protein BEH SEC Column (Waters, Cat#186007640) with isocratic 25 mM sodium phosphate, 200 mM NaCl, pH 6.8 as mobile phase buffer for SEC separation. The flow rate was 0.4 mL/min and the sample injection amount was 40 µg. Peaks were detected by absorbance at 280 nm. Before injection onto the SEC column, all samples were filtrated with 0.22 µm filter (Millipore, Cat#SLGP003RB) to remove any precipitated protein material. Data were analyzed by Empower 3 software. In summary, the purity of one-column Protein-A purified anti-p95HER2-CD137 antibodies are greater than 95% (**FIGURE 6A**).

[0188] The general structure of the bispecific antibodies is presented in **FIGURE 5**.

EXAMPLE 5

EVALUATION OF PROTEIN INTEGRITY OF ANTI- P95HER2-CD137 BISPECIFIC ANTIBODIES BY MCE-SDS-PAGE

[0189] All size variants analyses of purified antibody were performed on a LabChip GXII instrument (Perkin Elmer, Inc.). The assay was carried out with “Protein Clear IIR Assay” as manufacture’s protocol. Briefly, 2.5 μ l of protein sample at 1 mg/mL was mixed with 18 μ l of sample buffer. The sample buffer was prepared by mixing 700 μ l of Protein Clear HR sample buffer with either 24.5 μ l of 1M DTT (for the reducing assay) or 24.5 μ l of 0.25 M NEM (for the non-reducing assay). The samples were incubated at 70°C for 10 min. After cooling to room temperature, 35 μ l of water was added to each sample before loading onto the instrument. The samples were then analyzed using the Protein Clear HR Assay script. Meanwhile, the chip was prepared as instructed by the manufacturer and was maintained at 30°C throughout the analysis. The excitation and detection wavelengths are 630 nm and 700 nm, respectively. In summary, the purity and integrity of one-column Protein-A purified anti- p95HER2-CD137 antibodies reach about 95% (**FIGURE 6B**).

EXAMPLE 6

BINDING AFFINITY OF ANTI-P95HER2-CD137 BISPECIFIC ANTIBODY BY BIOLAYER INTERFEROMETRY

[0190] The affinity of anti-p95HER2-CD137 bsAbs were determined by biolayer interferometry analysis (ForteBIO, USA). To evaluate the binding affinity of anti-p95HER2-CD137 bsAbs against CD137, the bsAbs were loaded at 5 μ g/mL on AHC (Anti-Human IgG Fc Capture, ForteBIO, Cat. No.18-5060) for signal reach to approximate 0.5 nm. Sensors then were exposed to 7 series of concentration analytes which was CD137 (Cat#41B-H5256) extracellular domain fused with mouse Fc tag recombinant protein. To determine the affinity of anti-p95HER2-CD137 bsAbs against p95HER2, custom synthesis biotinylated Her2 p95 peptide (GenScript) was loaded at 5 ng/mL on SA (Streptavidin, ForteBIO, Cat#18-5019) biosensor for 1 min. Sensors then were exposed to 7 series of concentration analytes which was bsAbs. Measurements were performed as follows: 1 min baseline in kinetic buffer, 1 min baseline in kinetic buffer, 5 minutes association in 7 different concentration analytes and 5 minutes dissociation into kinetic buffer. The kinetic parameters were calculated and produced by Octet Data Acquisition and Analysis Software. In summary, the affinity (K_D) of anti-

p95HER2#R4-11-CD137#54 bsAb against p95HER2 and CD137 antigen was 0.189 and 1.12 nM, respectively (see **FIGURES 7A-7B**).

EXAMPLE 7

THE SIMULTANEOUS BINDING TO P95HER2 AND CD137 BY ANTI-P95HER2#R4-11-CD137#54 BSAB

[0191] The binding activities of anti- p95HER2-CD137 bispecific antibody was determined by ForteBIO® (Menlo Park, Calif.) biosensor analysis. In this method, the custom synthesis biotinylated Her2 p95 peptide (GenScript) was loaded onto SA (Streptavidin, ForteBIO, Cat#18-5019) biosensor at 50 µg/mL for 5 minutes. Sensors then were exposed to antibody samples at 100 nM for 5 minutes follow by associating with the mouse Fc tagged human CD137 protein (AcroBiosystems, Cat#41B-H5256) at 100 nM for 5 minutes. The binding chart were produced by Octet Data Acquisition and Analysis Software. In summary, these results show that anti-p95HER2-CD137 bispecific antibodies simultaneously recognize p95HER2 and CD137, as determined by ForteBIO® biosensor analysis (**FIGURE 8**).

EXAMPLE 8

p95HER2-DEPENDENT T CELL ACTIVATION INDUCED BY ANTI-P95HER2-CD137 BISPECIFIC ANTIBODY

[0192] Human T cells were isolated using a RosetteSep™ Human T Cell Enrichment Cocktail (STEMCELL Cat. No.15061). Purified T cells (1×10^5 cells) were cocultured with p95HER2-expressing NCI-H292 lung cancer cells (1×10^4 cells) in the presence of anti-CD3 (OKT3)-coated polybeads at T-cell:bead ratio 1:1. Three days later, T cell activation was measured by IFN-γ production as measured by ELISA.

[0193] The expression level of p95HER2 on NCI-H292 lung cancer cells were checked by flow cytometry (**FIGURE 9A**). More robust IFN-γ production was induced by anti- p95HER2-CD137 bsAb compared to the mono- or combination therapy with anti- p95HER2 and anti-CD137 #54 antibodies (**FIGURE 9B**).

EXAMPLE 9

ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY (ADCC) BY ANTI-P95HER2-CD137 BISPECIFIC ANTIBODY

[0194] For ADCC induction, CD16-NK92 effector cells were cocultured with CD137-expressing IIEK293 target cells (2×10^4 cells) at E/T ratio 1, 3 and 10 in a well of U-bottom 96-well plate. Two anti-p95HER2-CD137 bsAbs, #R4-11-#54 (with original IgG1 Fc) and #R4-11e5-#54 (with ADCC-enhancing IgG1 Fc, SEQ. NO. 42), were applied in this study. Six hours later, cytotoxicity activity was determined by CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega, G1780).

[0195] Compared to isotype control, #R4-11e5-#54, but not #R4-11 and #R4-11-#54, induced the ADCC in CD137-HEK293 cells. This was corresponding to the ADCC-enhancing mutations in the IgG1 Fc region of #R4-11e5-#54 (**FIGURE 10A**). #R4-11-#54 induced the ADCC activity against p95HER2-NCI-H292 cells as well as its parental #R4-11 mAb (**FIGURE 10B**).

[0196] In summary, these results indicated that #R4-11e5-#54 bsAb could be deleterious to CD137-expressing cells, like NK and T cells, which are critical effector cells in cancer. Therefore, #R4-11-#54 bsAb with normal IgG1 Fc was selected for further development.

EXAMPLE 10

INHIBITION OF TUMOR GROWTH BY ANTI-P95HER2-CD137 BISPECIFIC ANTIBODY *IN VIVO*.

[0197] To validate the anti-tumor activity of anti-p95HER2#4-11-CD137#54 bsAb, which does not cross-react with mouse CD137, human tumor cells (p95HER2-expressing MDA-MB-231) were premixed with human PBMC and xenografted orthotopically to forth mammary fat pad of female SCID-beige mice to evaluate anti-cancer activity *in vivo*. Ten days post tumor inoculation, equal moles of mAb (M.W.150 kDa, 7.7 mg/kg) and bsAb (M.W. 195 kDa, 10, 2, 0.4 mg/kg) were intraperitoneally injected twice per week. Tumor sizes (mm^3) were measured twice per week and calculated as $(\text{length} \times \text{width} \times \text{width})/2$. Tumor inhibition ratio was calculated by the following equations based on the tumor volume (TGIV) or tumor weight (TGIW). $\text{TGIv} (\%) = [1 - (V_t - V_0 \text{ of treatment group}) / (V_t - V_0 \text{ of control group})] \times 100\%$, which V_t : mean tumor volume after treatment; V_0 : mean tumor volume before treatment. $\text{TGIw} (\%)$

= (mean tumor weight of control group – mean tumor weight of treatment group) / mean tumor weight of control group × 100%.

[0198] In summary, these results show significant inhibition of tumor growth upon treatment with compared to the treatment with PBS (**FIGURES 11A-11C**). The greater tumor inhibition was shown by TGI_w as 95.6%, 91.5% and 93.7%, while TGI_v was 125.5%, 113% and 117%, at 10, 2 and 0.4 mg/kg, respectively.

EXAMPLE 11

PHARMACOKINETIC PROFILE OF ANTI- P95HER2-CD137 BISPECIFIC ANTIBODY IN MICE

[0199] The bispecific anti- p95HER2-CD137 antibody (5 mg per kg body weight) was administered via an intravenous bolus injection to SCI-beige mice. Peripheral blood was collected at 0.5, 24, 72, 168, 264, 336, 504, 672 and 840 hours post injection. Plasma concentrations of the antibody were determined by ELISA. MaxiSorp plates (Invitrogen) were coated with CD137-Fc fusion protein (AP Biosciences, 0.5 µg/mL), followed by application of serially diluted plasma samples and anti-CD73-CD137#54 bsAb as the standard curve. Bound antibodies were detected by CD73-Flag-His antigen (AP Biosciences) and HRP-conjugated anti-Flag antibody using TMB substrate. Plasma antibody concentrations were calculated by the interpolation method. PK parameters were calculated using PKSolver software. Anti-p95HER2-CD137 showed PK profile with t_{1/2} 336 hours and AUC 63896 µg/ml*h (**FIGURE 12**).

EXAMPLE 12

ANTIBODY GENERATION FROM THE OMNIMAB LIBRARY

[0200] To generate antibodies against CD73, selections with an OmniMab phagemid library were carried out. The phagemid library was built up by AP Biosciences Inc. (APBio Inc.) from a collection of peripheral blood mononuclear cells from over a hundred healthy donors.

[0201] First round panning was performed using Hyperphage (M13K07ΔpIII, Progen, Heidelberg, Germany). Solution phase panning was used for CD73 specific binders' selection and isolation from the OmniMab library. Solution phase panning was performed using recombinant CD73 protein carries flag tag and polyhistidine tag at the C-terminus which

produced by mammalian cell expression system used in first-round selection. Second round enrichment was also used solution phase panning. CD73-expressing Expi293 cells and breast cancer cell line MDA-MB-231 were used for third round enrichment separately. The specific CD73 binders were screened and isolated by direct ELISA and FACS after three round panning (**FIGURES 13A-13C**). The phage clones recognize human CD73 specifically after three rounds human CD73 specific enrichment process from OmniMab phage library. Phage-containing supernatant were applied to CD73 ELISA, and positive binders were numbered. MDA-MB-231 breast cancer cell line was stained with CD73 phages supernatant (50 μ l/well) which were isolated from MDA-MB-231 cell-based panning to examine CD73 binding activity. **FIGURE 13B** showed ranking of binders binding activity. Binders were isolated and sent for sequencing to confirm the sequence and diversity of the heavy chain.

EXAMPLE 13

SUBCLONING, EXPRESSION, AND PURIFICATION OF CD73-SPECIFIC BINDING PROTEINS IN THE FORM OF IGGs

[0202] To quickly screen candidates, the heavy chains and light chains of positive CD73 binders identified by ELISA and FACS were amplified, digested and subcloned into an IgG expression vector generated by APBio and carrying the engineered IgG1 constant region (SEQ ID NO:87). After sequence validation, plasmids were prepared and transfected into ExpiCHO cells (Invitrogen) for antibody production. After 6 days of culture, antibody secreted into serum-free medium was affinity purified from culture supernatant by Protein A chromatography. Purified antibody was concentrated, followed by dialysis in PBS buffer. The final concentration of dialyzed protein was determined using a NanoDrop2000 spectrophotometer and the purity and integrity were determined by SDS-PAGE with or without reducing reagent. Purified antibodies were analyzed by NuPAGE (ThermoFisher, Cat. No. NP0321BOX) under non-reducing or reducing conditions. Once the dye front has reached the bottom of the gel, turn off the voltage and remove the gel from the glass plates. Place the gel in Coomassie Brilliant Blue G-250 staining solution in a plastic container and rock for several hours. The destain can be poured off into the designated waste container in the fume hood and replaced several times until the bands can be clearly visualized against the background of the gel.

[0203] **FIGURE 14** shows representative PAGE gel analysis of different batches of purified anti-CD137 antibody leads. The purity and integrity of one-step Protein A-purified anti-CD73 antibody leads was analyzed by SDS-PAGE under non-reducing and reducing conditions. Results indicated that proteins have a molecular weight of about 145 kDa under non-reducing conditions, and heavy chain and light chain have a molecular weight of 55kDa and 25kDa, respectively, under reducing conditions. More than 90% purity could be obtained by one step of Protein A chromatography.

EXAMPLE 14

BLOCKADE OF CD73 ENZYMATIC ACTIVITY BY ANTI-CD73 ANTIBODY

[0204] MDA-MB-231 cells (2×10^4 cells) were incubated with anti-CD73 antibody in a well of 96-well tissue-cultured plate at 4°C for 30 minutes. AMP (12.5 μ M, Sigma, Cat. No. A1752) was added and incubated at 4°C for additional one hour. Supernatants were transferred to 96-well white plate, and residual AMP was detected by AMP-Glo assay followed manufacturer's instruction (Promega, Cat. No. V5011). The percentage of enzyme activity inhibition was calculated as $[(\text{Cells} + \text{AMP} + \text{antibody}) - (\text{Cells} + \text{AMP})] / [\text{AMP} - (\text{Cells} + \text{AMP})] \times 100$. To study the blockade activity of anti-CD73 antibody to soluble CD73, MDA-MB-231 cells (4.8×10^6 cells) seeded in a well of 24-well plate for 40 hours, then cultured medium were collected and incubated with antibodies at 37°C for 1 hour. AMP (12.5 μ M, Sigma, Cat. No. A1752) was added and incubated at 37°C for additional 2 hours, followed by the same procedures described above to detect residual AMP and calculate the percentage of enzyme inhibition.

[0205] In summary, the binding affinity of anti-CD73 antibody did not necessarily correspond to their blockade of enzymatic activity. Although possess the better binding activity than clone #3-4, the weaker blockade of CD73 enzymatic activity was observed in clone #3-6, #3-27, #3-34, #3-1-14, #3-1-21, and #3-1-31. Interestingly, clone #3-1-28 showed the most potent blockade of CD73 enzymatic activity even with a comparable binding activity to clone #3-4, and #3-1-28 was selected for affinity maturation (**FIGURE 15** and **FIGURE 16**). As illustrated in **FIGURE 15** the binding activity of anti-CD73 antibody leads was assessed by flow cytometer. MDA-MB-231 cells (1×10^5 cells) were surface stained with anti-CD73 antibody (4 μ g/ml) for 30 minutes, then incubated with A488-conjugated anti-human IgG secondary antibody for 15 minutes on ice. Cells were analyzed by flow cytometer, and A488

fluorescence intensity was shown by histograms (left) and number (right table). As illustrated in **FIGURE 16** the blockade of CD73 enzymatic activity by anti-CD73 antibody leads was assessed. The enzymatic activity of CD73 expressed by MDA-MB-231 on the cell surface was blocked by anti-CD73 antibody leads.

[0206] Corresponding to the improved binding affinity (**FIGURES 17A-17C** and **EXAMPLE 15**), the CD73 enzyme block activity of anti-CD73 clone #3-1-28-v1, #3-1-28-v2, and #3-1-28-v3 was better than their parental clone #3-1-28 (**FIGURE 18**). In the inhibition of cell membrane-bound CD73 enzyme, the IC_{50} of anti-CD73#3-1-28-v1 (0.213 nM) and anti-CD73#3-1-28-v2 (0.245 nM) was about 2 folds lower than that of parental #3-1-28 (0.49 nM) (**FIGURE 18A**). Interestingly, in the inhibition of soluble CD73 enzyme, the IC_{50} of anti-CD73#3-1-28-v1 (0.187 nM), anti-CD73#3-1-28-v2 (0.273 nM), and anti-CD73#3-1-28-v3 (0.241 nM) was even more than 5 folds lower than that of parental #3-1-28 (1.478 nM) (**FIGURE 18B**).

[0207] Furthermore, conversion of affinity-maturated anti-CD73 #3-1-28-v1, #3-1-28-v2, and #3-1-28-v3 to the bispecific antibody by linking CD137 scFv to their Fc C-terminal did not change their enzymatic blockade activities (**FIGURE 23**).

EXAMPLE 15

AFFINITY MATURATION OF ANTI-CD73#3-1-28

[0208] Affinity-matured antibodies can exhibit increased biological efficacy. Regardless of whether an antibody is isolated from a hybridoma or a human Fv phage library. An increased affinity may also allow for a reduced dosage of a therapeutic antibody and toxic side effects may be reduced. Germline hotspot residues are most likely to have a major impact on affinity based on statistical analysis of independent monoclonal antibodies derived from the same germline genes recognizing the same epitopes. Germline hotspots in the antibody complementarity determining regions (CDR) are naturally prone to hypermutations (Neuberger and Milstein, 1995). To improve #3-1-28 binding activity, we introduced random mutations into LCDR2/HCDR3 and made phage-display libraries with a diversity around 10^2 and 10^8 in these two CDR regions, respectively. Panning of these hotspot libraries has yielded mutant antibodies with increased affinity. CD73-expressing MDA-MB-231 breast cancer cells were used for three rounds cell-based panning enrichment. Binders with better binding activity than parental phage #3-1-28 were screened by FACS (**FIGURE 17A**), and 27 binders were

sequenced to confirm the diversity of the engineered CDR region. The heavy chains and light chains of positive CD73 binders identified by FACS were amplified, digested and subcloned into an IgG expression vector carrying the engineered IgG1 constant region. Vectors were transfected into the mammalian cell expression system for IgG production.

[0209] Based on the improved binding measured by ELISA and biolayer interferometry (**FIGURES 17B and 17C**), clone #1919-003, #1911-022, and #1911-030 were selected for further development of bispecific antibodies (having a general structure as illustrated in **FIGURE 19**). To emphasize the derivation from parental #3-1-28, clone #1919-003, #1911-022, and #1911-030 were re-named hereafter as #3-1-28-v1, #3-1-28-v2, and #3-1-28-v3, respectively. As illustrated in **FIGURE 17A**, CD73 binding activities of phage clones from small-scale affinity maturation library were determined by staining of CD73-expressing MDA-MB-231 cells and analyzed by flow cytometer. Clones with the better binding activity compared to parental phage clone #3-1-28 were numbered. As shown in **FIGURE 17B**, the binding activities of antibodies converted from phage clones numbered in **FIGURE 17A** were measured by CD73 ELISA. The clones in bold #1919-002, #1919-003, #1919-004, #1911-022, and #1911-030 showed the better binding activity than parental #3-1-28 antibody. As illustrated in **FIGURE 17C**, binding kinetics of antibodies selected in **FIGURE 17B** were measured by biolayer interferometry. To emphasize the derivation from clone #3-1-28, #1919-003, #1911-022, and #1911-030 were named hereafter as #3-1-28-v1, #3-1-28-v2, and #3-1-28-v3, respectively. Based on the detected K_D value, the affinities of #3-1-28-v1, #3-1-28-v2, and #3-1-28-v3 were improved about 2 folds compared to parental #3-1-28.

EXAMPLE 16

BINDING AFFINITY OF ANTI-CD73 MONOCLONAL ANTIBODY AND ANTI-CD73-CD137 BISPECIFIC ANTIBODY BY BIOLAYER INTERFEROMETRY

[0210] The affinity of anti-CD73 mAbs and bsAbs were determined by biolayer interferometry analysis (ForteBIO, USA). In this method, anti-CD73 antibodies were loaded at 5 $\mu\text{g/mL}$ on AHC (Anti-Human IgG Fc Capture, ForteBIO, Cat. No.18-5060) for signal reach to approximate 0.5 nm. Sensors then were exposed to his-tagged human CD73 (AcroBiosystems, Cat. No. CD3-H52H7)-containing solutions that 2-fold serial diluted from the top concentration 30 nM for total 7 points. Measurements were performed as follows: 1 minute baseline in kinetic buffer, 5 minutes association in hCD73-His-containing solutions,

and 5 minutes dissociation into kinetic buffer. The kinetic parameters were calculated and produced by Octet Data Acquisition and Analysis Software.

[0211] In summary, binding affinity of anti-CD73 #3-1-28-v1, #3-1-28-v2, and #3-1-28-v3 show about 2-fold improvement after affinity maturation compared to parental #3-1-28 (**FIGURE 17C**). The improved CD73 binding affinities of #3-1-28-v1, #3-1-28-v2, and #3-1-28-v3 remains after conversion to anti-CD73-CD137 bispecific antibodies (**FIGURES 21A-21B**).

EXAMPLE 17

EVALUATION OF PROTEIN AGGREGATION OF ANTI-CD73-CD137 SCFV BISPECIFIC ANTIBODY BY SEC-HPLC

[0212] Purified and concentrated antibody (>1mg/ml) was applied to determine variants and aggregations by SEC-HPLC analysis. SEC-HPLC was performed using a Waters ACQUITY Arc system with Waters 2489 UV/Vis detector. Samples were load into XBridge Protein BEH SEC Column (Waters, Cat#186007640) with isocratic 25 mM sodium phosphate, 200 mM NaCl, pH 6.8 as mobile phase buffer for SEC separation. The flow rate was 0.4 mL/min and the sample injection amount was 40 µg. Peaks were detected by absorbance at 280 nm. Before injection onto the SEC column, all samples were filtrated with 0.22 µm filter (Millipore, Cat#SLGP003RB) to remove any precipitated protein material. Data were analyzed by Empower 3 software. In summary, the purity of one-column Protein-A purified anti-CD73-CD137 antibodies are greater than 90% (**FIGURES 20A-20B**).

EXAMPLE 18

EVALUATION OF PROTEIN INTEGRITY OF ANTI-CD73-CD137 BISPECIFIC ANTIBODIES BY MCE-SDS-PAGE

[0213] All size variants analyses of purified antibody were performed on a LabChip GXII instrument (Perkin Elmer, Inc.). The assay was carried out with “Protein Clear HR Assay” as manufacture’s protocol. Briefly, 2.5 µl of protein sample at 1 mg/mL was mixed with 18 µl of sample buffer. The sample buffer was prepared by mixing 700 µl of Protein Clear HR sample buffer with either 24.5 µl of 1M DTT (for the reducing assay) or 24.5 µl of 0.25 M NEM (for the non-reducing assay). The samples were incubated at 70°C for 10 min. After cooling to room temperature, 35 µl of water was added to each sample before loading onto the instrument. The

samples were then analyzed using the Protein Clear HR Assay script. Meanwhile, the chip was prepared as instructed by the manufacturer and was maintained at 30°C throughout the analysis. The excitation and detection wavelengths are 630 nm and 700 nm, respectively. In summary, the purity and integrity of one-column Protein-A purified anti-CD73-CD137 antibodies reach about 95% (**FIGURES 20A-20B**).

EXAMPLE 19

ANTIGEN RECOGNITION BY ANTI-CD73-CD137 BISPECIFIC ANTIBODIES

[0214] The binding activities of the AP601 was determined by ForteBIO® (Menlo Park, Calif.) biosensor analysis. In this method, the His tagged human CD73 protein (AcroBiosystems, Cat. No. CD3-H52H7) were loaded onto HIS1K (Anti-Penta-HIS) biosensor (Cat. No.18-5120) at 5 µg/mL for 5 minutes. Sensors then were exposed to antibody samples at 100 nM for 5 minutes follow by associating with the mouse Fc tagged human CD137 protein (AcroBiosystems, Cat. No. 41B-H5256) at 100 nM for 5 minutes. The binding chart were produced by Octet Data Acquisition and Analysis Software.

