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HUMANIZED ANTI-MUC1* ANTIBODIES

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention:

[0002] The present application relates to humanized anti-MUC1* antibodies and methods of making and using them.

[0003] 2. General Background and State of the Art:

[0004] We previously discovered that a cleaved form of the MUC1 (SEQ ID NO:1) transmembrane protein is a growth factor receptor that drives the growth of over 75% of all human cancers. The cleaved form of MUC1, which we called MUC1* (pronounced muk 1 star), is a powerful growth factor receptor. Cleavage and release of the bulk of the extracellular domain of MUC1 unmasks a binding site for activating ligands dimeric NME1, NME6 or NME7. It is an ideal target for cancer drugs as it is aberrantly expressed on over 75% of all cancers and is likely overexpressed on an even higher percentage of metastatic cancers (Fessler SP, Wotkowicz MT, Mahanta SK and Bamdad C. (2009). MUC1* is a determinant of trastuzumab (Herceptin) resistance in breast cancer cells. *Breast Cancer Res Treat.* 118(1):113-124). After MUC1 cleavage most of its extracellular domain is shed from the cell surface. The remaining portion has a truncated extracellular domain that at least comprises the primary growth factor receptor sequence, PSMGFR (SEQ ID NO:2).

[0005] Antibodies are increasingly used to treat human diseases. Antibodies generated in non-human species have historically been used as therapeutics in humans, such as horse antibodies. More recently, antibodies are engineered or selected so that they contain mostly human sequences in order to avoid a generalized rejection of the foreign antibody. The process of engineering recognition fragments of a non-human antibody into a human antibody is generally called 'humanizing'. The amount of non-human sequences that are used to replace the human antibody sequences determines whether they are called chimeric, humanized or fully human.

[0006] Alternative technologies exist that enable generation of humanized or fully human antibodies. These strategies involve screening libraries of human antibodies or antibody fragments and identifying those that bind to the target antigen, rather than immunizing an animal with the antigen. Another approach is to engineer the variable region(s) of an antibody into an antibody-like molecule. The present invention is intended to also encompass these approaches for use with recognition fragments of antibodies that the inventors have determined bind to the extracellular domain of MUC1*.

[0007] In addition to treating patients with an antibody, cancer immunotherapies have recently been shown to be effective in the treatment of cancers. T-cell based cancer immunotherapy is an attractive approach to overcome the cancer cells evasion from the immune system. A first immunotherapy, called CAR T (chimeric antigen receptor T cell) therapy relies on the expression of a CAR on the surface of the patient T cells for adoptive T-cell therapy (Dai H, Wang Y, Lu X, Han W. (2016) Chimeric Antigen Receptors Modified T-Cells for Cancer Therapy. J Natl Cancer Inst. 108(7): djv439). Such receptor is composed of an anti cancer scFv linked to a T cell transmembrane and signaling domains. Upon binding of the receptor to a cancer associated antigen, a signal is transmitted resulting in T-cell activation, propagation and the targeted killing of the cancer cells. In practice, a patient's T cells are isolated and transduced with a CAR, expanded and then injected back into the patient. When the patient's CAR T cells bind to the antigen on a cancer cell, the CAR T cells expand and attack the cancer cells. A drawback of this method is the risk of activating the patient's immune system to destroy cells bearing the target antigen, when most cancer antigens are expressed on some healthy tissues, but overexpressed on cancerous tissues. To minimize the risk of off-tumor/on-target effects, the cancer antigen should be minimally expressed on healthy tissues.

[0008] A second cancer immunotherapy involves BiTEs (Bi-specific T cell Engagers). The BiTE approach attempts to eliminate the CAR T associated risk of off-tumor/on-target effects. Unlike CAR T, BiTEs are bispecific antibodies that should not pose any greater risk than regular antibody-based therapies. However, unlike typical anti-cancer antibodies that bind to and block a cancer antigen, BiTEs are designed to bind to an antigen on the tumor cell and simultaneously bind to an antigen on an immune cell, such as a T cell. In this way, a BiTE recruits the T cell to the tumor. BiTEs are engineered proteins that simultaneously bind to a cancer associated antigen and a T-cell surface protein such as CD3-epsilon. BiTEs are antibodies made by genetically linking the scFv's of an antibody that binds to a T cell antigen, like anti-CD3-epsilon to a scFv of a therapeutic monoclonal antibody that binds to a cancer antigen (Patrick A. Baeuerle, and Carsten Reinhardt (2009) Bispecific T-cell engaging antibodies for cancer therapy. Cancer Res. 69(12):4941-4944).

SUMMARY OF THE INVENTION

[0009] In one aspect, the present invention is directed to a human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein that binds to a region on extracellular domain of MUC1 isoform or cleavage product that is devoid of the tandem repeat domains. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein may specifically bind to

- [0