[0215] In summary, these results show that anti-CD73-CD137 bispecific antibodies simultaneously recognize CD73 and CD137, as determined by ForteBIO® biosensor analysis (**FIGURE 22**).

EXAMPLE 20

RESCUE OF AMP-SUPPRESSED T-CELL PROLIFERATION BY ANTI-CD73-CD137 BISPECIFIC ANTIBODY

[0216] Human T cells were isolated using a RosetteSep™ Human T Cell Enrichment Cocktail (STEMCELL Cat. No.15061). Purified T cells were labeled with CFSE (ThermoFisher, Cat. No. C34554) and stimulated with human T-Activator CD3/CD28 dynabeads (ThermoFisher, Cat. No. 11131D) at T-cell:bead ratio 1:0.5 in the presence of AMP (400 µM, Sigma, Cat. No. A1752). Three days later, T-cell activation was determined by IFN-γ production measured by ELISA and cell proliferation analyzed by flow cytometry.

[0217] In summary, corresponding to the binding affinity, affinity-improved anti-CD73-CD137 bispecific antibodies showed the better rescue of AMP-suppressed T-cell activation (**FIGURE 24**).

EXAMPLE 21**CD73-DEPENDENT T CELL ACTIVATION INDUCED BY ANTI-CD73-CD137
BISPECIFIC ANTIBODY**

[0218] Human T cells were isolated using a RosetteSep™ Human T Cell Enrichment Cocktail (STEMCELL Cat. No.15061). Purified T cells (1×10^5 cells) were cocultured with CD73-expressing MDA-MB-231 breast and NCI-H292 lung cancer cells (1×10^4 cells) in the presence of anti-CD3 (OKT3)-coated polybeads at T-cell:bead ratio 1:1. Three days later, T cell activation was measured by IFN- γ production as measured by ELISA.

[0219] CD73 expression level on MDA-MB-231 breast cancer cells and NCI-H292 lung cancer cells were checked by flow cytometry (**FIGURE 25A**). More robust IFN- γ production was induced by anti-CD73-CD137 bsAb compared to the mono- or combination therapy with anti-CD73 and anti-CD137 #54 antibodies (**FIGURES 25B-25C**).

EXAMPLE 22**INHIBITION OF TUMOR GROWTH BY ANTI-CD73#3-1-28-V2-CD137#54
BISPECIFIC ANTIBODY *IN VIVO***

[0220] To validate the anti-tumor activity of anti-CD73#3-1-28-v2-CD137#54 bsAb, which does not cross-react with mouse CD73 and mouse CD137, human tumor cells (MDA-MB-231) were premixed with human PBMC and xenografted orthotopically to forth mammary fat pad of female SCID-beige mice to evaluate anti-cancer activity *in vivo*. Ten days post tumor inoculation, equal moles of mAb (M.W.150 kDa, 10 mg/kg) and bsAb (M.W. 195 kDa, 13 mg/kg) were intraperitonally injected twice per week. Tumor sizes (mm³) were measured twice per week and calculated as (length×width×width)/2. Tumor inhibition ratio was calculated by the following equations based on the tumor volume (TGI_v) or tumor weight (TGI_w). $TGI_v (\%) = [1 - (V_t - V_0 \text{ of treatment group}) / (V_t - V_0 \text{ of control group})] \times 100\%$, which V_t: mean tumor volume after treatment; V₀: mean tumor volume before treatment. $TGI_w (\%) = (\text{mean tumor weight of control group} - \text{mean tumor weight of treatment group}) / \text{mean tumor weight of control group} \times 100\%$. For isolation of tumor-infiltrating lymphocytes (TILs), dissected tumors were minced and digested with RPMI-1640 medium containing collagenase IV (Worthington, Cat. No. LS004188), hyaluronidase (Sigma, Cat. No. H6254), and DNase I (Sigma, Cat. No. D5025) by gentleMACS (Miltenyi Biotec Inc.), followed by density gradient

separation with Lymphoprep (STEMCELL, Cat. No. 07811). Isolated TILs were stained with cell-type specific markers and analyzed by flow cytometer.

[0221] In summary, these results show significant inhibition of tumor growth upon treatment with 10 mg/kg anti-CD73#3-1-28-v2-CD137#54 bsAb compared to the treatment with anti-CD73 ref mAb (**FIGURE 26**). The greater tumor inhibition was shown by TGI_w as 40.9% and 17.5%, while TGI_v was 27% and 10%, at 10 and 3 mg/kg, respectively. Corresponding to the antitumor activity, anti-CD73#3-1-28-v2-CD137#54 bsAb specifically induced expansion of CD8+, while decreased CD4+, T-cells in tumors (**FIGURES 26A-26E**).

EXAMPLE 23

PHARMACOKINETIC PROFILE OF ANTI-CD73-CD137 BISPECIFIC

ANTIBODY IN MICE

[0222] The bispecific anti-CD73#3-1-28-v2-CD137#54 antibody (5 mg per kg body weight) was administered via an intravenous bolus injection to SCI-beige mice. Peripheral blood was collected at 0.5, 24, 72, 168, 240, 336, 504, and 672 hours post injection. Plasma concentrations of the antibody were determined by ELISA. MaxiSorp plates (Invitrogen) were coated with CD137-Fc fusion protein (AP Biosciences, 0.5 µg/mL), followed by application of serially diluted plasma samples and anti-CD73-CD137#54 bsAb as the standard curve. Bound antibodies were detected by CD73-Flag-His antigen (AP Biosciences) and HRP-conjugated anti-Flag antibody using TMB substrate. Plasma antibody concentrations were calculated by the interpolation method. PK parameters were calculated using PKSolver software. Anti-CD73#3-1-28-v3 showed a worse PK profile with t_{1/2} 103 hours and AUC 16438 µg/ml*h, while anti-CD73 #3-1-28, #3-1-28-v1, #3-1-28-v2-CD137#54 bsAbs showed a comparable t_{1/2} and AUC (**FIGURE 27**).

[0223] Sequences

SEQ ID NO	Name	SEQ (CDR defined by Kabat)
Anti-p95HER2 monoclonal antibody		
SEQ ID NO:1	Anti-p95HER2 clone R4-3 heavy chain	QITLKESGPTLVKPTQTLTLTCTFSGFSLSSGGVGVGWIRQPPGKALEWLALIIYWDDDDKRYSPSLKSRLTITKDTSKKQVVLMTNMDPVDATYYCAHLDPENYFDQWGKGLTVSS
SEQ ID NO:2	Anti-p95HER2 clone R4-3 light chain	DIQMTQSPSSLSASVGDRTITCRASQISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFRSGSGSDFTLTISLQPEDFATYYCQOSYSTPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEKHKLYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO:3	CDR-H1 of anti-p95HER2 clone R4-3	SGGVGVG

SEQ ID NO:4	CDR-H2 of anti-p95HER2 clone R4-3	LIYWDDDKRYSPSLKS
SEQ ID NO:5	CDR-H3 of anti-p95HER2 clone R4-3	LDYPENYFDQ
SEQ ID NO:6	CDR-L1 of anti-p95HER2 clone R4-3	RASQSISSYLN
SEQ ID NO:7	CDR-L2 of anti-p95HER2 clone R4-3	AASSLQS
SEQ ID NO:8	CDR-L3 of anti-p95HER2 clone R4-3	QQSYSTPT
SEQ ID NO:9	Anti-p95HER2 clone R4-5 heavy chain	QITLKESGPALVKPTQTLTLCTFSGFSLSTSGVGVGWIRQPPGKALE WLAL LIYWDDDKRYSPSLKS SRLTITKDTSKNQVVLMTNMDPVDAT YYCAH SRLPYNSHGY WGQGTITVTVSS
SEQ ID NO:10	Anti-p95HER2 clone R4-5 light chain	DIQMTQSPSSLSASVGDRTITC QASQAIGNYLS WYQQKPGRAPKLLI Y AAS TLHSGVPSRFSGSGSGTDFTLTITSLQPDDFATYYC QQSYSPV T FGLGKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO:11	CDR-H1 of anti-p95HER2 clone R4-5	TSGVGVG
SEQ ID NO:12	CDR-H2 of anti-p95HER2 clone R4-5	LIYWDDDKRYSPSLKS
SEQ ID NO:13	CDR-H3 of an anti-p95HER2 clone R4-5	SRLPYNSHGY
SEQ ID NO:14	CDR-L1 of anti-p95HER2 clone R4-5	QASQAIGNYLS
SEQ ID NO:15	CDR-L2 of anti-p95HER2 clone R4-5	AASTLHS
SEQ ID NO:16	CDR-L3 of anti-p95HER2 clone R4-5	QQSYSPVPT
SEQ ID NO:17	Anti-p95HER2 clone R4-11 heavy chain	QITLKESGPTLVKPTQTLTLCTFSGFSLSTSGVGVGWIRQPPGKALE WLAL LIYWDDDKRYSPSLKS SRLTITKDTSKNQVVLMTNMDPVDAT YYCAH AHPFSGSYNF WGQGTITVTVSS
SEQ ID NO:18	Anti-p95HER2 clone R4-11 light chain	DIVMTQSPDLSAVSLGERATINC KSSQSVLFSSNNKDYL AWYQQRPG QPPKLLIH WASTREP GVDPDRFSGSGSGTDFTLTISLQAEDVAVYY CL QSYSNPPT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKAD YEKIKVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO:19	CDR-H1 of anti-p95HER2 clone R4-11	TSGVGVG
SEQ ID NO:20	CDR-H2 of anti-p95HER2 clone R4-11	LIYWDDDKRYSPSLKS
SEQ ID NO:21	CDR-H3 of anti-p95HER2 clone R4-11	AHPFSGSYNF
SEQ ID NO:22	CDR-L1 of anti-p95HER2 clone R4-11	KSSQSVLFSSNNKDYLA
SEQ ID NO:23	CDR-L2 of anti-p95HER2 clone R4-11	WASTREP
SEQ ID NO:24	CDR-L3 of anti-p95HER2 clone R4-11	LQSYSNPPT

SEQ ID NO:25	Anti-p95HER2 clone R4-15 heavy chain	QITLKESGPTLVKPTQTLTLTCTFSGFSLSSGGVGVGWIRQPPGKALE WLA LIYWDDDDKRYSPSLKS RLTITKDTSKKQVVLMTNMDPVDAT YYCAH LDYPENYFDQ WGQGLTVTVSS
SEQ ID NO:26	Anti-p95HER2 clone R4-15 light chain	DIQMTQSPSSLSASVGDRTFTCR RASQSISTYLN WYQQKPGKAPKLLI Y AAS TLQSGVPSRFSGSGSGTDFTLAISSLQPEDFATYFC QQSYSFPT GGGTRLEIKRTMAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKSDSTYLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO:27	CDR-III of anti-p95HER2 clone R4-15	SGGVGVG
SEQ ID NO:28	CDR-II2 of anti-p95HER2 clone R4-15	LIYWDDDDKRYSPSLKS
SEQ ID NO:29	CDR-H3 of anti-p95HER2 clone R4-15	LDYPENYFDQ
SEQ ID NO:30	CDR-L1 of anti-p95HER2 clone R4-15	RASQSISTYLN
SEQ ID NO:31	CDR-L2 of anti-p95HER2 clone R4-15	AASTLQS
SEQ ID NO:32	CDR-L3 of anti-p95HER2 clone R4-15	QQSYSFPT
SEQ ID NO:33	Anti-p95HER2 clone R4-38 heavy chain	QITLKESGPTLVKPTQTLTLTCTFSGFSLSS TSGVGVG WIRQHPGKALE WLA LIYWDDDDKRYSPSLKS RLTITKDTSKNQVVLMTNMDPVDAT YYCAH STVDVHYFDY WGQGLTVTVSS
SEQ ID NO:34	Anti-p95HER2 clone R4-38 light chain	DIQMTQSPSSLSASVGDRTITCR RASQSISSYLN WYQQKPGKAPKLLI Y AASS LQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC QQSYSTPT GGGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKSDSTYLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO:35	CDR-III of anti-p95HER2 clone R4-38	TSGVGVG
SEQ ID NO:36	CDR-H2 of anti-p95HER2 clone R4-38	LIYWDDDDKRYSPSLKS
SEQ ID NO:37	CDR-H3 of anti-p95HER2 clone R4-38	STVDVHYFDY
SEQ ID NO:38	CDR-L1 of anti-p95HER2 clone R4-38	RASQSISSYLN
SEQ ID NO:39	CDR-L2 of anti-p95HER2 clone R4-38	AASSLQS
SEQ ID NO:40	CDR-L3 of anti-p95HER2 clone R4-38	QQSYSTPT
SEQ ID NO:41	constant domain in heavy chain (IgG1)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTIKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG SFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG
SEQ ID NO:42	constant domain in heavy chain e5 (hlgG1e5)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGP DV FLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKAL PLP EKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG SFFLYSKLTVDKSRW QQGN VFCFSVMHEALHNHYTQKSLSLSPG
SEQ ID NO:43	SP1	METDTLLLWVLLLWVPGSTG
SEQ ID NO:44	GS linker	GGGGG
SEQ ID NO:45	(G4S)4 linker	GGGGSGGGGSGGGGSGGGGS
Anti-p95HER2-CD137 bispecific antibody		

SEQ ID NO:46	Anti-p95HER2#4-11-CD137#54 bsAb heavy chain	QITLKESGPTLVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALEW LALIYWDDDKRYSPLKSRLLTITKDTSKNQVVLMTNMDPVDATATYY CAHAHPFSGSYNFWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPCPAPELLGG PSVFLFPPKPKDTLMISRTPETVCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSS VMHEALHNHYTQKSLSLSPGSGGGGSGGGGSGVQLVQSGAEVKKPGS TVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGRIIPILGIANYAQKF QGRVTITADKSTSTAYMELSSLRSEDTAVYYCASPPYYDSSGYYPPLGA FDIWGQGTMTVSSAGGGGSGGGGSGGGGSGGGGSSYELTQPPSVSV SPGQTASITCSGDKLGEKYASWYQQKAGQSPILVIYQDSKRPSGIPERF SGSNSGNTATLTISGLQAGDEADYYCQAWDGSSTYVFGTGKVTVLG
SEQ ID NO:47	Anti-p95HER2#4-11-CD137#54 bsAb heavy chain (hIgG1e5)	QITLKESGPTLVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALEW LALIYWDDDKRYSPLKSRLLTITKDTSKNQVVLMTNMDPVDATATYY CAHAHPFSGSYNFWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPCPAPELLGG P <u>DV</u> FLFPPKPKDTLMISRTPETVCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP <u>L</u> <u>PE</u> EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSS VMHEALHNHYTQKSLSLSPGSGGGGSGGGGSGVQLVQSGAEVKKPGS TVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGRIIPILGIANYAQKF QGRVTITADKSTSTAYMELSSLRSEDTAVYYCASPPYYDSSGYYPPLGA FDIWGQGTMTVSSAGGGGSGGGGSGGGGSGGGGSSYELTQPPSVSV SPGQTASITCSGDKLGEKYASWYQQKAGQSPILVIYQDSKRPSGIPERF SGSNSGNTATLTISGLQAGDEADYYCQAWDGSSTYVFGTGKVTVLG
SEQ ID NO:48	CD137#54-scFv	QVQLVQSGAEVKKPGSTVKVSCKASGGTFSSYAISWVRQAPGQGLE WMGRIIPILGIANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVY YCASPPYYDSSGYYPPLGAFDIWGQGTMTVSSAGGGGSGGGGSGGGG SSYELTQPPSVSVSPGQTASITCSGDKLGEKYASWYQQKAG QSPILVIYQDSKRPSGIPERFSGSNSGNTATLTISGLQAGDEADYYCQ WDGSSTYVFGTGKVTVLG
Anti-CD73 monoclonal antibody		
SEQ ID NO:49	Anti-CD73 clone CR3-4 heavy chain	QVQLVQSGAEVKKPGSSVKVSCKASGYTFT <u>GYVMH</u> WVRQAPGQGL EWMG <u>WINPNSGGTNYAQEFQ</u> GRVTMTRDTSISTAYMELSRRLSDDT AVYYCARE <u>ESMVRGVSGAFDI</u> WGQGTMTVSSA
SEQ ID NO:50	Anti-CD73 clone CR3-4 light chain	QSALTQPASVSGSPGQSITISCT <u>TGTSSDVGSYNLVS</u> WYQHPGKAPKL MIY <u>EGSKRPS</u> GVSNNRFGSKSGNTASLTISGLQAEDEADYYC <u>CSYAG</u> <u>SSGWD</u> IGTGTKVTVLGQPKANPTVTLFPPSSEELQANKATLVCLISDF YPGAVTVAWKADGSPVKAGVETTKPSKQSNKYAASSYLSLTPEQW KSHRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO:51	CDR-H1 of anti-CD73 clone CR3-4	GYVMH
SEQ ID NO:52	CDR-H2 of anti-CD73 clone CR3-4	WINPNSGGTNYAQEFQG
SEQ ID NO:53	CDR-H3 of anti-CD73 clone CR3-4	ESMVRGVSGAFDI
SEQ ID NO:54	CDR-L1 of anti-CD73 clone CR3-4	TGTSSDVGSYNLVS
SEQ ID NO:55	CDR-L2 of anti-CD73 clone CR3-4	EGSKRPS
SEQ ID NO:56	CDR-L3 of anti-CD73 clone CR3-4	CSYAGSSGWD
SEQ ID NO:57	Anti-CD73 clone CR3-16 heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFT <u>DFYIH</u> WVRQAPGQGLE WMG <u>WINPYSGSTNYPQKFQ</u> GRVTMTRDTSISTAYMDLSSLTSDDTA VYLCVR <u>GRWFE</u> <u>DFDY</u> WGQGTMTVSSA

SEQ ID NO:58	Anti-CD73 clone CR3-16 light chain	QSALTQPPSVSGSPGQSITISC TGTSSDVGTYNFVS WYQQHPGKVPKLIIY DVSNRPS GVSIRFSGSKSGNTASLTISGLQAEDEADYYC SSFRSGST YV FGTGTRVTVLGQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPAEC
SEQ ID NO:59	CDR-H1 of anti-CD73 clone CR3-16	DFYIH
SEQ ID NO:60	CDR-II2 of anti-CD73 clone CR3-16	WINPYSGSTNYPQKFQG
SEQ ID NO:61	CDR-H3 of anti-CD73 clone CR3-16	GRWFDFDY
SEQ ID NO:62	CDR-L1 of anti-CD73 clone CR3-16	TGTSSDVGTYNFVS
SEQ ID NO:63	CDR-L2 of anti-CD73 clone CR3-16	DVSNRPS
SEQ ID NO:64	CDR-L3 of anti-CD73 clone CR3-16	SSFRSGSTYV
SEQ ID NO:65	Anti-CD73 clone CR3-1-28 heavy chain	QVQLVQSGAEVKKPGSSVKVSCKASGGTF SSYAIS WVRQAPGQGLEWMG RIIPILGIANYAOKFOG RVITITADKSTSTAYMELSSLRSEDTAVYYCARD DSPVHYH YSGSYRDAFDIWGQGTMTVTVSS
SEQ ID NO:66	Anti-CD73 clone CR3-1-28 light chain	QSVLTQPPSASGTPGQRVISC SGSSSNIGSNSVY WYQQLPGTAPKLLIY SNNQRPS GVPGRFSGSKSGTSASLAISGLRSEDEADYYC AAWDDSL RGPV FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQWKS HRSYSCQVTHEGSTVEKTVAPTECS
SEQ ID NO:67	CDR-H1 of anti-CD73 clone CR3-1-28	SYAIS
SEQ ID NO:68	CDR-H2 of anti-CD73 clone CR3-1-28	RIIPILGIANYAQKFQG
SEQ ID NO:69	CDR-H3 of anti-CD73 clone CR3-1-28	DSPVHYHSGSYRDAFDI
SEQ ID NO:70	CDR-L1 of anti-CD73 clone CR3-1-28	SGSSSNIGSNSVY
SEQ ID NO:71	CDR-L2 of anti-CD73 clone CR3-1-28	SNNQRPS
SEQ ID NO:72	CDR-L3 of anti-CD73 clone CR3-1-28	AAWDDSLRGPV
SEQ ID NO:73	Anti-CD73 clone CR3-1-37 heavy chain	QVQLVQSGAEVKRPGSSVKVSCKASGGTF SSYAIS WVRQAPGQGLEWMG GIIPIFGTANYAOKFOG RVITITADESTSTAYMELSSLRSEDTAVYYCARG GVSYYYGMHV WGQGTITVTVSS
SEQ ID NO:74	Anti-CD73 clone CR3-1-37 light chain	SYVLTQPPSVSVTPGKTARIA CGGNNLES KIVSWYQQKPGQAPVLVIF YD TD RPS GISERFSGSNSGNTATLTISRVEAGDEADYYC QVWDSNSD HVI FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPAEC
SEQ ID NO:75	CDR-H1 of anti-CD73 clone CR3-1-37	SYAIS
SEQ ID NO:76	CDR-H2 of anti-CD73 clone CR3-1-37	GIIPIFGTANYAQKFQG
SEQ ID NO:77	CDR-H3 of anti-CD73 clone CR3-1-37	GVSYYYGMHV

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		PILGLIANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCASPP YYDSSGYYPGLGAFDIWGQGTMTVTVSSAGGGGSGGGGSGGGGSGGGG SSYELTQPPSVSVSPGQTASITCSGDKLGEKYASWYQQKAGQSPILVIY QDSKRPSGIPERFSGSNSGNTATLTISGLQAGDEADYYCQAWDGSSTY VFGTGKVTVLG
SEQ ID NO:90	Anti-CD73 #CR3-1-28- v3-CD137#54 heavy chain	QVQLVQSGAEVKKPKGSSSVKVSCKASGGTFSSYAIWVRQAPGQGLE WMGRIIPILGLIANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVY YCARDSPVHYGDTYYRDSFDIWGQGTMTVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVTPSSSLGTQTYICNVNIKPSNTKVDKKVEPKSCDKTIITC PPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGVQLVQ SGAEVKKPGSTVKVSCKASGGTFSSYAIWVRQAPGQGLEWMGRIPI LGLIANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCASPPY DSSGYYPGLGAFDIWGQGTMTVTVSSAGGGGSGGGGSGGGGSGGGGSS YELTQPPSVSVSPGQTASITCSGDKLGEKYASWYQQKAGQSPILVIYQ DSKRPSGIPERFSGSNSGNTATLTISGLQAGDEADYYCQAWDGSSTYV FGTGKVTVLG
SEQ ID NO:91	CD137#54-scFv	QVQLVQSGAEVKKPGSTVKVSCKASGGTFSSYAIWVRQAPGQGLE WMGRIIPILGLIANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVY YCASPPYDSSGYYPGLGAFDIWGQGTMTVTVSSAGGGGSGGGGSGGGG SGGGGSSYELTQPPSVSVSPGQTASITCSGDKLGEKYASWYQQKAG QSPILVIYQDSKRPSGIPERFSGSNSGNTATLTISGLQAGDEADYYCQ AWDGSSTYVFGTGKVTVLG

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[0225] Although the invention has been described with reference to the above examples, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:**1.** An antibody or antigen binding fragment thereof comprising:

(i) a V_H region comprising complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:3, CDR-H2 is set forth in SEQ ID NO:4, and CDR-H3 is set forth in SEQ ID NO:5 or sequences having 90% identity to SEQ ID NO:3, 4 or 5 and the antigen binding specificity thereof; and a V_L region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:6, CDR-L2 is set forth in SEQ ID NO:7, and CDR-L3 is set forth in SEQ ID NO:8 or sequences having 90% identity to SEQ ID NO:6, 7 or 8 and the antigen binding specificity thereof;

(ii) a V_H region comprising CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:11, CDR-H2 is set forth in SEQ ID NO:12, and CDR-H3 is set forth in SEQ ID NO:13 or sequences having 90% identity to SEQ ID NO:11, 12 or 13 and the antigen binding specificity thereof; and a V_L region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:14, CDR-L2 is set forth in SEQ ID NO:15, and CDR-L3 is set forth in SEQ ID NO:16 or sequences having 90% identity to SEQ ID NO:14, 15 or 16 and the antigen binding specificity thereof;

(iii) a V_H region comprising CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:19, CDR-H2 is set forth in SEQ ID NO:20, and CDR-H3 is set forth in SEQ ID NO:21 or sequences having 90% identity to SEQ ID NO:19, 20 or 21 and the antigen binding specificity thereof; and a V_L region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:22, CDR-L2 is set forth in SEQ ID NO:23, and CDR-L3 is set forth in SEQ ID NO:24 or sequences having 90% identity to SEQ ID NO:22, 23 or 24 and the antigen binding specificity thereof;

(iv) a V_H region comprising CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:27, CDR-H2 is set forth in SEQ ID NO:28, and CDR-H3 is set forth in SEQ ID NO:29 or sequences having 90% identity to SEQ ID NO:27, 28 or 29 and the antigen binding specificity thereof; and a V_L region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:30, CDR-L2 is set forth in SEQ ID NO:31, and CDR-L3 is set forth in SEQ ID NO:32 or sequences having 90% identity to SEQ ID NO:30, 31 or 32 and the antigen binding specificity thereof; or

(v) a V_H region comprising CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:35, CDR-H2 is set forth in SEQ ID NO:36, and CDR-H3 is set forth in SEQ ID NO:37 or sequences having 90% identity to SEQ ID NO:35, 36 or 37 and the antigen binding specificity thereof; and a V_L region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:38, CDR-L2 is set forth in SEQ ID NO:39, and CDR-L3 is set forth in SEQ ID NO:40 or sequences having 90% identity to SEQ ID NO:38, 39 or 40 and the antigen binding specificity thereof, wherein the antibody or antigen binding fragment binds to p95HER2.

2. The antibody or antigen binding fragment of claim 1, wherein:

(i) the V_H region has an amino acid sequence having at least 80% identity to SEQ ID NO:1 and an antigen binding specificity of SEQ ID NOs:3-5 and the V_L region has an amino acid sequence having at least 80% identity to SEQ ID NO:2 and an antigen binding specificity of SEQ ID NOs:6-8;

(ii) the V_H region has an amino acid sequence having at least 80% identity to SEQ ID NO:9 and an antigen binding specificity of SEQ ID NOs:11-13 and the V_L region has an amino acid sequence having at least 80% identity to SEQ ID NO:10 and an antigen binding specificity of SEQ ID NOs:14-16;

(iii) the V_H region has an amino acid sequence having at least 80% identity to SEQ ID NO:17 and an antigen binding specificity of SEQ ID NOs:19-21 and the V_L region has an amino acid sequence having at least 80% identity to SEQ ID NO:18 and an antigen binding specificity of SEQ ID NOs:22-24;

(iv) the V_H region has an amino acid sequence having at least 80% identity to SEQ ID NO:25 and an antigen binding specificity of SEQ ID NOs:27-29 and the V_L region has an amino acid sequence having at least 80% identity to SEQ ID NO:26 and an antigen binding specificity of SEQ ID NOs:30-32; or

(v) the V_H region has an amino acid sequence having at least 80% identity to SEQ ID NO:33 and an antigen binding specificity of SEQ ID NOs:35-37 and the V_L region has an amino acid sequence having at least 80% identity to SEQ ID NO:34 and an antigen binding specificity of SEQ ID NOs:38-40.

3. The antibody or antigen binding fragment of claim 1, wherein:

(i) the V_H region is set forth in SEQ ID NO:1 and the V_L region is set forth in SEQ ID NO:2 or a sequence having 90% identity thereto and the binding specificity thereof;

(ii) the V_H region is set forth in SEQ ID NO:9 and the V_L region is set forth in SEQ ID NO:10 or a sequence having 90% identity thereto and the binding specificity thereof;

(iii) the V_H region is set forth in SEQ ID NO:17 and the V_L region is set forth in SEQ ID NO:18 or a sequence having 90% identity thereto and the binding specificity thereof;

(iv) the V_H region is set forth in SEQ ID NO:25 and the V_L region is set forth in SEQ ID NO:26 or a sequence having 90% identity thereto and the binding specificity thereof; or

(v) the V_H region is set forth in SEQ ID NO:33 and the V_L region is set forth in SEQ ID NO:34 or a sequence having 90% identity thereto and the binding specificity thereof.

4. The antibody or antigen binding fragment of claim 1, wherein the antibody comprises an Fc domain.

5. The antibody or antigen binding fragment of claim 4, wherein the Fc domain is an IgG domain, an IgE domain, an IgM domain, and IgD domain, an IgA domain, or an IgY domain.

6. The antibody or antigen binding fragment of claim 5, wherein the IgG domain is an IgG1 domain, an IgG2 domain, an IgG3 domain, or an IgG4 domain.

7. The antibody or antigen binding fragment of claim 6, wherein the IgG1 domain comprises an amino acid sequence of SEQ ID NO:41.

8. The antibody or antigen binding fragment of claim 6, wherein the IgG1 domain comprises point mutations compared to a wild-type IgG1 domain that modify or enhance antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC).

9. The antibody or antigen binding fragment of claim 8, wherein the point mutation is S241D, A332L, or I334E.

10. A bispecific antibody comprising a first antigen binding region and a second antigen binding region, wherein the first antigen binding region binds to p95HER2.

11. The bispecific antibody of claim 10, wherein the antibody comprises a V_H region comprising complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:19, CDR-H2 is set forth in SEQ ID NO:20, and CDR-H3 is set forth in SEQ ID NO:21 or sequences having 90% identity to SEQ ID NO:19, 20 or 21 and the antigen binding specificity thereof; and a V_L region comprising CDR-L1, CDR-L2,

and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:22, CDR-L2 is set forth in SEQ ID NO:23, and CDR-L3 is set forth in SEQ ID NO:24 or sequences having 90% identity to SEQ ID NO:19, 20 or 21 and the antigen binding specificity thereof.

12. The bispecific antibody of claim 10, wherein the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:17 and an antigen binding specificity of SEQ ID NOs:19-21 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:18 and an antigen binding specificity of SEQ ID NOs:22-24.

13. The bispecific antibody of claim 10, wherein the second antigen binding region binds to an immune checkpoint molecule, or an immune stimulatory molecule.

14. The bispecific antibody of claim 10, wherein the antibody comprises a heavy chain sequence having the amino acid sequence of SEQ ID NO:46 or 47 or a sequence having 90% identity thereto and the binding specificity thereof.

15. The bispecific antibody of claim 14, further comprising a light chain having the amino acid sequence of SEQ ID NO:18.

16. The bispecific antibody of claim 10, wherein the bispecific antibody comprises an Fc domain.

17. The bispecific antibody of claim 16, wherein the Fc domain is an IgG domain, an IgE domain, an IgM domain, and IgD domain, an IgA domain, or an IgY domain.

18. The bispecific antibody of claim 16, wherein the Fc domain is an IgG domain.

19. The bispecific antibody of claim 18, wherein the IgG domain is an IgG1 domain, an IgG2 domain, an IgG3 domain, or an IgG4 domain.

20. The bispecific antibody of claim 16, wherein a scFv is linked to the C-terminus of the Fc domain.

21. The bispecific antibody of claim 20, comprising a linker between the Fab domain and the scFv domain.

22. The bispecific antibody of claim 21, wherein the Fab fragment is linked to the N-terminus of the Fc domain.

- 23.** The bispecific antibody of claim 22, wherein the Fab includes a p95HER2 binding site, and the scFv includes a CD137 binding site.
- 24.** An antibody-drug conjugate comprising: a therapeutic agent, and the antibody or an antigen binding fragment thereof of any one of claims 1-9, or the bispecific antibody of any one of claim 10-23.
- 25.** The antibody drug conjugate of claim 24, wherein the therapeutic agent is covalently linked to the antibody, antigen binding fragment or bispecific antibody via a linker.
- 26.** A pharmaceutical composition comprising the antibody or antigen binding fragment of any one of claims 1-9, the bispecific antibody of any one of claim 10-23, or the antibody-drug conjugate of any one of claims 24-25 and at least one pharmaceutically acceptable carrier.
- 27.** The pharmaceutical composition of claim 26, wherein the pharmaceutically acceptable carrier is conjugated to the C-terminus of the amino acid sequence of the antibody, antigen binding fragment, bispecific antibody, or antibody-drug conjugate.
- 28.** A method of treating cancer in a subject comprising:
administering to the subject a therapeutically effective amount of the antibody or antigen binding fragment of any one of claims 1-9, the bispecific antibody of any one of claims 10-23, or the antibody-drug conjugate of any one of claims 24-25, thereby treating the cancer.
- 28.** The method of claim 28, wherein the cancer is selected from prostate cancer, lung cancer, Non-Small Cell Lung Cancer (NSCLC), melanoma, lymphoma, breast cancer, head and neck cancer, renal cell carcinoma (RCC), ovarian cancer, kidney cancer, urinary bladder cancer, uterine cancer, cervical cancer, ovarian cancer, liver cancer, stomach cancer, colon cancer, rectal cancer, oral cavity cancer, pharynx cancer, pancreatic cancer, thyroid cancer, skin cancer, brain cancer, bone cancer, hematopoietic cancer, or leukemia.
- 30.** An isolated amino acid sequence as set forth in SEQ ID NOs:1-42 or 46-47.
- 31.** An isolated nucleic acid sequence encoding the antibody or an antigen-binding fragment thereof of any one of claims 1-9, or the bispecific antibodies of any one of claims 10-23.
- 32.** An isolated nucleic acid encoding any one of SEQ ID NOs:1-42 or 46-47.

33. An antibody or antigen binding fragment thereof comprising:

(i) a VH region comprising complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:51, CDR-H2 is set forth in SEQ ID NO:52, and CDR-H3 is set forth in SEQ ID NO:53 or sequences having 90% identity to SEQ ID NO:51, 52 or 53 and the antigen binding specificity thereof; and a VL region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:54, CDR-L2 is set forth in SEQ ID NO:55, and CDR-L3 is set forth in SEQ ID NO:56 or sequences having 90% identity to SEQ ID NO:55, 56 or 57 and the antigen binding specificity thereof;

(ii) a VH region comprising CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:59, CDR-H2 is set forth in SEQ ID NO:60, and CDR-H3 is set forth in SEQ ID NO:61 or sequences having 90% identity to SEQ ID NO:59, 60 or 61 and the antigen binding specificity thereof; and a VL region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:62, CDR-L2 is set forth in SEQ ID NO:63, and CDR-L3 is set forth in SEQ ID NO:64 or sequences having 90% identity to SEQ ID NO:62, 63 or 64 and the antigen binding specificity thereof;

(iii) a VH region comprising CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof; and a VL region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:71, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 71 or 72 and the antigen binding specificity thereof;

(iv) a VH region comprising CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:75, CDR-H2 is set forth in SEQ ID NO:76, and CDR-H3 is set forth in SEQ ID NO:77 or sequences having 90% identity to SEQ ID NO:75, 76 or 77 and the antigen binding specificity thereof; and a VL region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:78, CDR-L2 is set forth in SEQ ID NO:79, and CDR-L3 is set forth in SEQ ID NO:80 or sequences having 90% identity to SEQ ID NO:78, 79 or 80 and the antigen binding specificity thereof;

(v) a VH region comprising CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen

binding specificity thereof; and a VL region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:82, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 82 or 72 and the antigen binding specificity thereof;

(vi) a VII region comprising CDR-II1, CDR-II2, and CDR-II3, wherein CDR-II1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof; and a VL region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:84, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 84 or 72 and the antigen binding specificity thereof; or

(vii) a VH region comprising CDR-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof; and a VL region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:86, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 86 or 72 and the antigen binding specificity thereof; wherein the antibody or antigen binding fragment binds to CD73.

34. The antibody or antigen binding fragment of claim 33, wherein:

(i) the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:49 and an antigen binding specificity of SEQ ID NOs:51-53 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:50 and an antigen binding specificity of SEQ ID NOs:54-56;

(ii) the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:57 and an antigen binding specificity of SEQ ID NOs:59-61 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:58 and an antigen binding specificity of SEQ ID NOs:62-64;

(iii) the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:66 and an antigen binding specificity of SEQ ID NOs:70-72;

(iv) the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:73 and an antigen binding specificity of SEQ ID NOs:75-77 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:74 and an antigen binding specificity of SEQ ID NOs:78-80;

(v) the VII region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:81 and an antigen binding specificity of SEQ ID NOs:70, 82 and 72;

(vi) the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:83 and an antigen binding specificity of SEQ ID NOs: 70, 84 and 72; or

(vii) the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:85 and an antigen binding specificity of SEQ ID NOs: 70, 86 and 72.

35. The antibody or antigen binding fragment of claim 33, wherein:

(i) the VH region is set forth in SEQ ID NO:49 and the VL region is set forth in SEQ ID NO:50 or a sequence having 90% identity thereto and the binding specificity thereof;

(ii) the VH region is set forth in SEQ ID NO:57 and the VL region is set forth in SEQ ID NO:58 or a sequence having 90% identity thereto and the binding specificity thereof;

(iii) the VH region is set forth in SEQ ID NO:65 and the VL region is set forth in SEQ ID NO:66 or a sequence having 90% identity thereto and the binding specificity thereof;

(iv) the VH region is set forth in SEQ ID NO:73 and the VL region is set forth in SEQ ID NO:74 or a sequence having 90% identity thereto and the binding specificity thereof;

(v) the VH region is set forth in SEQ ID NO:65 and the VL region is set forth in SEQ ID NO:81 or a sequence having 90% identity thereto and the binding specificity thereof;

(vi) the VH region is set forth in SEQ ID NO:65 and the VL region is set forth in SEQ ID NO:83 or a sequence having 90% identity thereto and the binding specificity thereof; or

(vii) the VII region is set forth in SEQ ID NO:65 and the VL region is set forth in SEQ ID NO:85 or a sequence having 90% identity thereto and the binding specificity thereof.

- 36.** The antibody or antigen binding fragment of claim 33, wherein the antibody comprises an Fc domain.
- 37.** The antibody or antigen binding fragment of claim 36, wherein the Fc domain is an IgG domain, an IgE domain, an IgM domain, and IgD domain, an IgA domain, or an IgY domain.
- 38.** The antibody or antigen binding fragment of claim 37, wherein the IgG domain is an IgG1 domain, an IgG2 domain, an IgG3 domain, or an IgG4 domain.
- 39.** The antibody or antigen binding fragment of claim 38, wherein the IgG1 domain comprises an amino acid sequence of SEQ ID NO:87.
- 40.** The antibody or antigen binding fragment of claim 38, wherein the IgG1 comprises point mutations compared to wild-type IgG1 that modify or enhance antibody-dependent cellular cytotoxicity (ADCC) and/ or complement-dependent cytotoxicity (CDC).
- 41.** The antibody or antigen binding fragment of claim 40, wherein the point mutation is K297A, or K322A.
- 42.** A bispecific antibody comprising a first antigen binding region and a second antigen binding region, wherein the first antigen binding region binds to CD73.
- 43.** The bispecific antibody of claim 42, wherein the antibody comprises:
- (i) a VH region comprising complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof; and a VL region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:71, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 71 or 72 and the antigen binding specificity thereof;
 - (ii) a VII region comprising complementarity-determining region (CDR)-II1, CDR-II2, and CDR-II3, wherein CDR-II1 is set forth in SEQ ID NO:67, CDR-II2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof; and a VL region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:82, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 82 or 72 and the antigen binding specificity thereof;

(iii) a VH region comprising complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof; and a VL region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:84, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 84 or 72 and the antigen binding specificity thereof; or

(iv) a VH region comprising complementarity-determining region (CDR)-H1, CDR-H2, and CDR-H3, wherein CDR-H1 is set forth in SEQ ID NO:67, CDR-H2 is set forth in SEQ ID NO:68, and CDR-H3 is set forth in SEQ ID NO:69 or sequences having 90% identity to SEQ ID NO:67, 68 or 69 and the antigen binding specificity thereof; and a VL region comprising CDR-L1, CDR-L2, and CDR-L3, wherein CDR-L1 is set forth in SEQ ID NO:70, CDR-L2 is set forth in SEQ ID NO:86, and CDR-L3 is set forth in SEQ ID NO:72 or sequences having 90% identity to SEQ ID NO:70, 86 or 72 and the antigen binding specificity thereof.

44. The bispecific antibody of claim 42, wherein:

(i) the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:66 and an antigen binding specificity of SEQ ID NOs:70-72;

(ii) the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:81 and an antigen binding specificity of SEQ ID NOs:70, 82 and 72;

(iii) the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:82 and an antigen binding specificity of SEQ ID NOs:70, 84 and 72; or

(iv) the VH region has an amino acid sequence having at least 80% identity to SEQ ID NO:65 and an antigen binding specificity of SEQ ID NOs:67-69 and the VL region has an amino acid sequence having at least 80% identity to SEQ ID NO:85 and an antigen binding specificity of SEQ ID NOs:70, 86 and 72.

- 45.** The bispecific antibody of claim 42, wherein the second antigen binding region binds to an immune checkpoint molecule, or an immune stimulatory molecule.
- 46.** The bispecific antibody of claim 42, wherein the antibody comprises a heavy chain sequence having the amino acid sequence of SEQ ID NO:88, 89, 90 or 91 or a sequence having 90% identity thereto and the binding specificity thereof.
- 47.** The bispecific antibody of claim 42, further comprising a light chain having the amino acid sequence of SEQ ID NO:66.
- 48.** The bispecific antibody of claim 42, wherein the bispecific antibody comprises an Fc domain.
- 49.** The bispecific antibody of claim 48, wherein the Fc domain is an IgG domain, an IgE domain, an IgM domain, and IgD domain, an IgA domain, or an IgY domain.
- 50.** The bispecific antibody of claim 42, wherein the Fc domain is an IgG domain.
- 51.** The bispecific antibody of claim 50, wherein the IgG domain is an IgG1 domain, an IgG2 domain, an IgG3 domain, or an IgG4 domain.
- 52.** The bispecific antibody of claim 48, wherein a scFv is linked to the C-terminus of the Fc domain.
- 53.** The bispecific antibody of claim 52, comprising a linker between the Fab domain and the scFv domain.
- 54.** The bispecific antibody of claim 53, wherein the Fab fragment is linked to the N-terminus of the Fc domain.
- 55.** The bispecific antibody of claim 54, wherein the Fab includes a CD73 binding site, and the scFv includes a CD137 binding site.
- 56.** An antibody-drug conjugate comprising: a therapeutic agent, and the antibody or an antigen binding fragment thereof of any one of claims 33-41, or the bispecific antibody of any one of claims 42-55.
- 57.** The antibody-drug conjugate of claim 56, wherein the therapeutic agent is covalently linked to the antibody, antigen binding fragment or bispecific antibody via a linker.

58. A pharmaceutical composition comprising the antibody or antigen binding fragment of any one of claims 33-41, the bispecific antibody of any one of claims 42-55, or the antibody-drug conjugate of any one of claims 56-57 and a at least one pharmaceutically acceptable carrier.

59. The pharmaceutical composition of claim 58, wherein the pharmaceutically acceptable carrier is conjugated to the C-terminus of the amino acid sequence of the antibody, antigen binding fragment, bispecific antibody, or antibody-drug conjugate.

60. A method of treating cancer in a subject comprising:
administering to the subject a therapeutically effective amount of the antibody or antigen binding fragment of any one of claims 33-41, the bispecific antibody of any one of claims 42-55, or the antibody-drug conjugate of any one of claims 56-57, thereby treating the cancer.

61. The method of claim 60, wherein the cancer is selected from prostate cancer, lung cancer, Non-Small Cell Lung Cancer (NSCLC), melanoma, lymphoma, breast cancer, head and neck cancer, renal cell carcinoma (RCC), ovarian cancer, kidney cancer, urinary bladder cancer, uterine cancer, cervical cancer, ovarian cancer, liver cancer, stomach cancer, colon cancer, rectal cancer, oral cavity cancer, pharynx cancer, pancreatic cancer, thyroid cancer, skin cancer, brain cancer, bone cancer, hematopoietic cancer, or leukemia.

62. An isolated amino acid sequence as set forth in SEQ ID NOs:49-86 or 88-91.

63. An isolated amino acid sequence encoding the antibody or antigen-binding fragment thereof of any one of claims 32-40, or the bispecific antibody of any one of claims 41-54.

64. An isolated nucleic acid encoding any one of SEQ ID NOs:49-86 or 88-91.

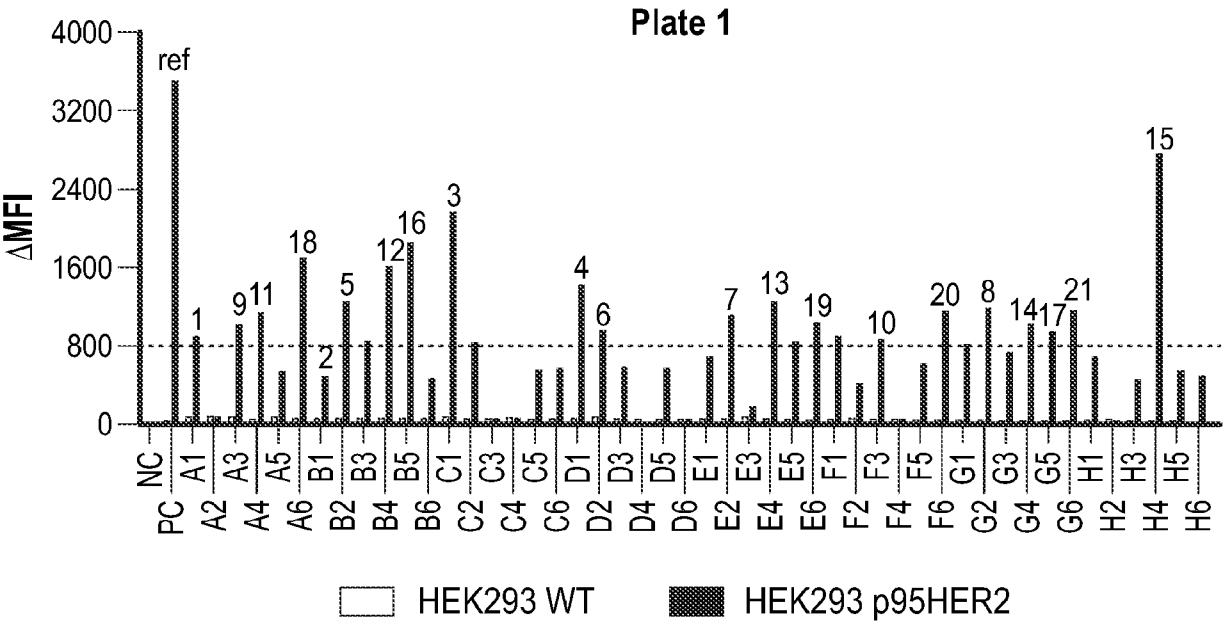


FIGURE 1A

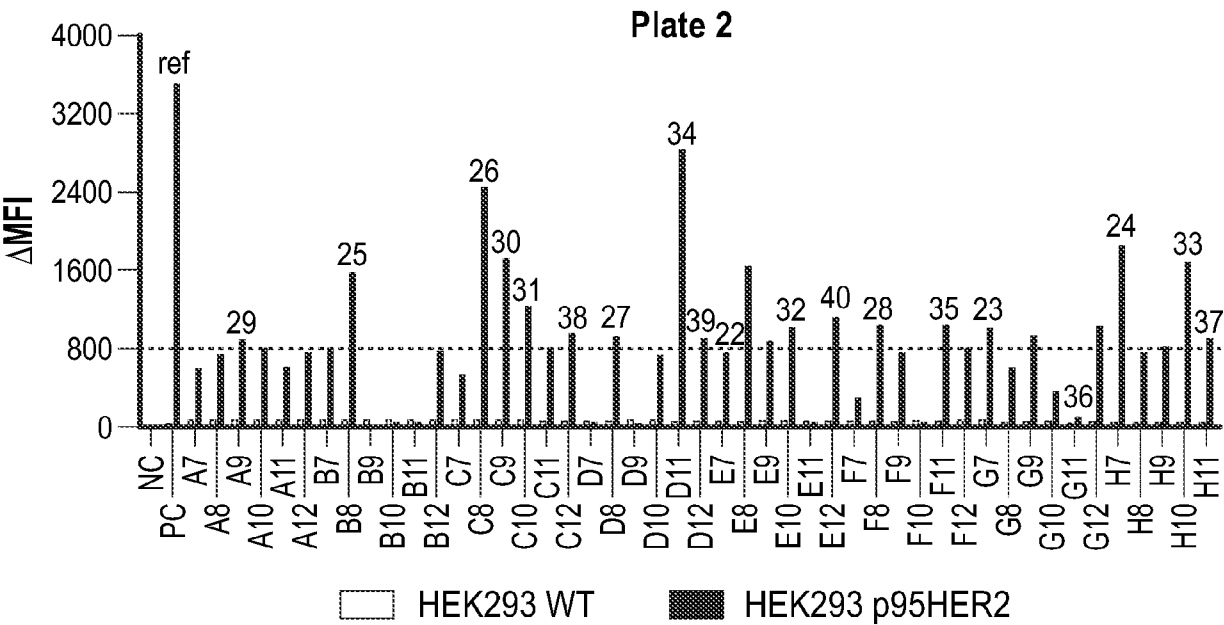


FIGURE 1B

	1	2	3	4	5	6	7	8	9	10	11	12
A	R4-1		R4-9	R4-11		R4-18			R4-29			
B	R4-2	R4-5		R4-12	R4-16			R4-25				
C	R4-3							R4-26	R4-30	R4-31		R4-38
D	R4-4	R4-6						R4-27			R4-34	R4-39
E		R4-7		R4-13		R4-19	R4-22			R4-32		R4-40
F			R4-10			R4-20		R4-28			R4-35	
G		R4-8		R4-14	R4-17	R4-21	R4-23				R4-36	
H				R4-15			R4-24			R4-33	R4-37	

FIGURE 1C

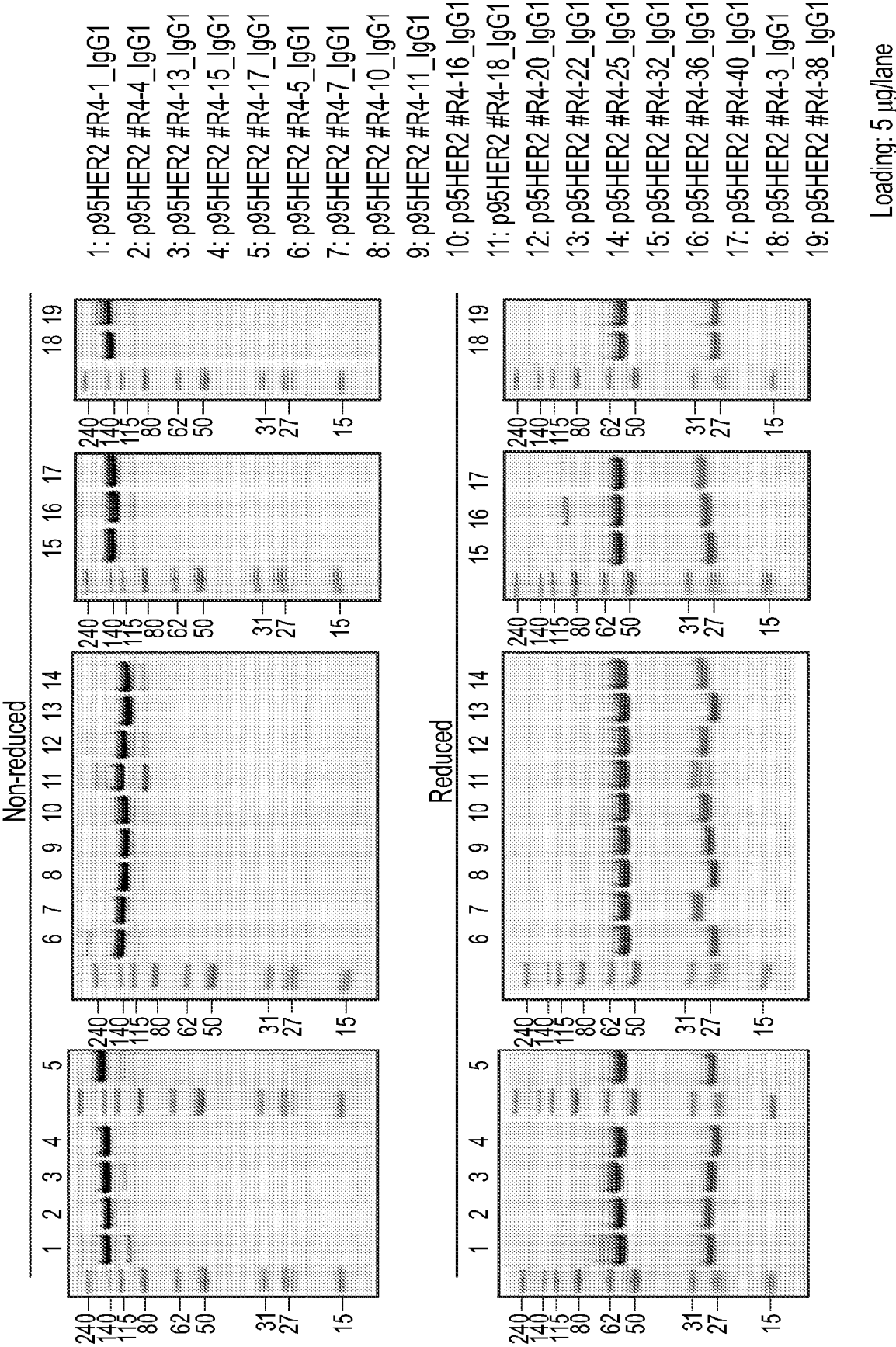


FIGURE 2

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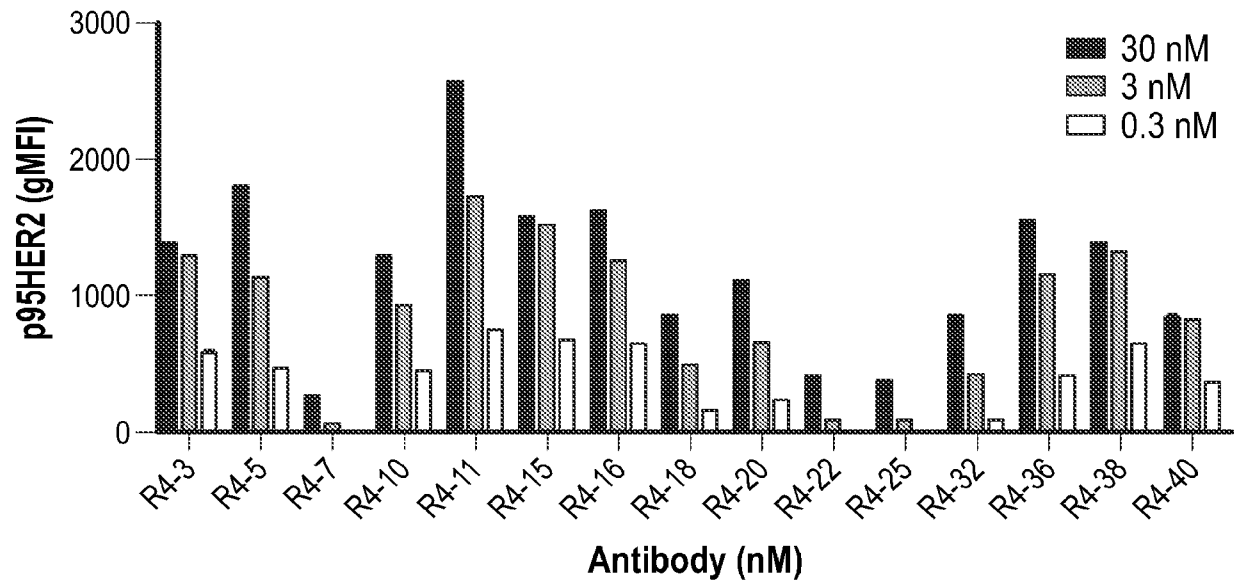
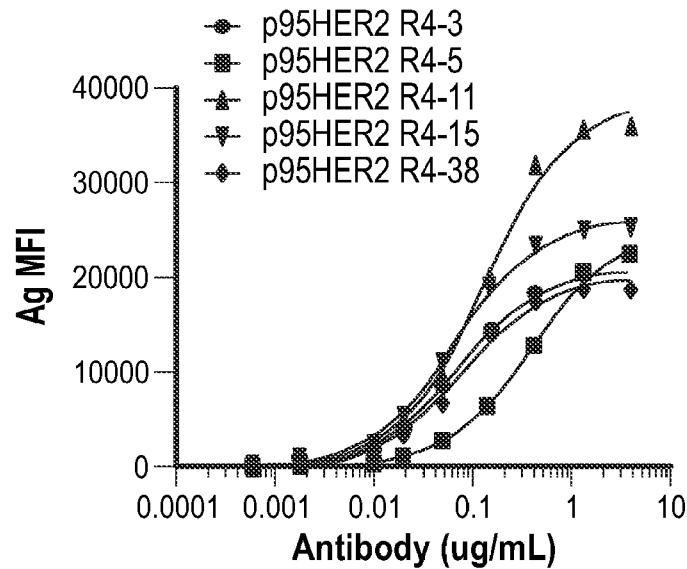


FIGURE 3A



Clone Name	EC50
p95HER2 R4-3	0.0708
p95HER2 R4-5	0.3989
p95HER2 R4-11	0.1316
p95HER2 R4-15	0.0659
p95HER2 R4-38	0.0781

FIGURE 3B

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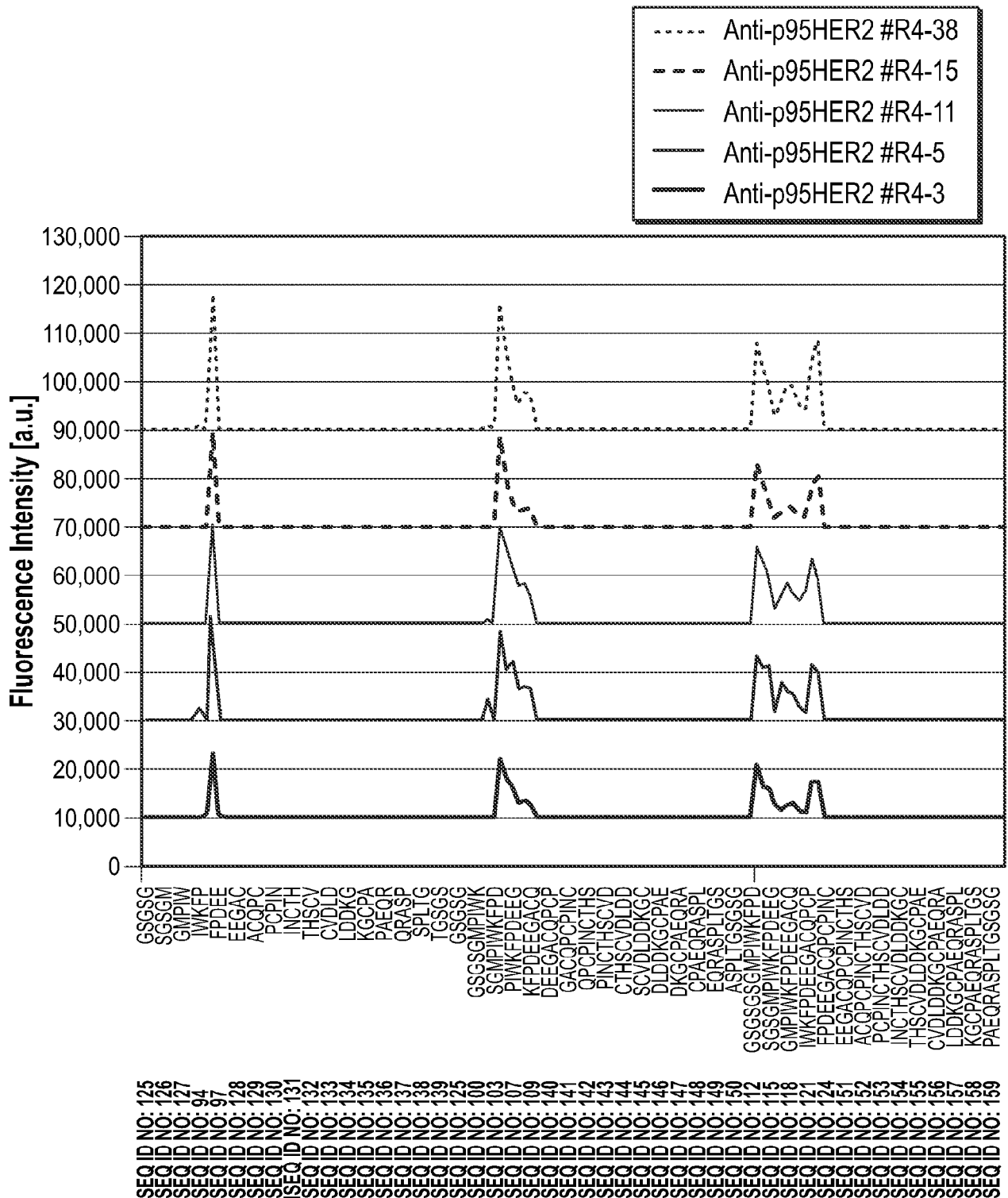


FIGURE 4A

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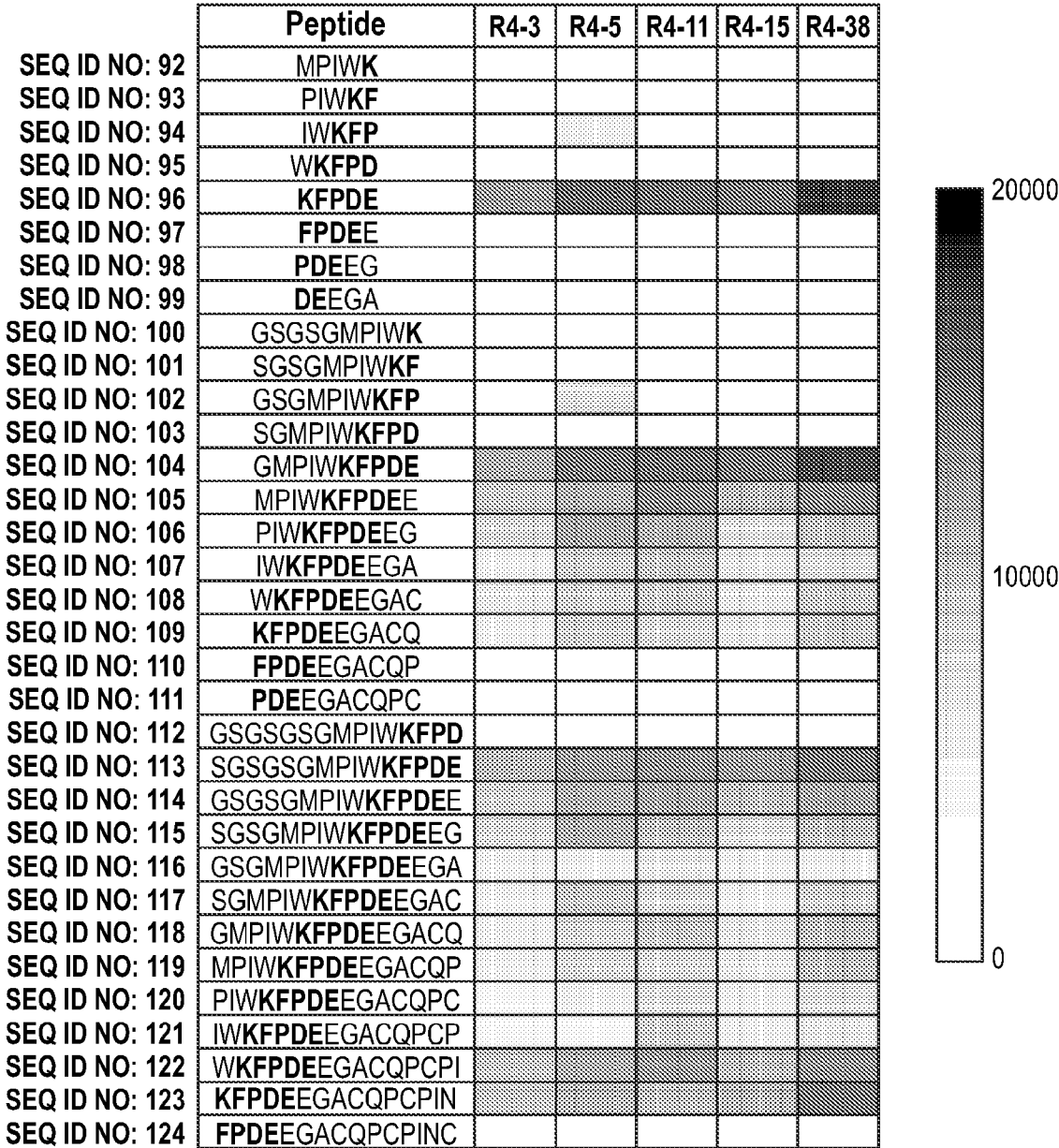


FIGURE 4B

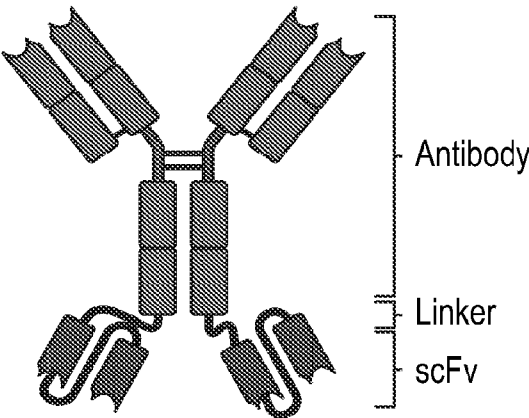


FIGURE 5

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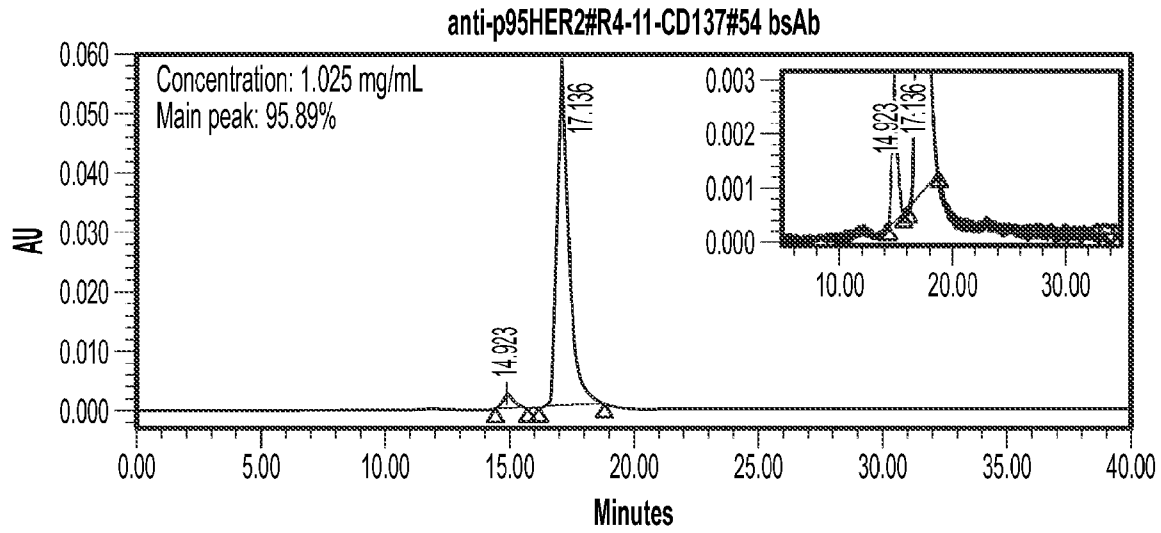


FIGURE 6A

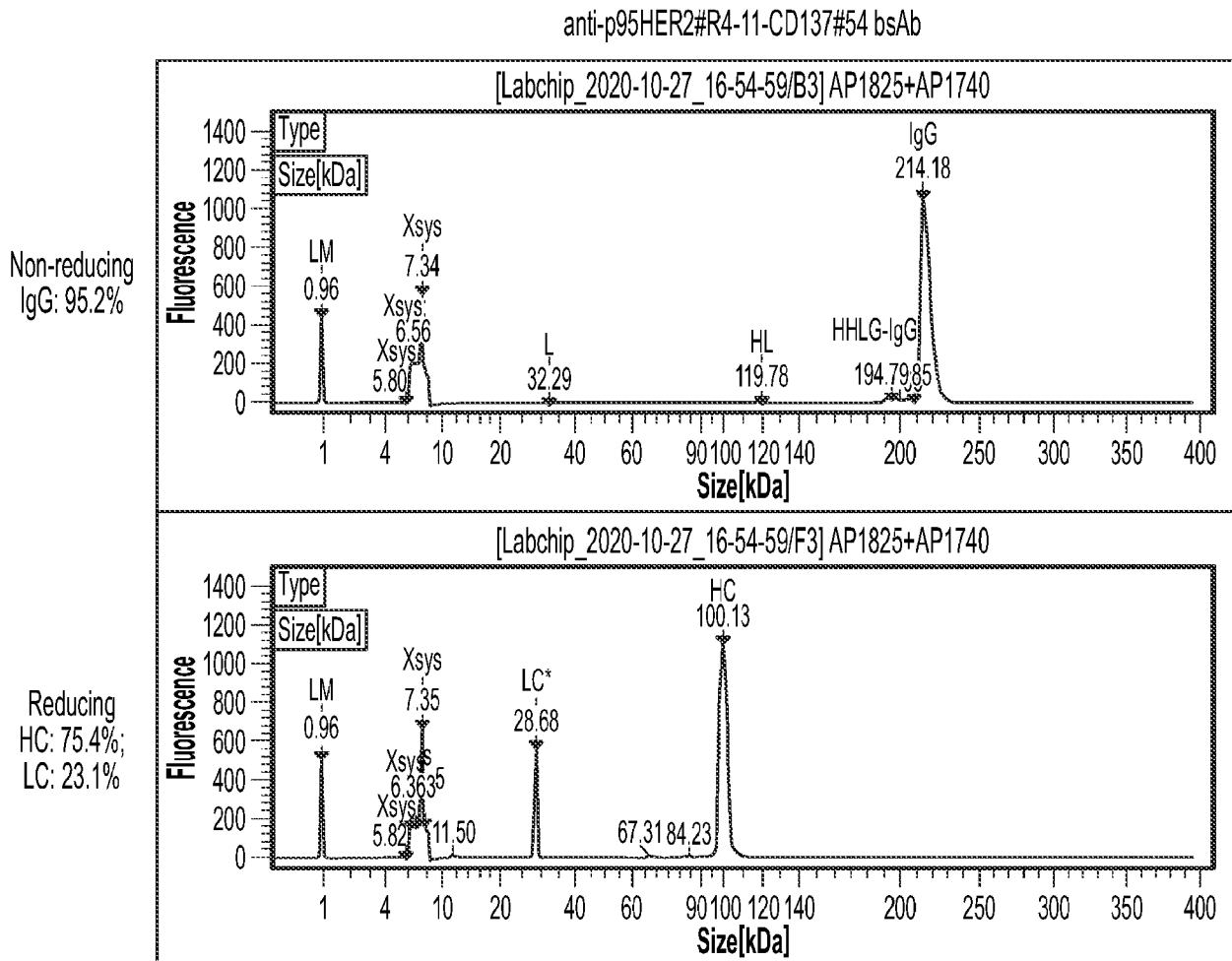


FIGURE 6B

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anti-p95HER2#R4-11-CD137#54 bsAb
 K_D (M): $1.89\text{E-}10$

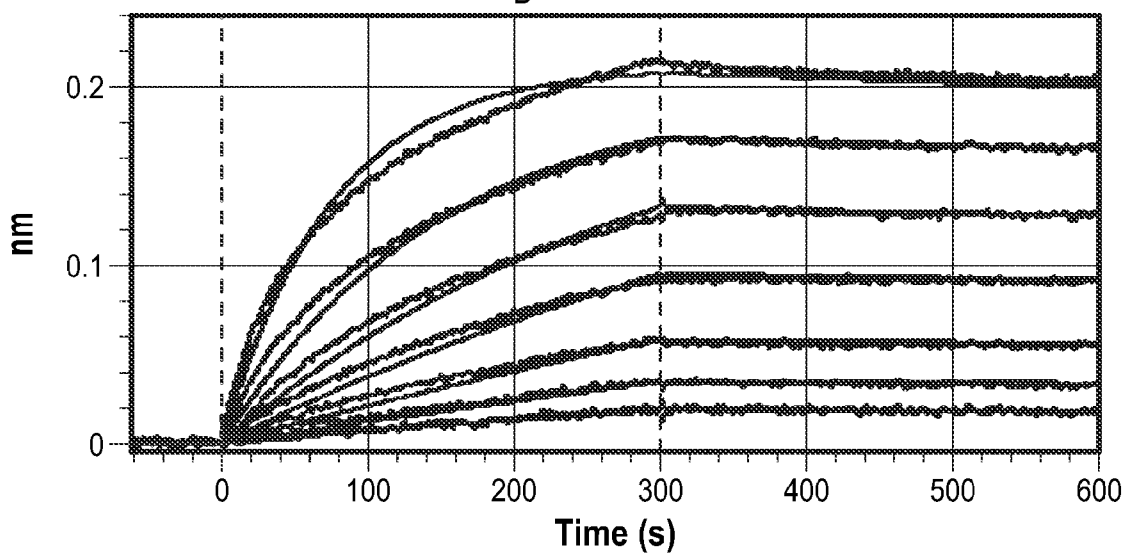


FIGURE 7A

anti-p95HER2#R4-11-CD137#54 bsAb
 K_D (M): $1.12\text{E-}09$

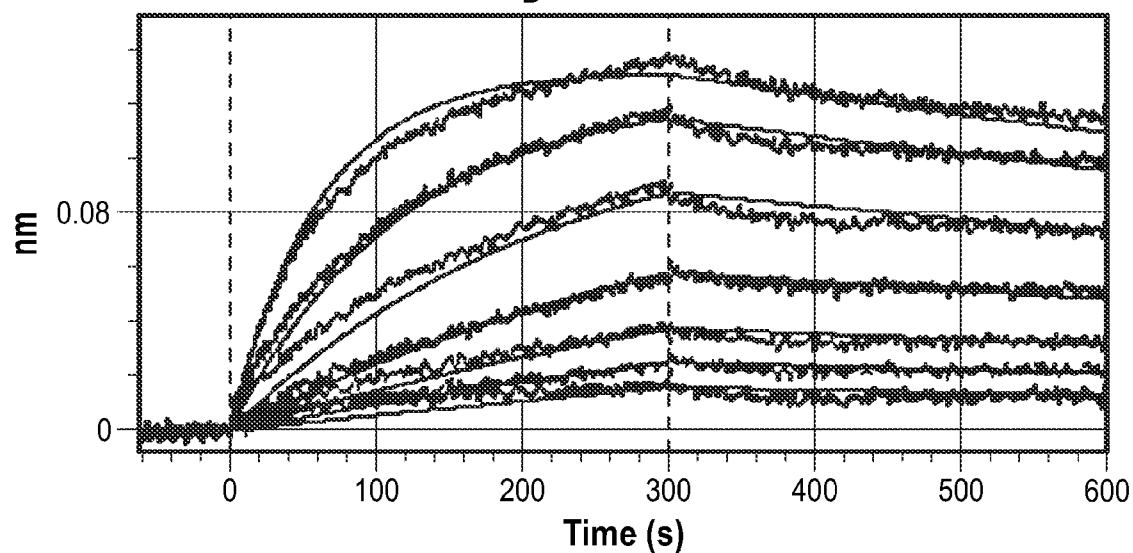


FIGURE 7B

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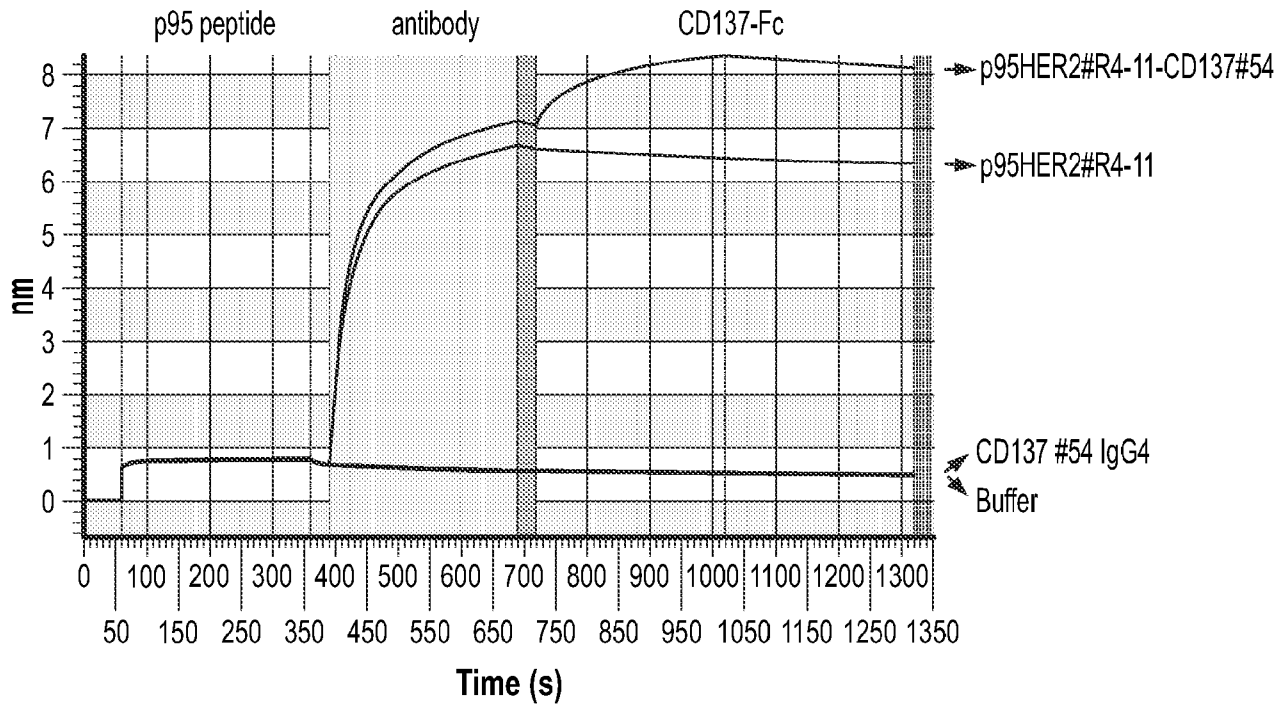


FIGURE 8

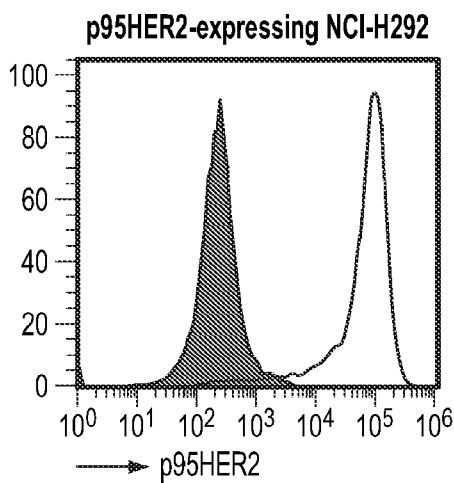


FIGURE 9A

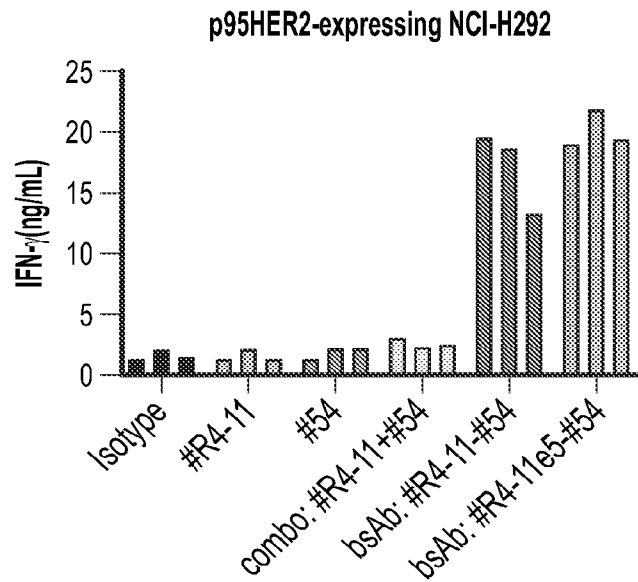


FIGURE 9B

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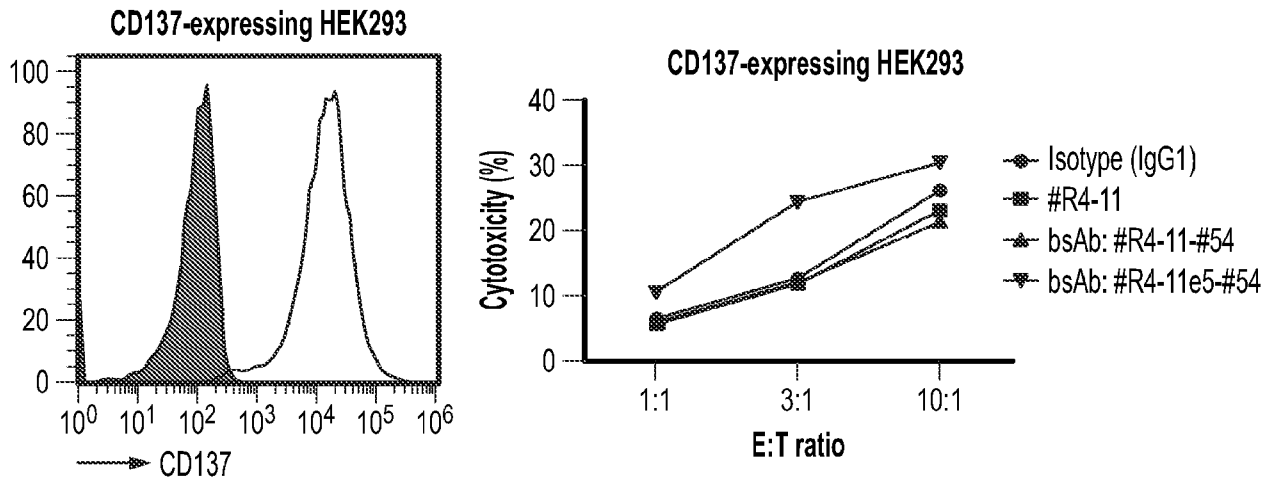


FIGURE 10A

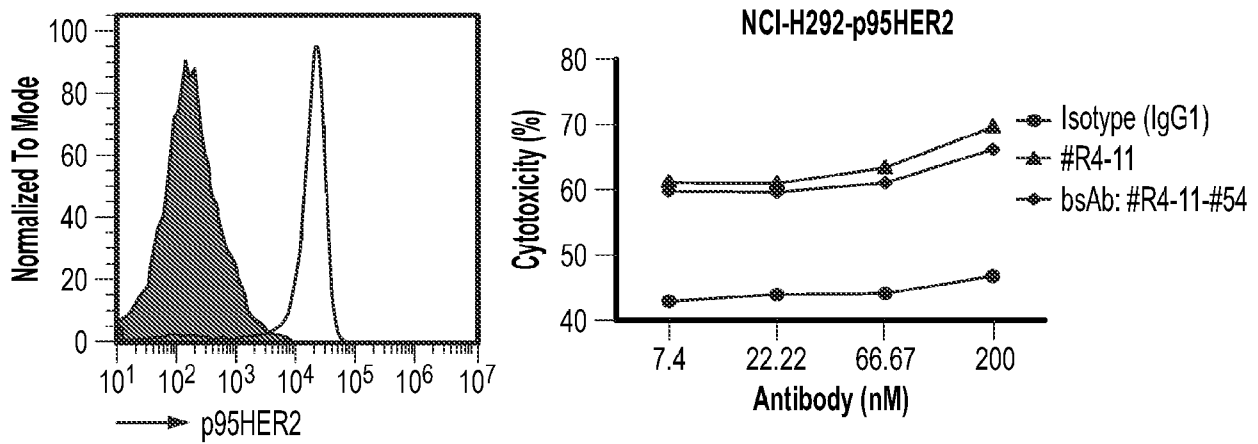


FIGURE 10B

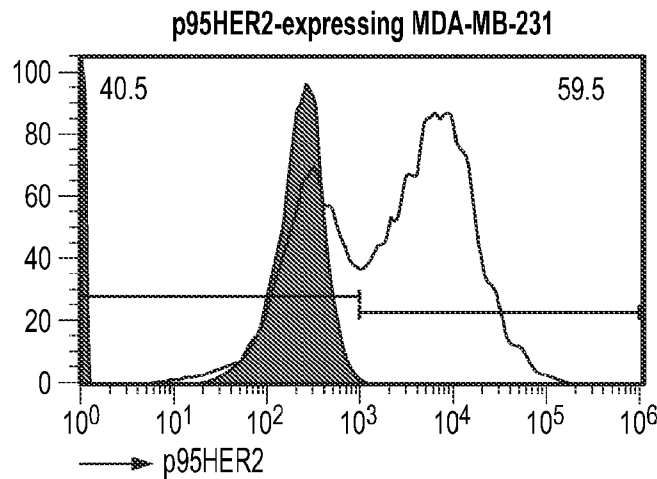


FIGURE 11A

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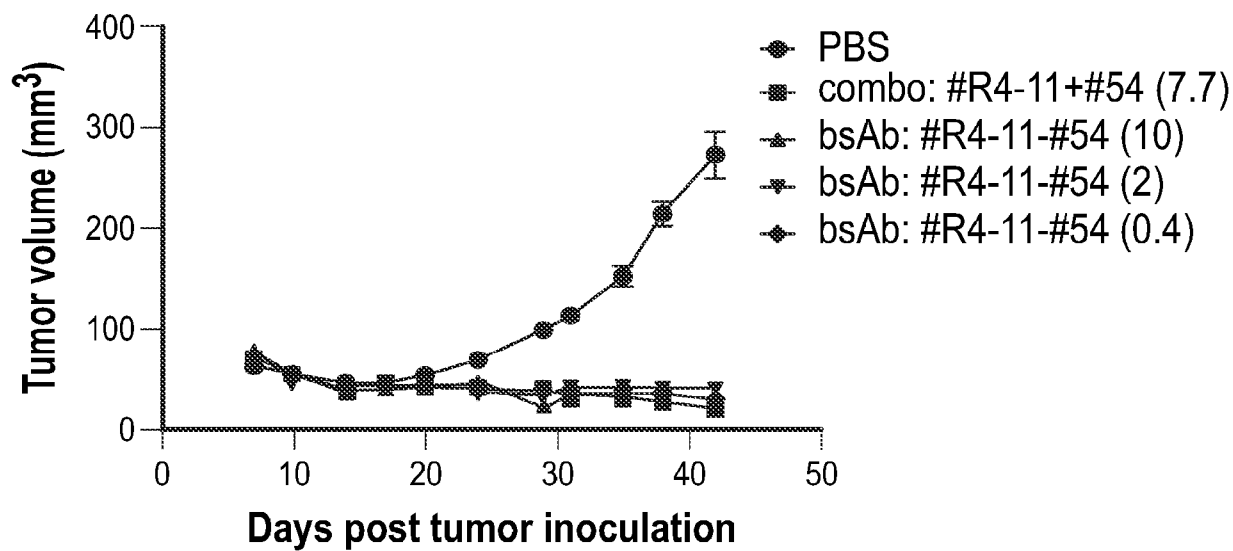


FIGURE 11B

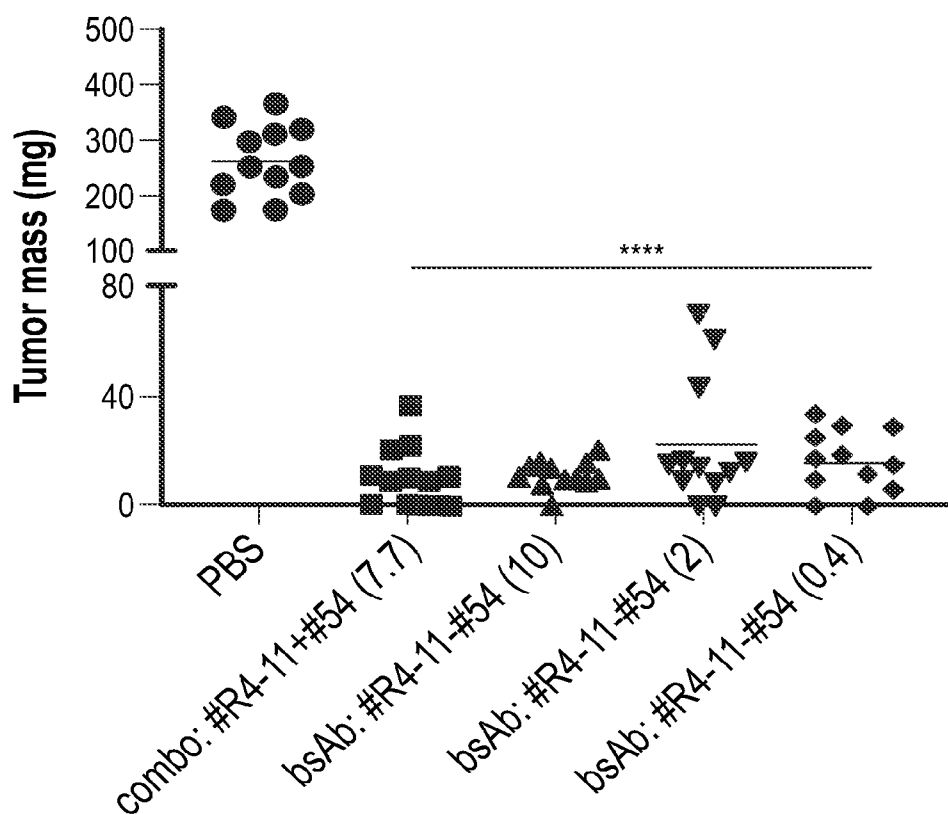
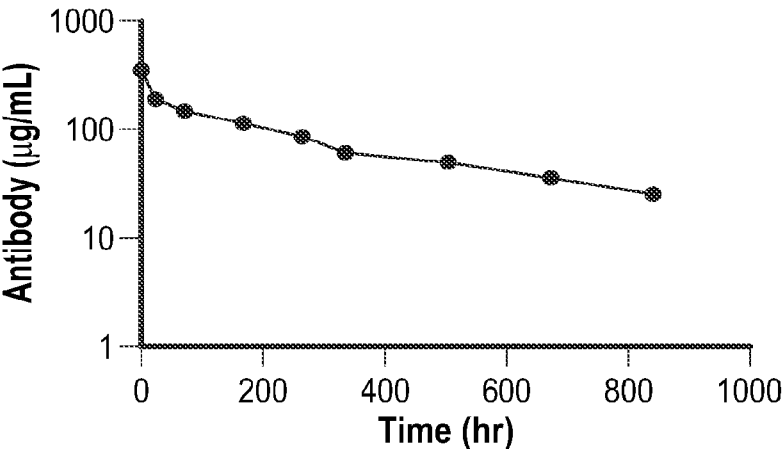


FIGURE 11C

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Parameter	Unit	#R4-11-#54
Lambda_z	1/h	0.002061747
t1/2	h	336.194049
Tmax	h	0.5
Cmax	µg/ml	354.88
C0	µg/ml	359.5411154
Clast_obs/Cmax		0.070446348
AUC 0-t	µg/ml*h	63896.23778
AUC 0-inf_obs	µg/ml*h	76021.87496
AUC 0-t/0-inf_obs		0.840498052
AUMC 0-inf_obs	µg/ml*h^2	33233919.58
MRT 0-inf_obs	h	437.1625876
Vz_obs	(mg/kg)/(µg/ml)	5.263564299
Cl_obs	(mg/kg)/(µg/ml)/h	0.01085214
Vss_obs	(mg/kg)/(µg/ml)	4.744149431

FIGURE 12

3rd round single clone screening

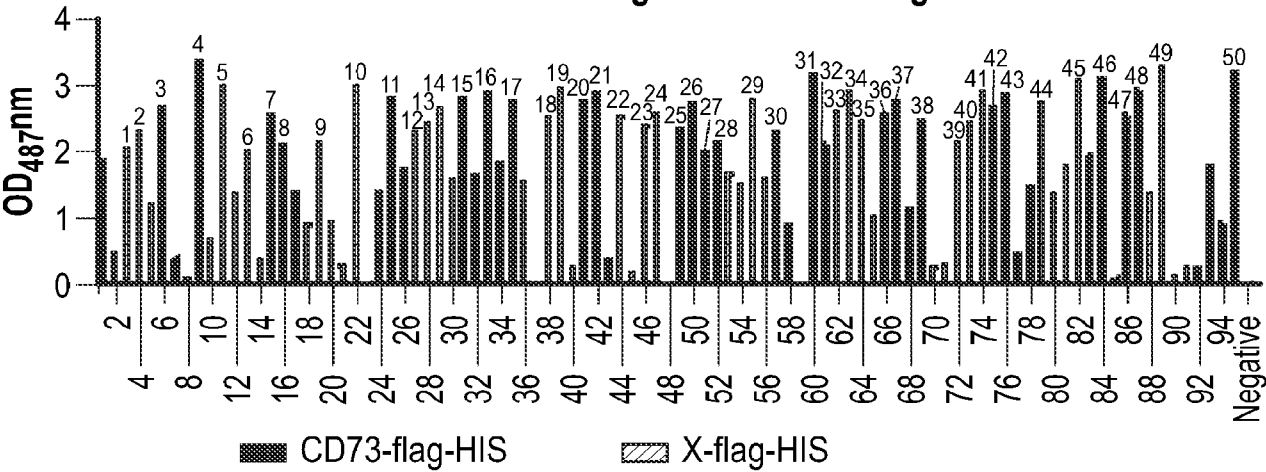


FIGURE 13A

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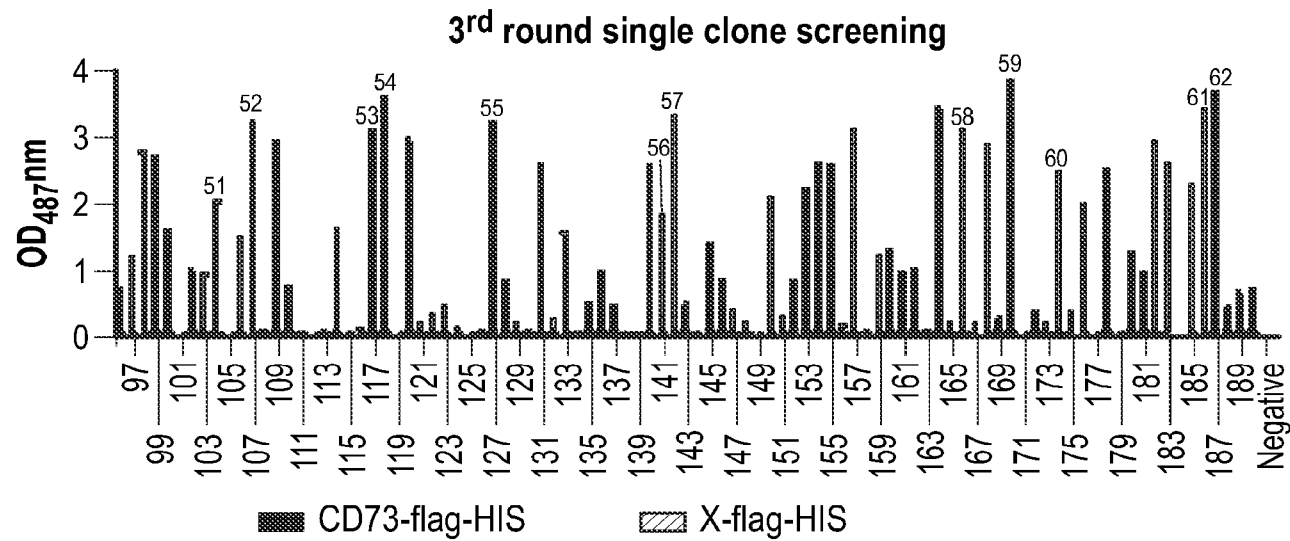


FIGURE 13B

No.	Clone name	No.	Clone name	No.	Clone name	No.	Clone name
1	CR3-1-1	16	CR3-1-16	31	CR3-1-31	46	CR3-1-46
2	CR3-1-2	17	CR3-1-17	32	CR3-1-32	47	CR3-1-47
3	CR3-1-3	18	CR3-1-18	33	CR3-1-33	48	CR3-1-48
4	CR3-1-4	19	CR3-1-19	34	CR3-1-34	49	CR3-1-49
5	CR3-1-5	20	CR3-1-20	35	CR3-1-35	50	CR3-1-50
6	CR3-1-6	21	CR3-1-21	36	CR3-1-36	51	CR3-4
7	CR3-1-7	22	CR3-1-22	37	CR3-1-37	52	CR3-6
8	CR3-1-8	23	CR3-1-23	38	CR3-1-38	53	CR3-9
9	CR3-1-9	24	CR3-1-24	39	CR3-1-39	54	CR3-10
10	CR3-1-10	25	CR3-1-25	40	CR3-1-40	55	CR3-12
11	CR3-1-11	26	CR3-1-26	41	CR3-1-41	56	CR3-16
12	CR3-1-12	27	CR3-1-27	42	CR3-1-42	57	CR3-17
13	CR3-1-13	28	CR3-1-28	43	CR3-1-43	58	CR3-24
14	CR3-1-14	29	CR3-1-29	44	CR3-1-44	59	CR3-26
15	CR3-1-15	30	CR3-1-30	45	CR3-1-45	60	CR3-27
						61	CR3-33
						62	CR3-34

FIGURE 13C

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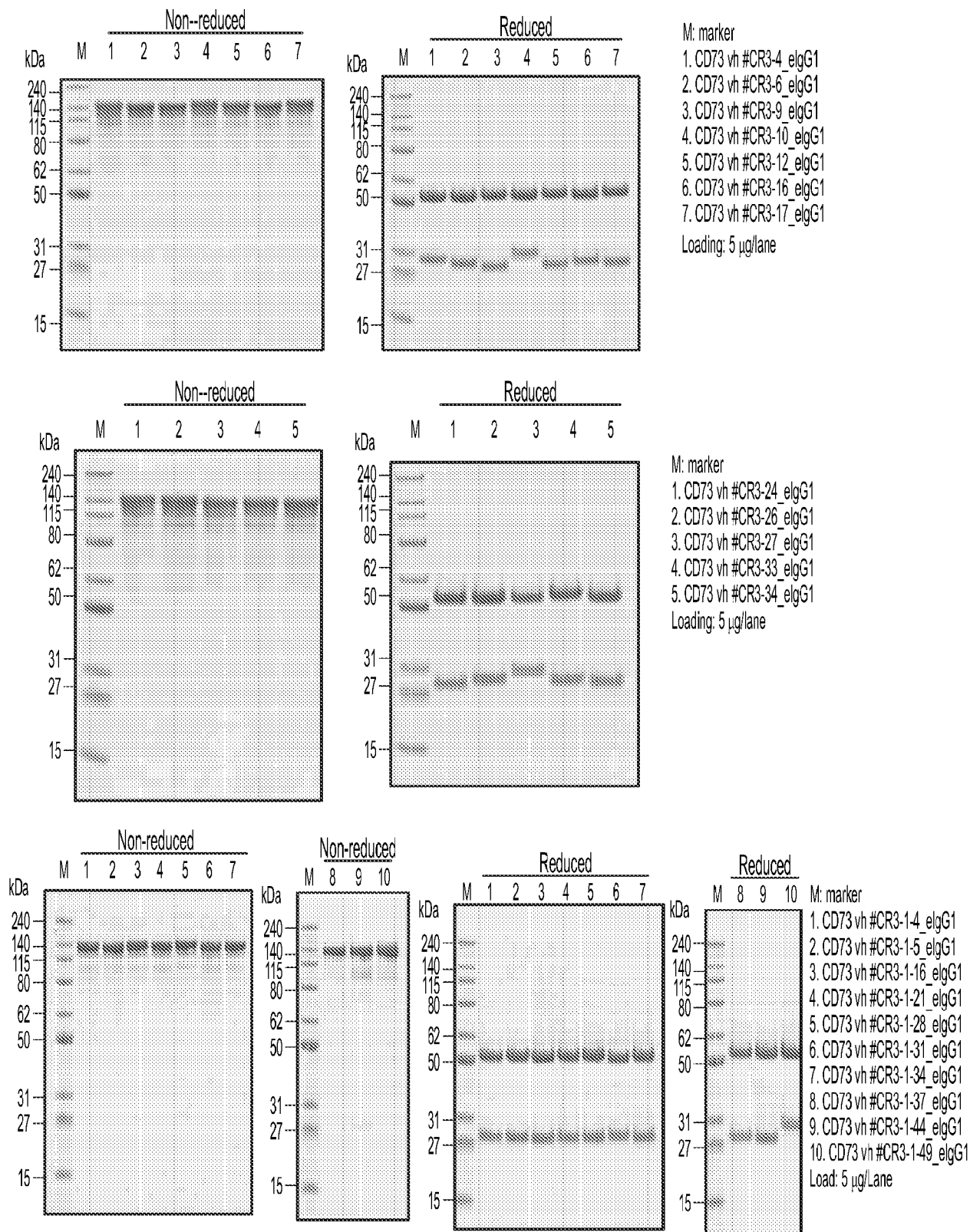


FIGURE 14

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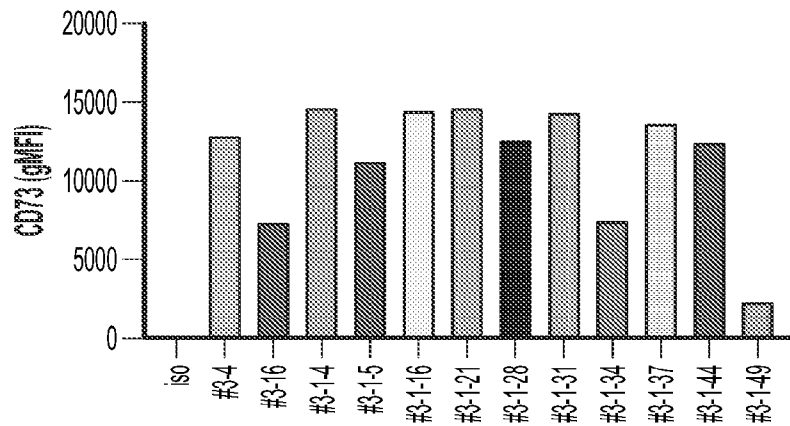
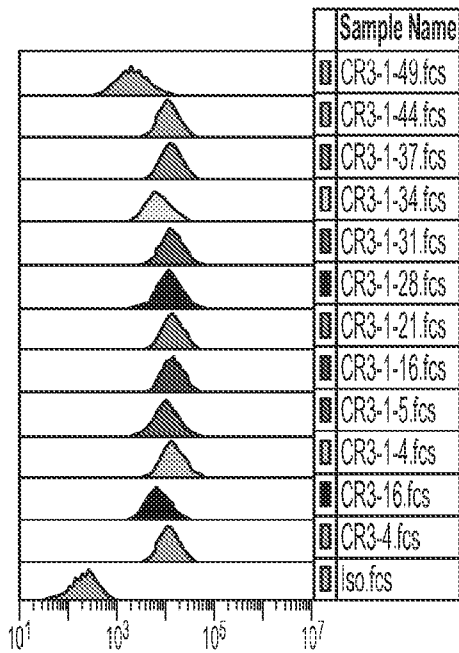
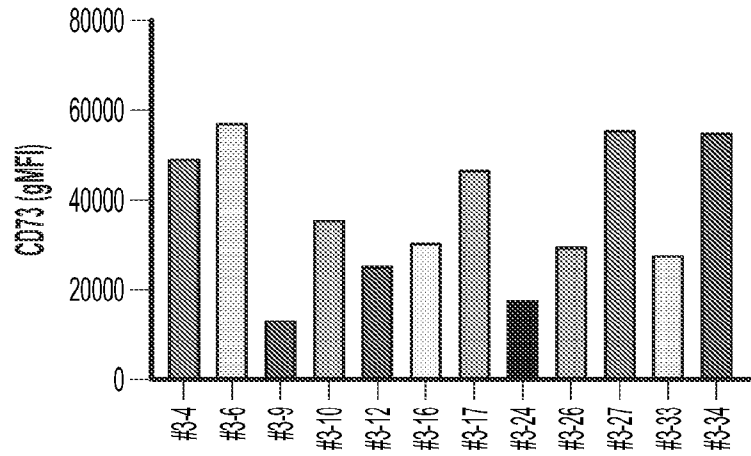
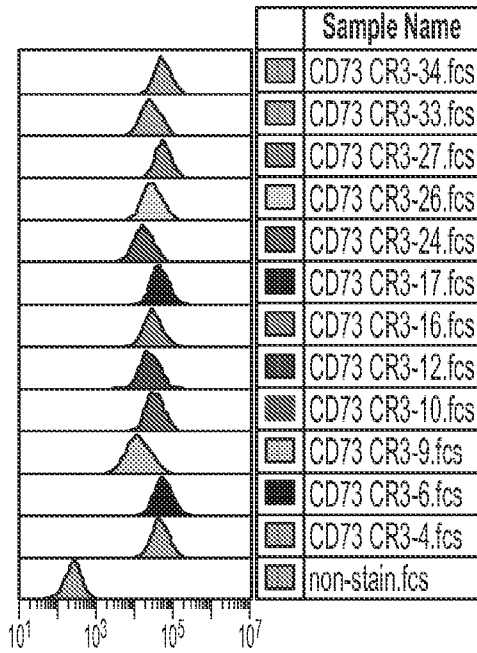


FIGURE 15

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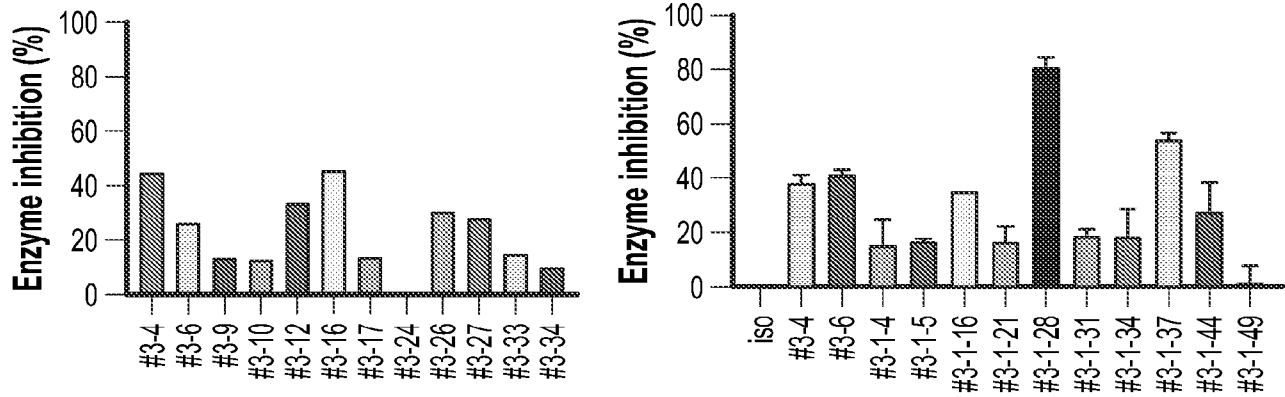
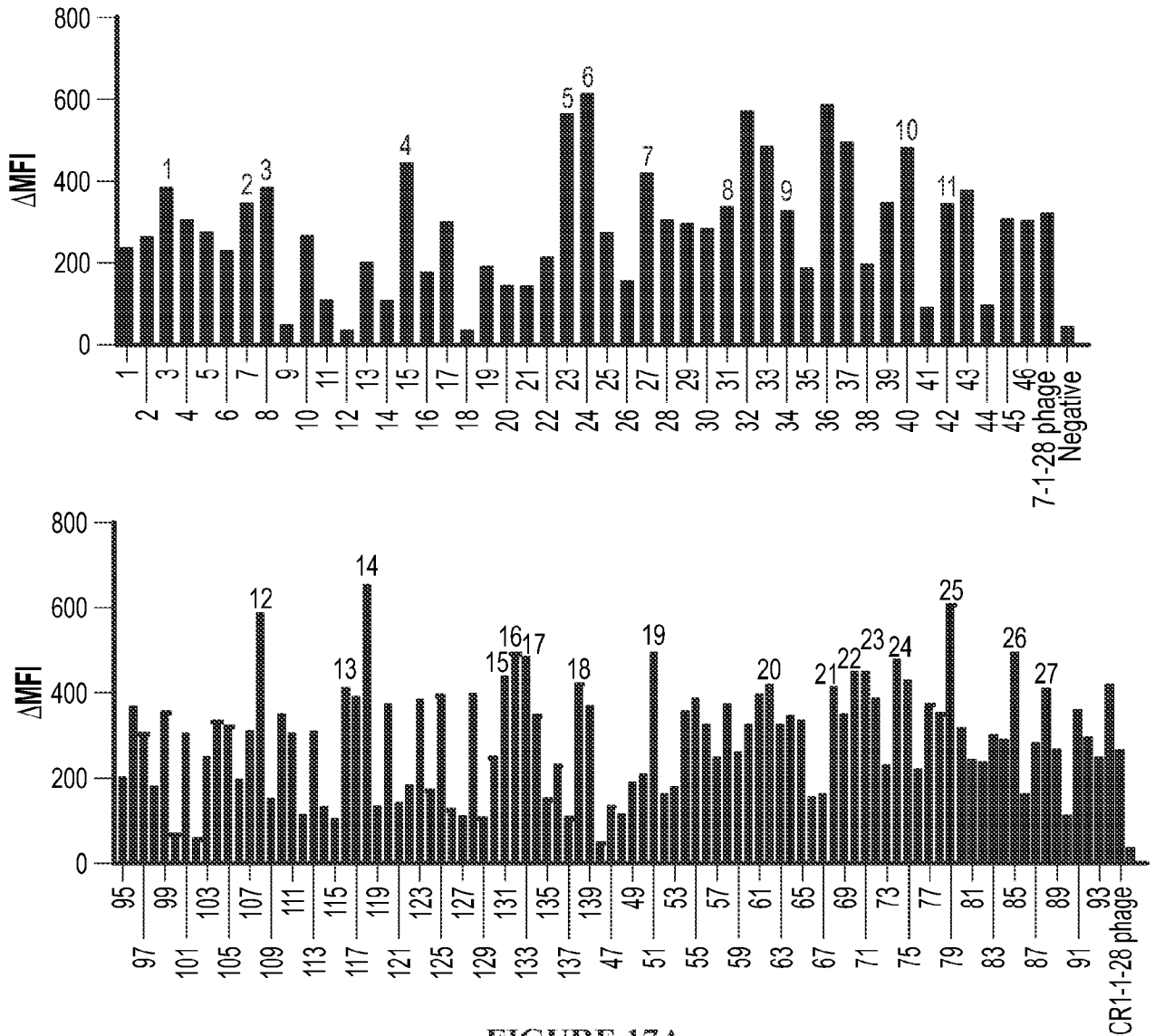


FIGURE 16



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			Antibody (nM)		
			0.37	3.33	30
1st batch	No.	Clone name			
		#3-1-28	2.009	3.464	3.811
	1	1919-001	1.067	2.081	3.096
	2	1919-002	3.034	3.816	3.961
	3	1919-003	3.598	3.956	4.000
	4	1919-004	3.101	3.852	4.000
	5	1919-005	1.141	2.286	3.342
	6	1919-006	1.796	3.000	3.760
	7	1919-007	1.366	2.417	3.276
	8	1919-008	0.928	1.616	2.433
	9	1919-009	2.535	3.729	3.945
	10	1919-010	1.972	3.264	3.912
	11	1919-011	0.509	1.229	2.771
2nd batch		#3-1-28	1.558	3.063	3.790
	13	1911-020	0.684	1.624	2.952
	14	1911-021	0.312	0.520	1.347
	16	1911-023	1.311	2.635	3.622
	17	1911-024	2.469	3.506	3.877
	20	1911-027	1.840	3.191	3.815
	21	1911-028	2.065	3.103	3.772
	23	1911-030	2.721	3.770	4.000
	24	1911-031	2.146	3.341	3.917
	26	1911-033	2.534	3.769	3.880
3rd batch		#3-1-28	2.343	3.734	3.934
	12	1911-019	2.755	3.788	3.891
	15	1911-022	3.393	3.947	3.962
	18	1911-025	1.856	3.271	3.878
	19	1911-026	2.291	3.740	3.909
	22	1911-029			
	25	1911-032	2.849	3.801	3.973

FIGURE 17B

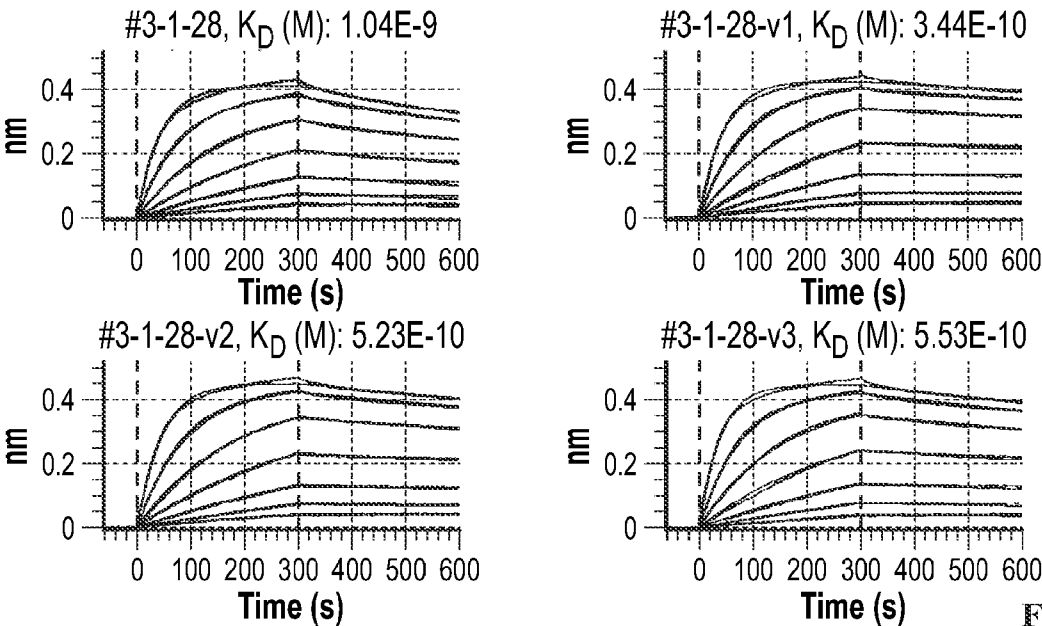


FIGURE 17C

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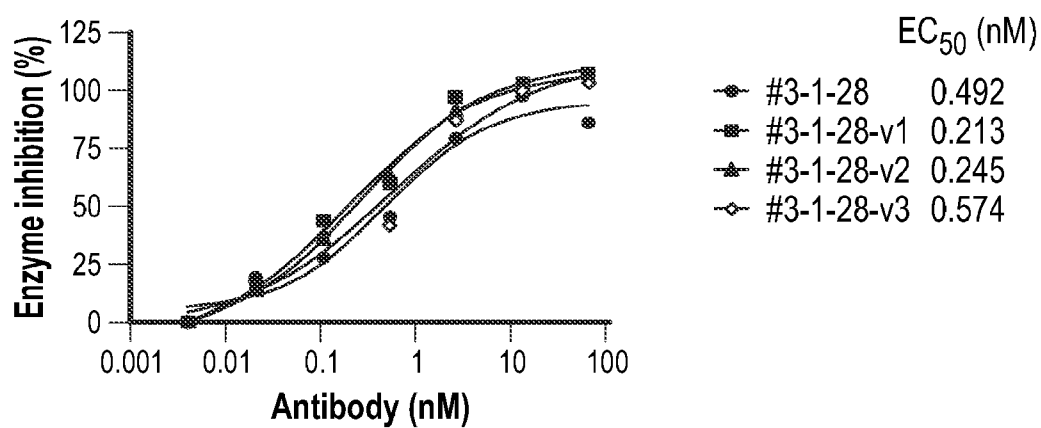


FIGURE 18A

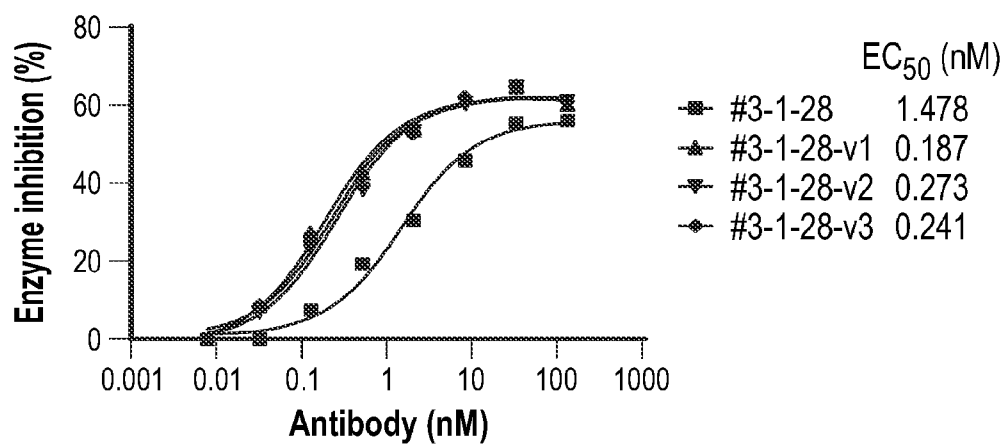


FIGURE 18B

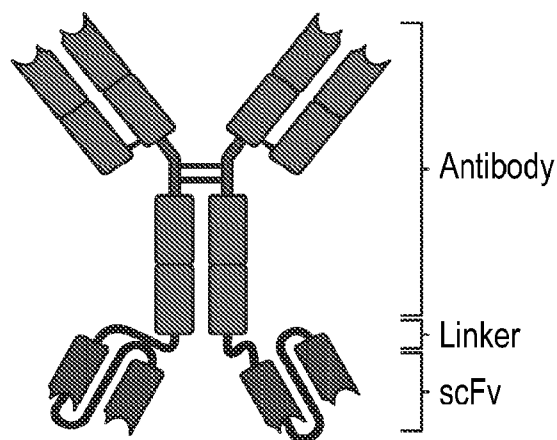


FIGURE 19

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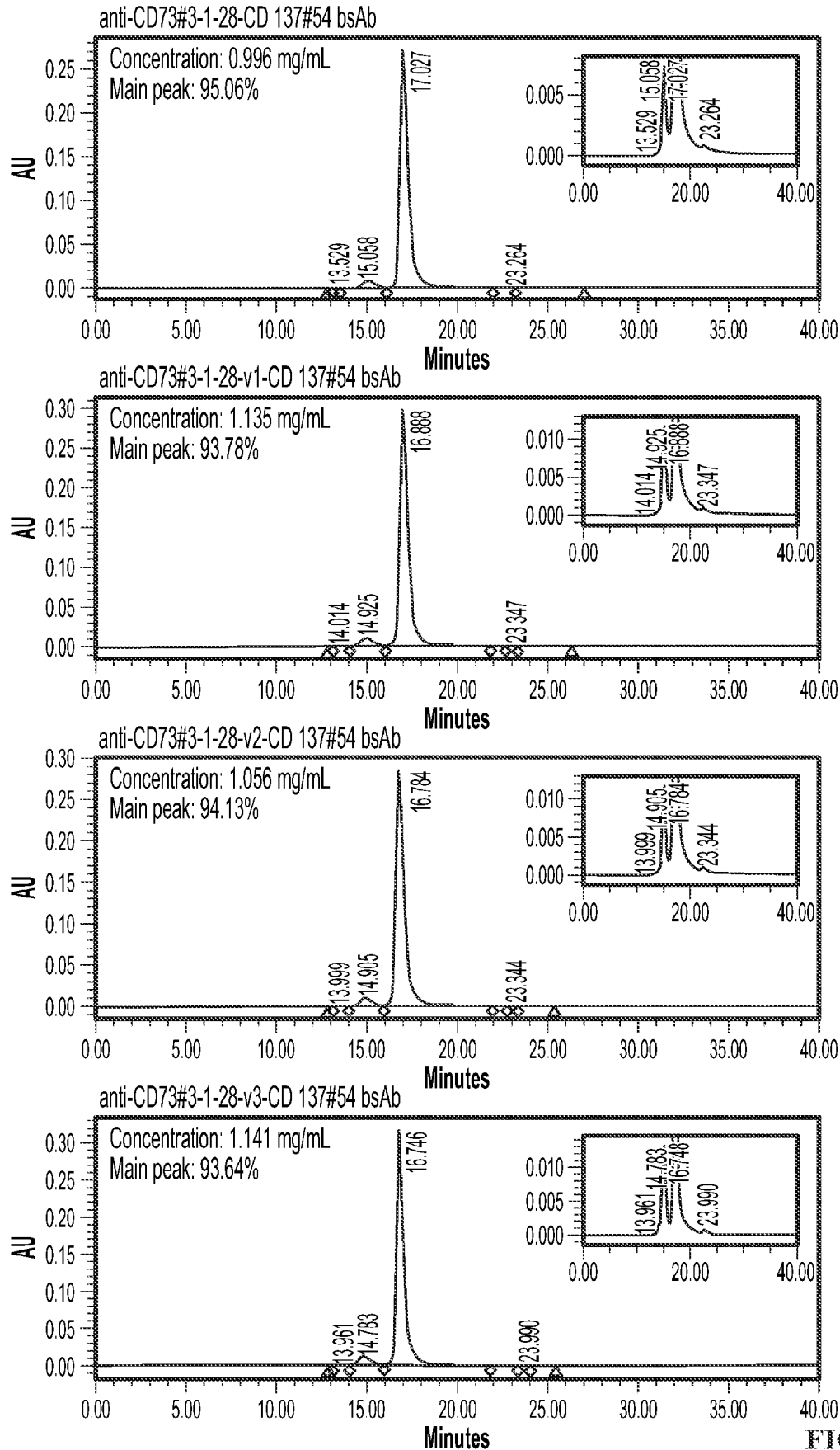


FIGURE 20A

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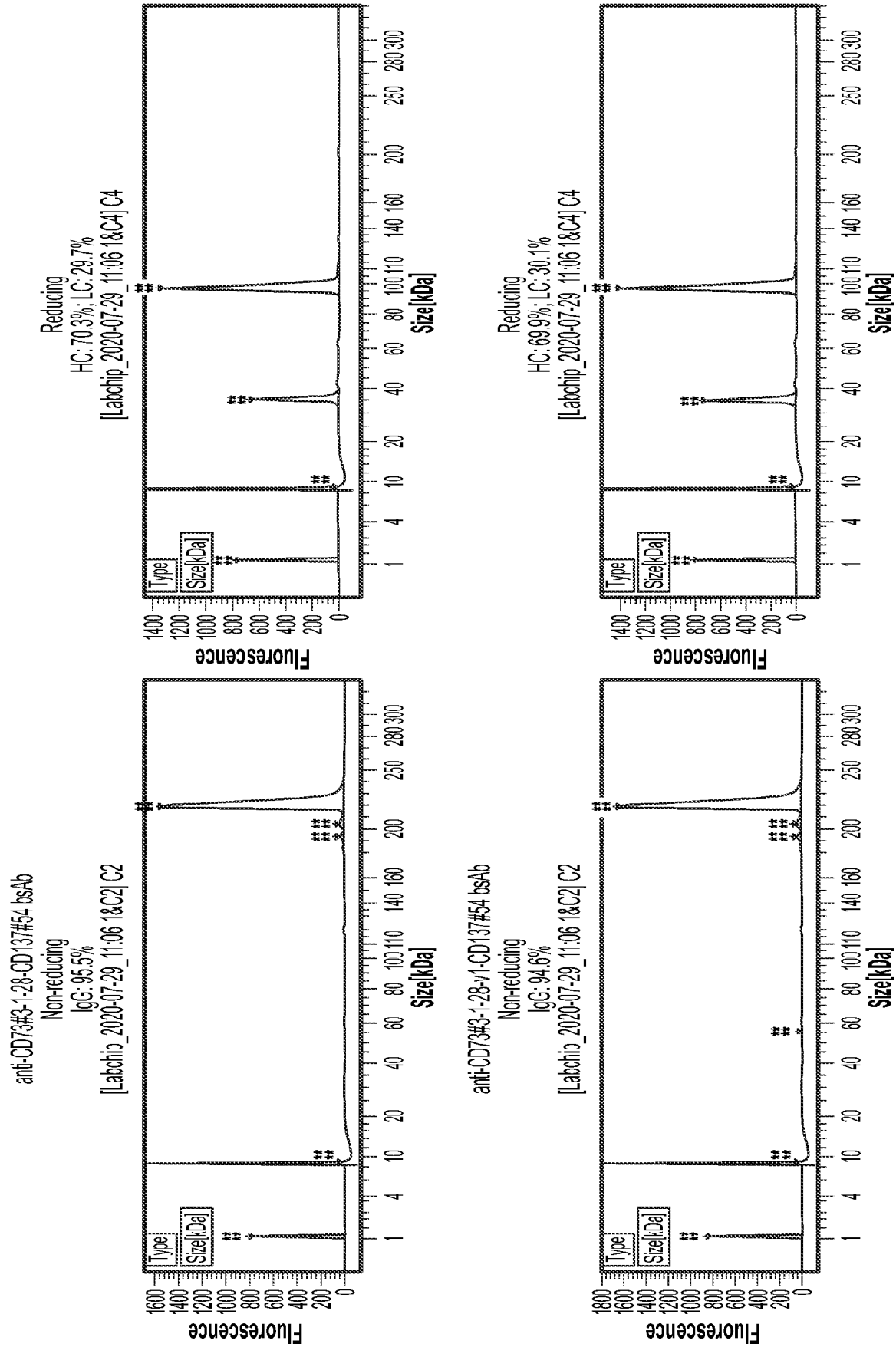


FIGURE 20B

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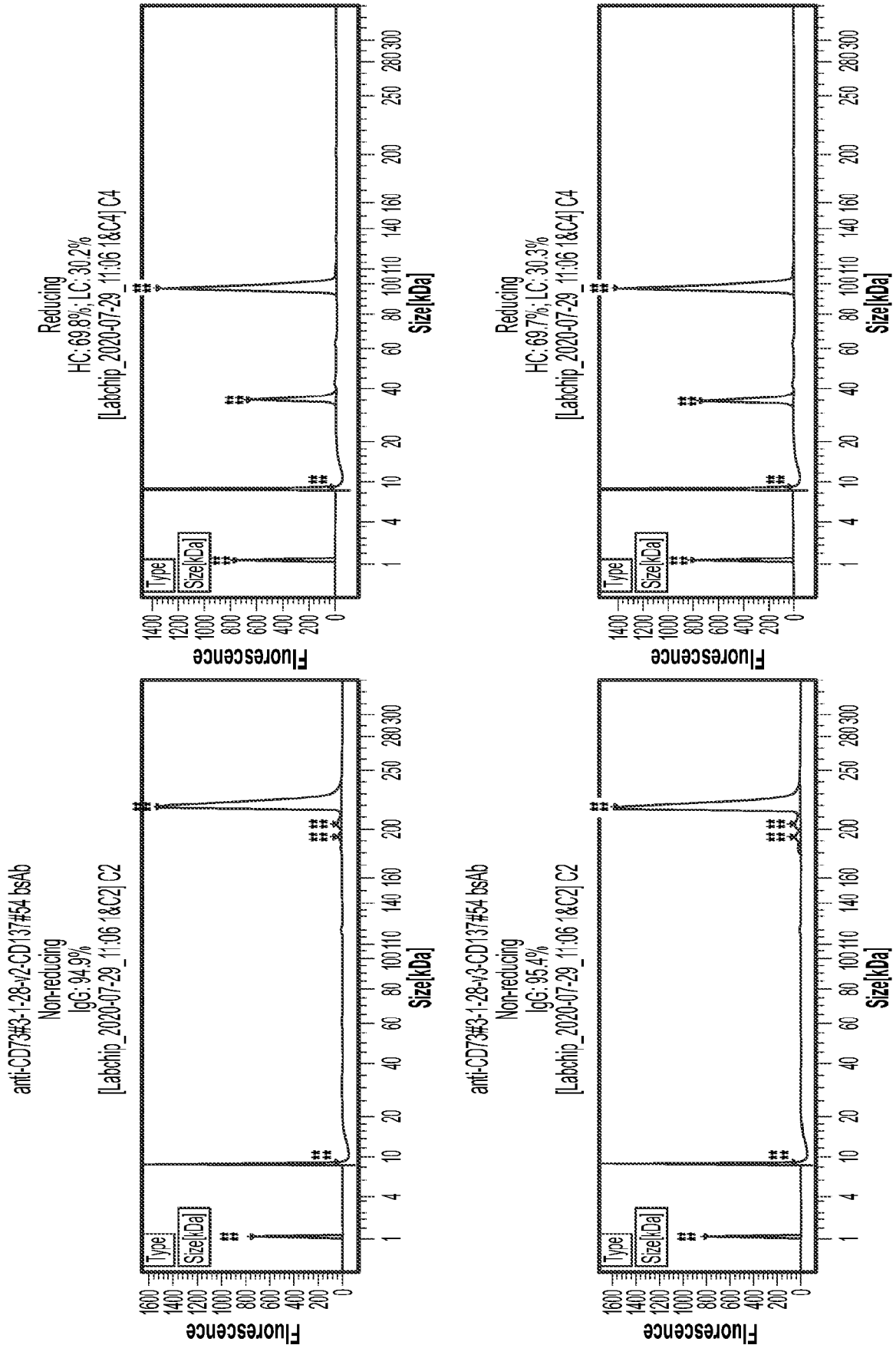


FIGURE 20B (Cont.)

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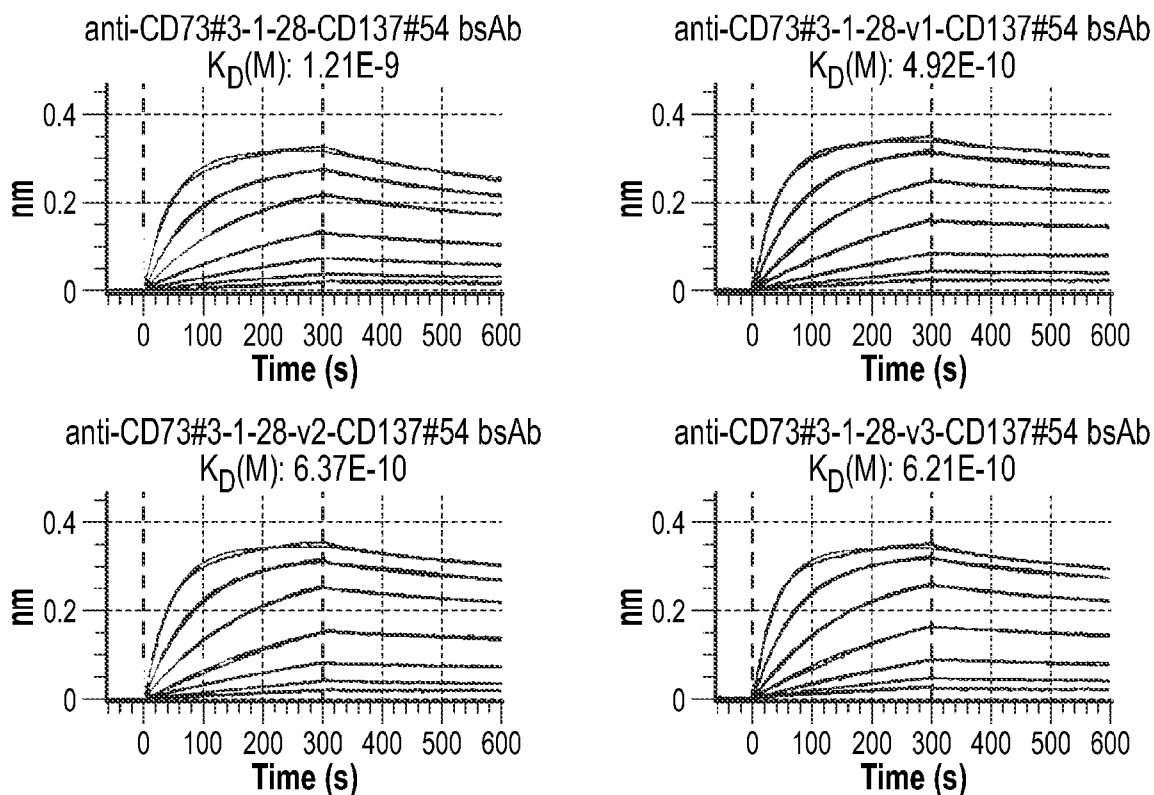


FIGURE 21A

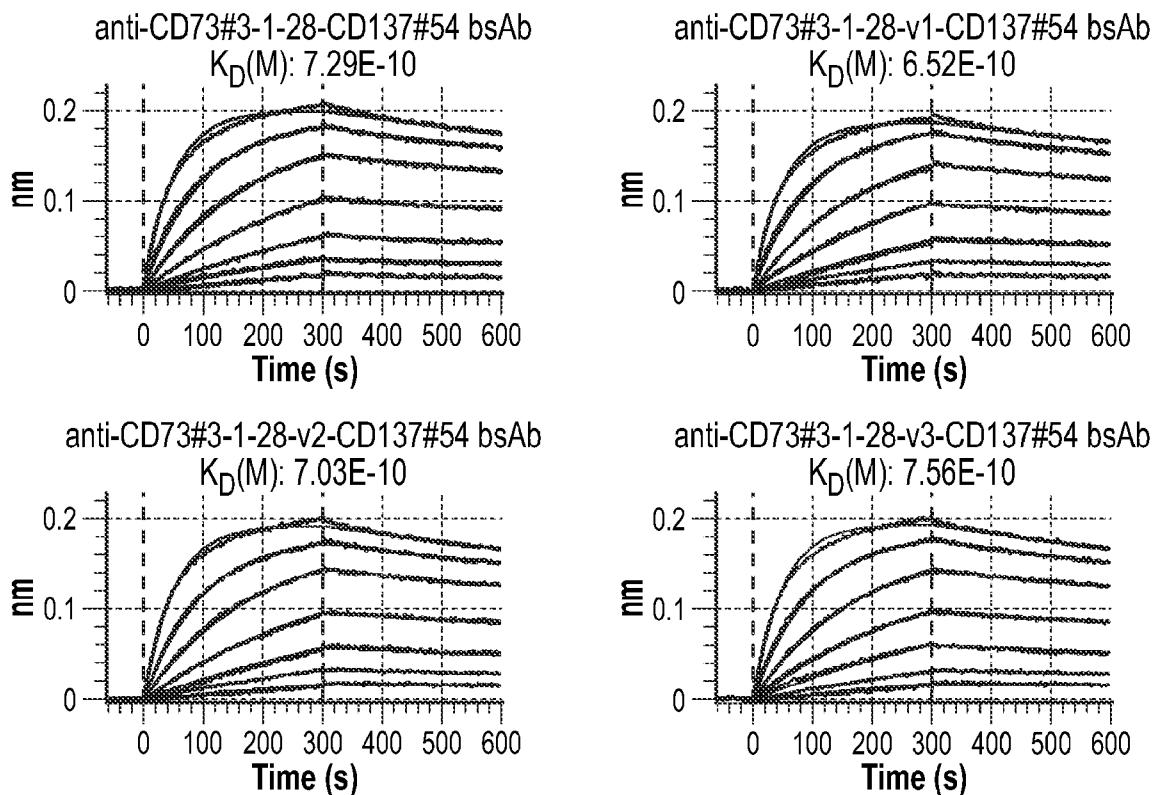


FIGURE 21B

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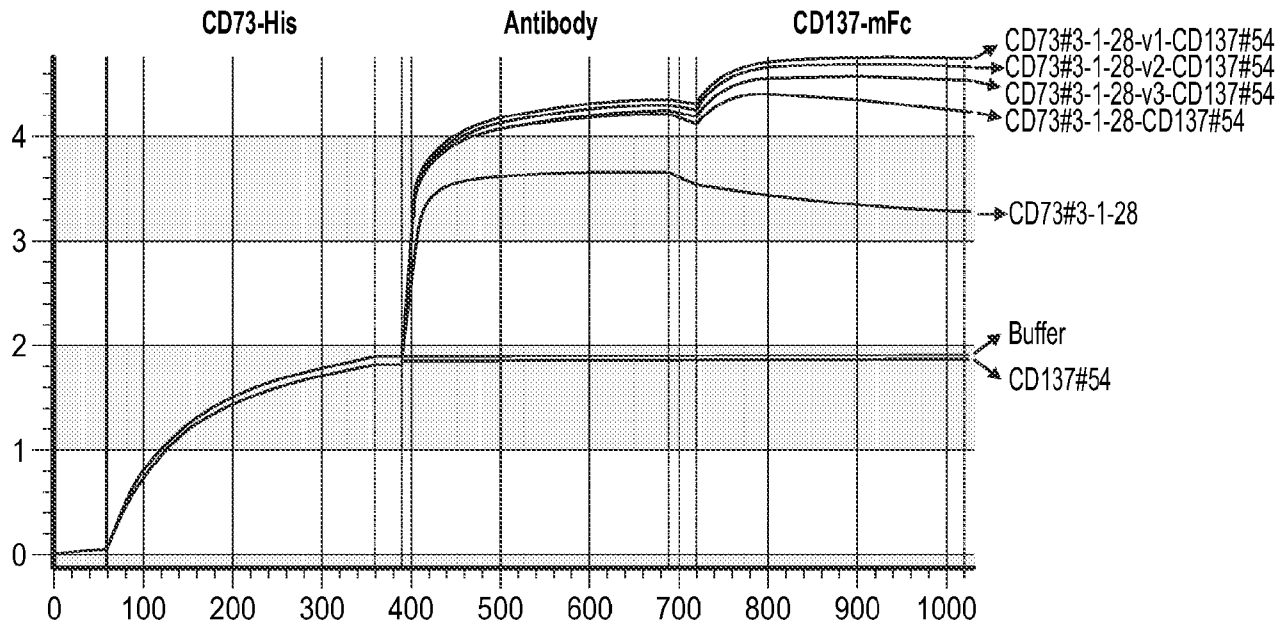


FIGURE 22

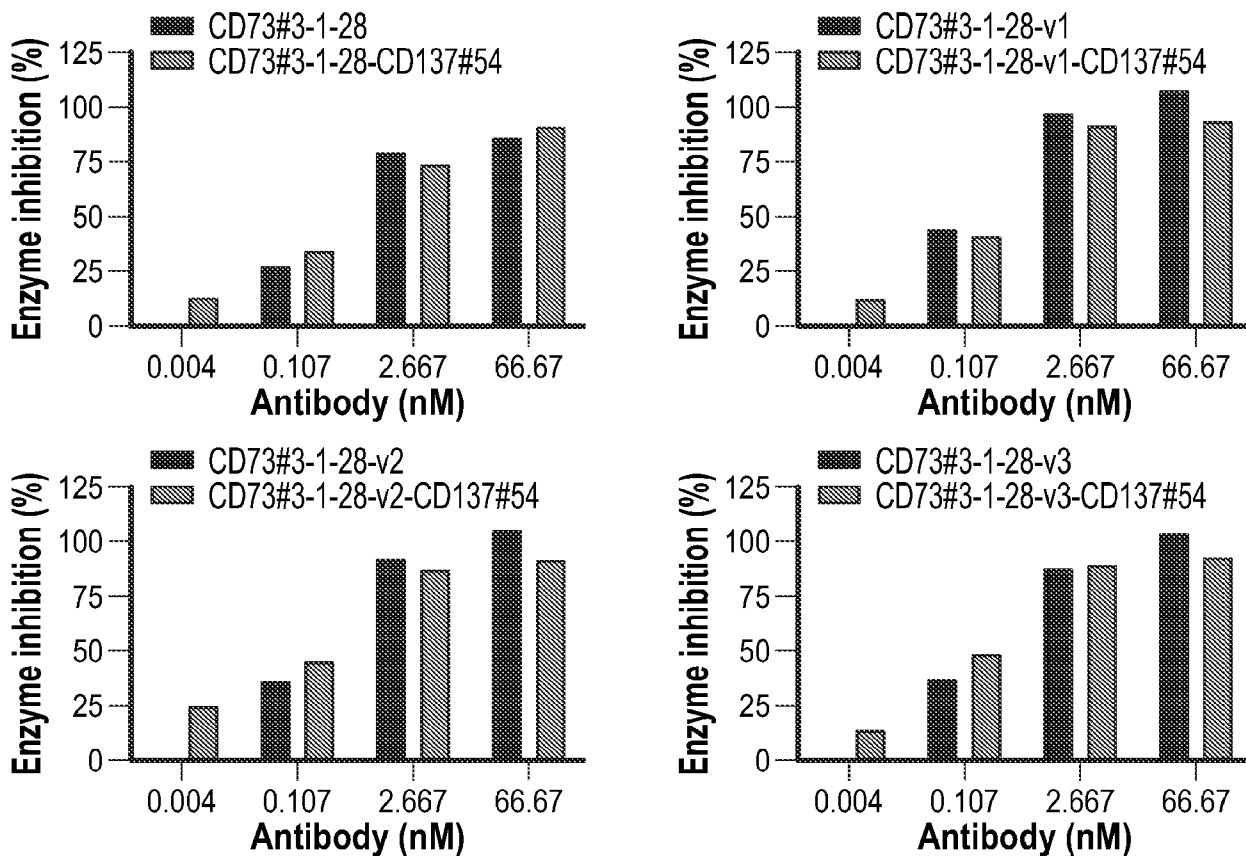


FIGURE 23

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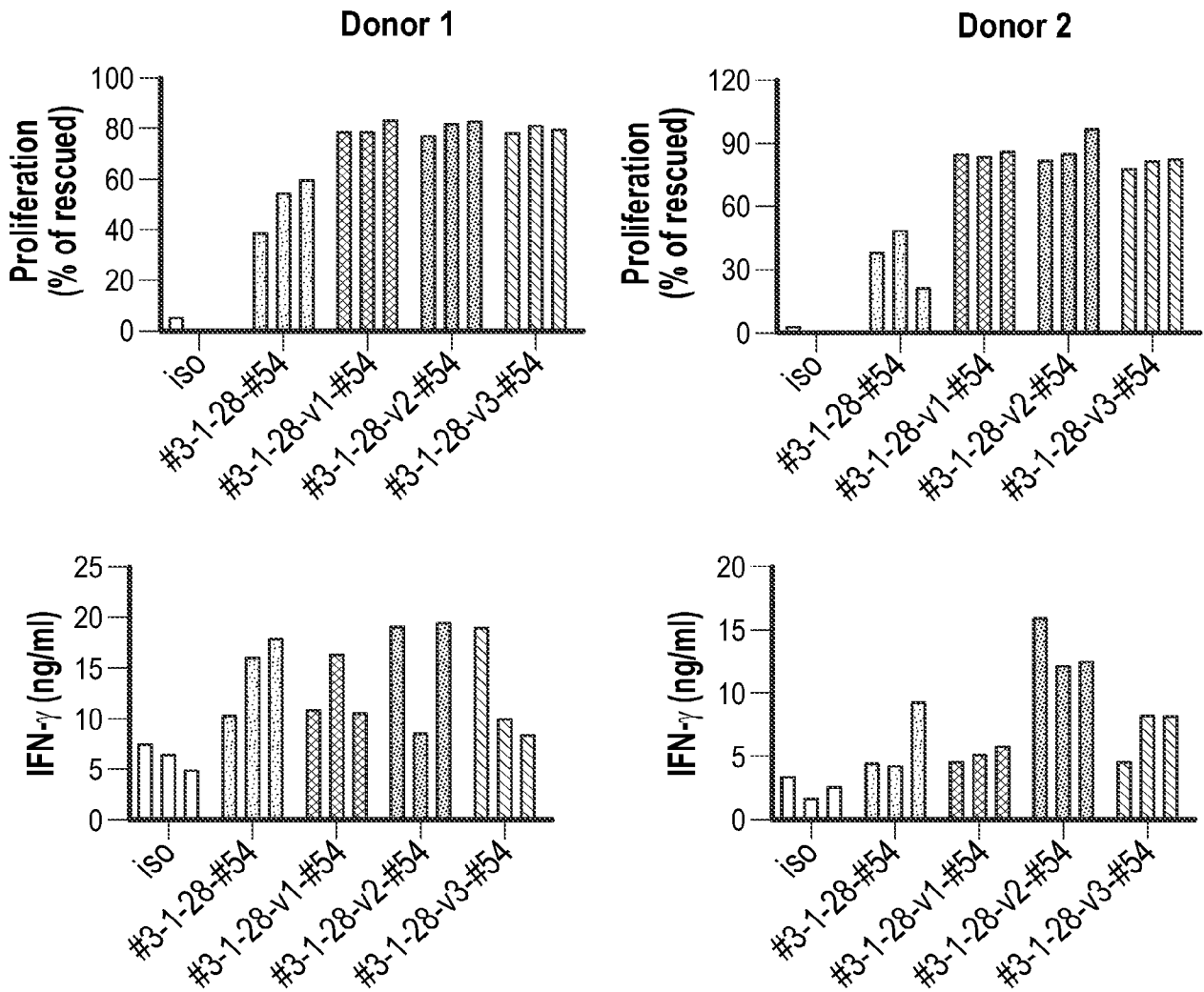


FIGURE 24

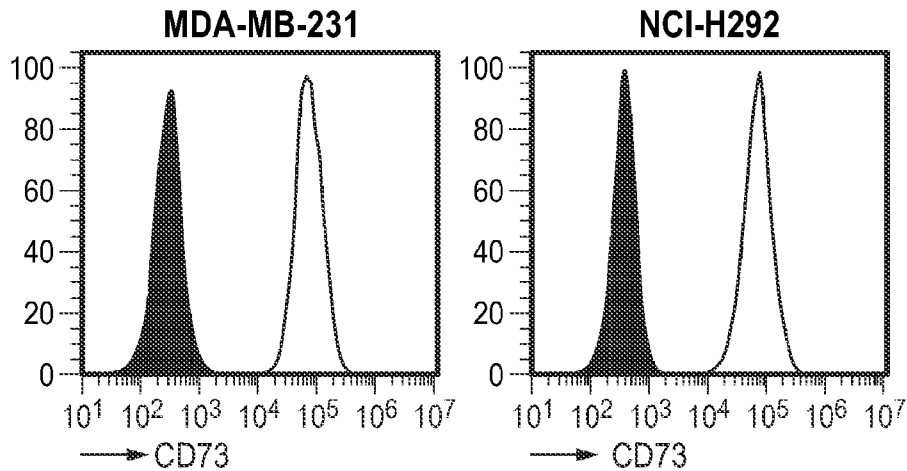


FIGURE 25A

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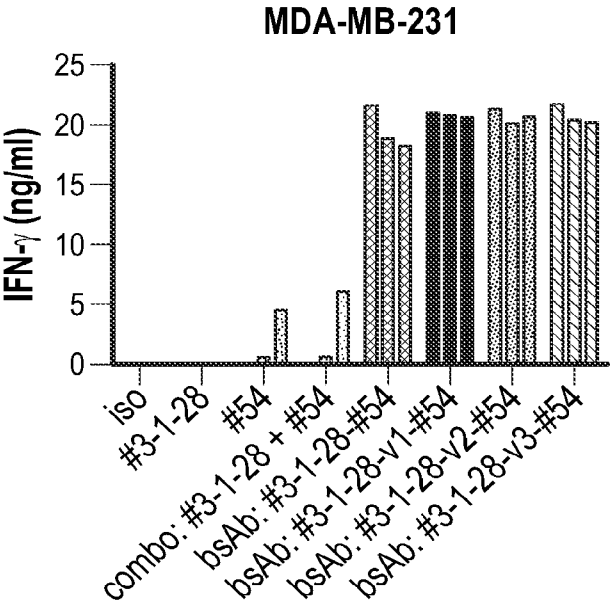


FIGURE 25B

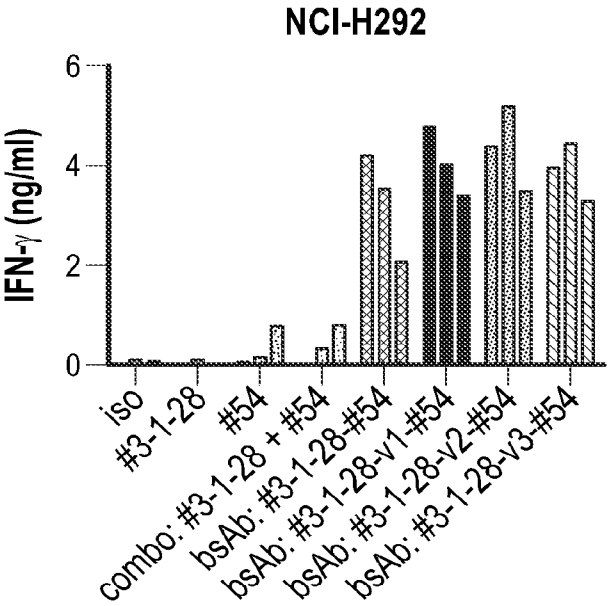


FIGURE 25C

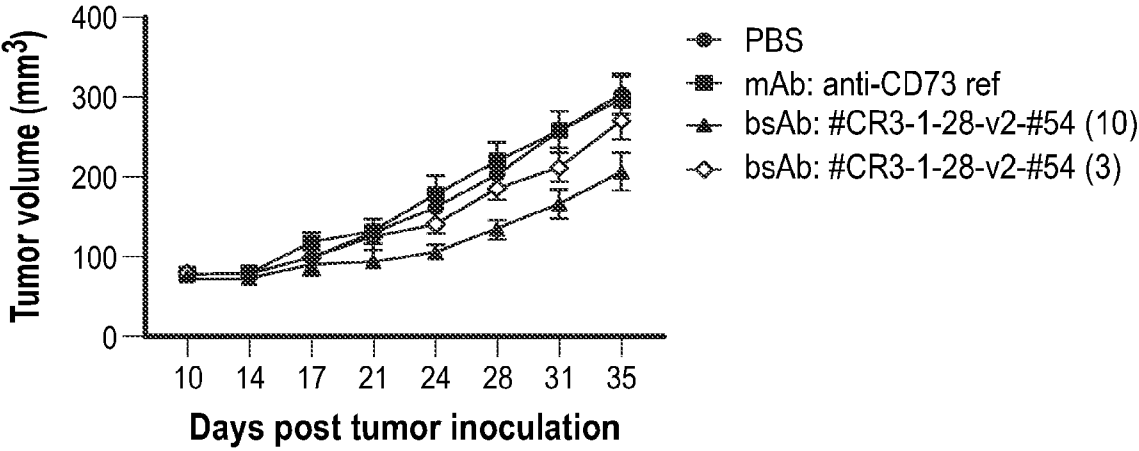


FIGURE 26A

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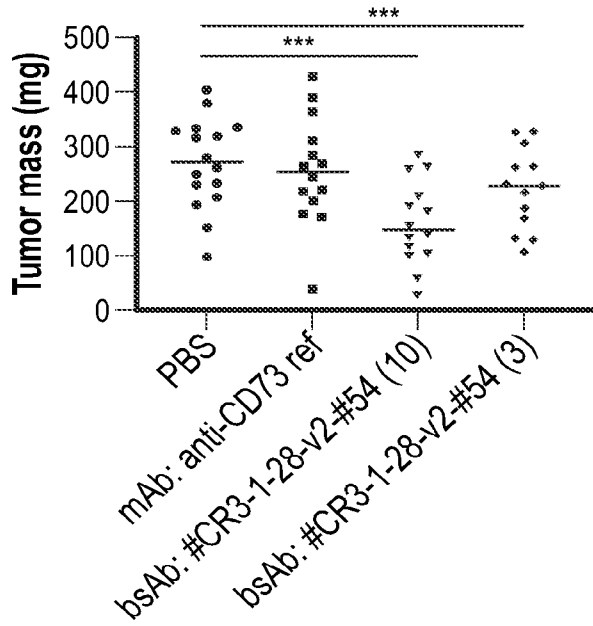


FIGURE 26B

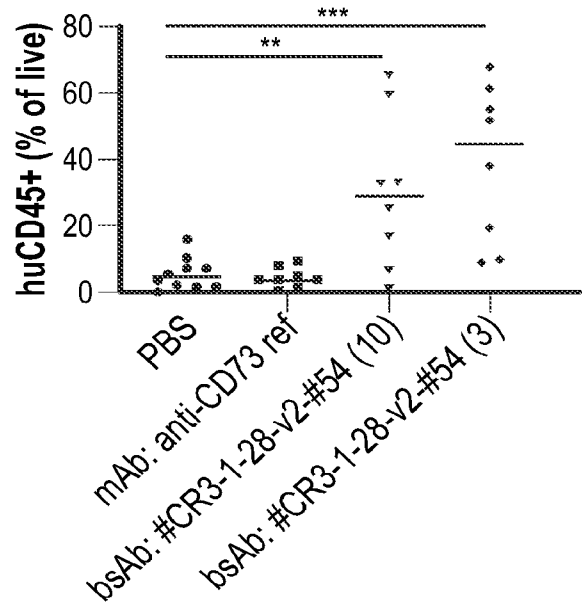


FIGURE 26C

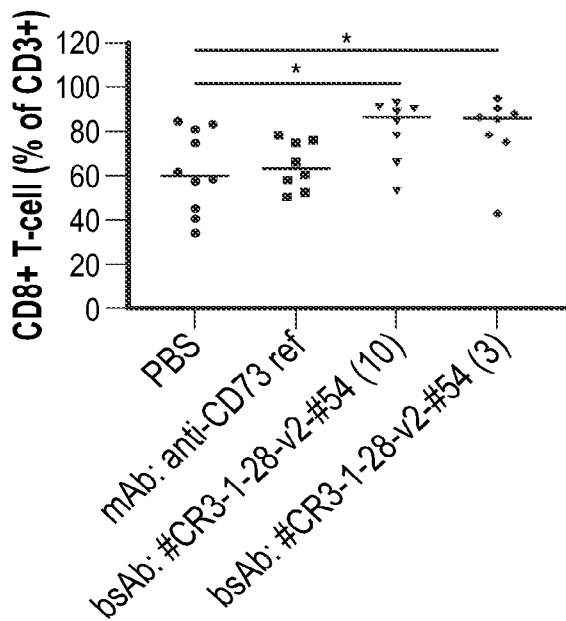


FIGURE 26D

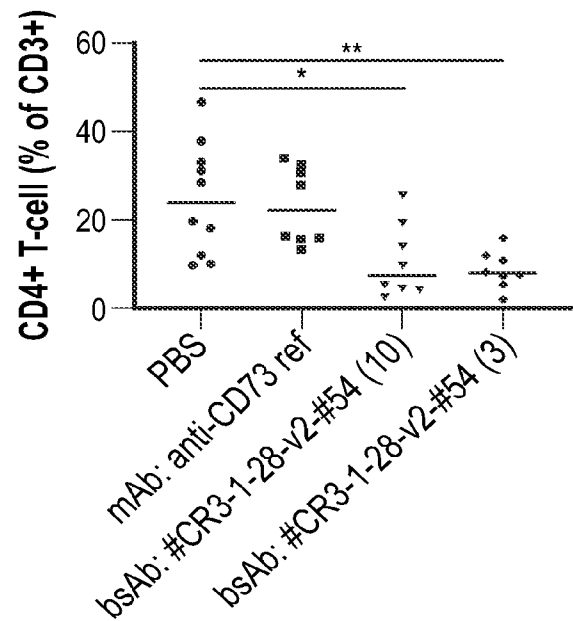
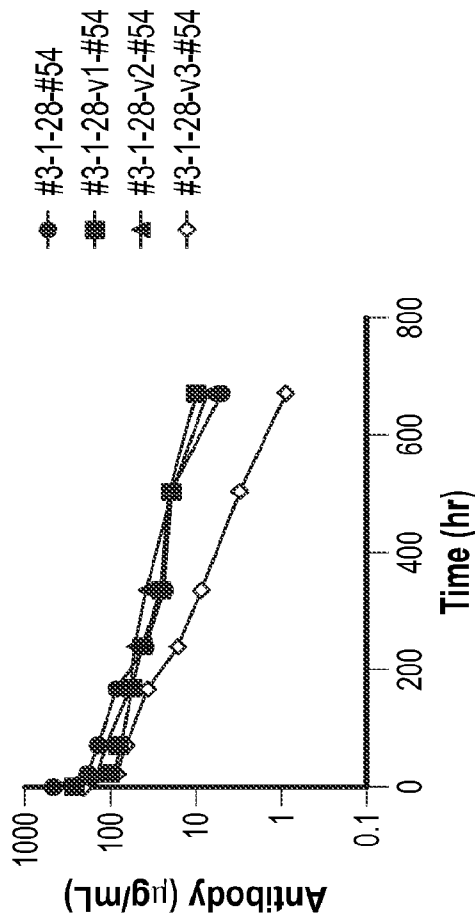


FIGURE 26E

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Parameter	Unit	AP2000+AP1919	AP2000+AP1919-003	AP2205+AP1919	AP2206+AP1919
Lambda_z	1/h	0.005260149	0.003342802	0.004362062	0.00670365
t1/2	h	131.7733067	207.3551107	158.9035673	103.3984697
Tmax	h	0.5	0.5	0.5	0.5
Cmax	µg/ml	471.72	258.1	256.06	206.547
C0	µg/ml	481.4150498	263.7697552	259.4161276	210.5499723
Clast_obs/Cmax		0.010663105	0.038550949	0.027805983	0.004212116
AUC 0-t	µg/ml*h	39494.42626	27808.78494	34115.06153	16438.38899
AUC 0-inf_obs	µg/ml*h	40450.673	30785.32933	35747.31718	16568.16903
AUC 0-t/0-inf_obs		0.976360177	0.903312894	0.954339073	0.992166905
AUMC 0-inf_obs	µg/ml*h^2	6679391.251	8443159.19	7697892.023	2051976.76
MRT 0-inf_obs	h	165.1243541	274.2591804	215.3418111	123.8505448
Vz_obs	(mg/kg)/(µg/ml)	0.02349883	0.048586485	0.032065258	0.045017787
Cl_obs	(mg/kg)/(µg/ml)/h	0.000123607	0.000162415	0.000139871	0.000301783
Vss_obs	(mg/kg)/(µg/ml)	0.020410582	0.044543811	0.030119996	0.037376051

FIGURE 27

Plate 1

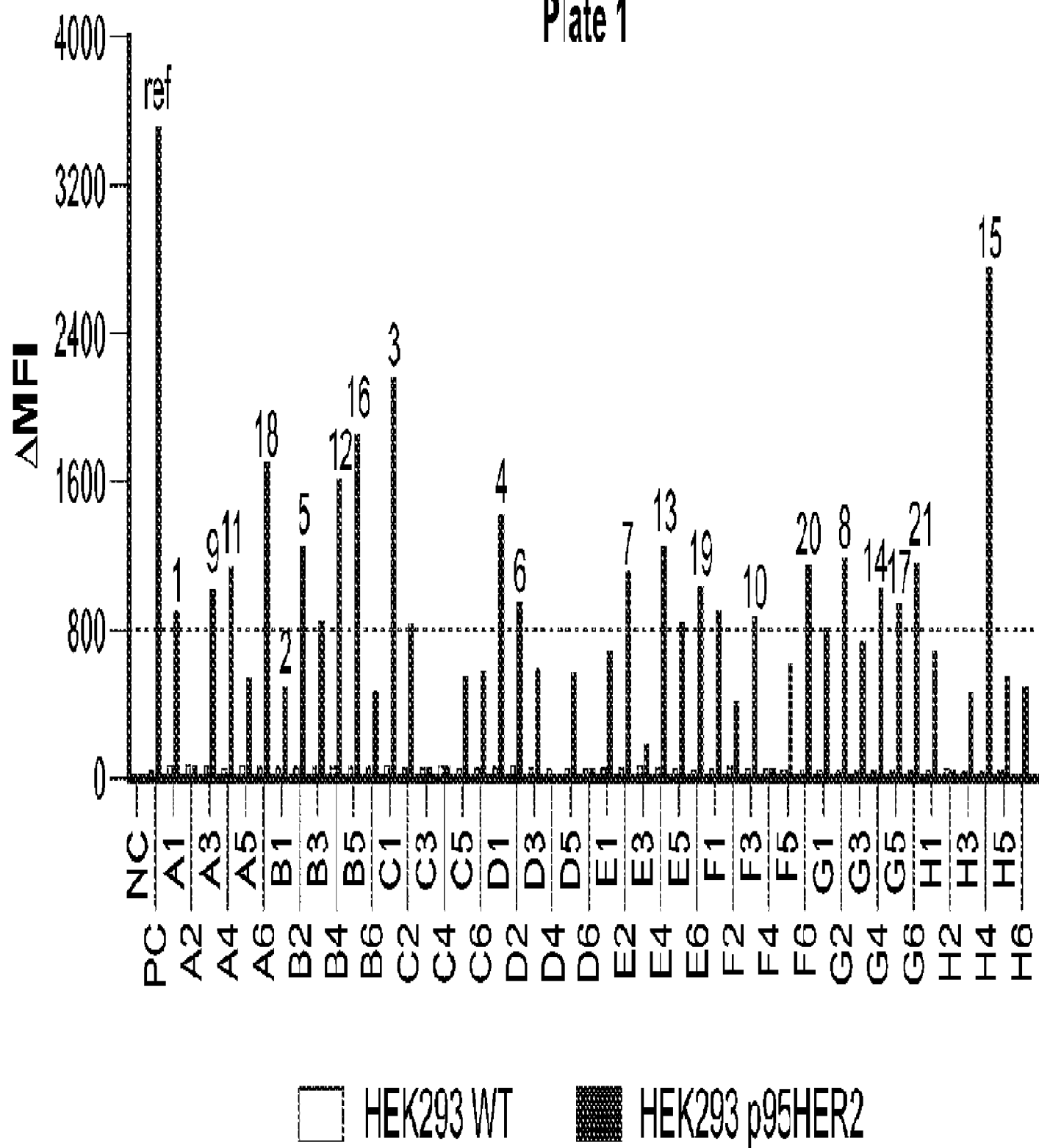


FIGURE 1A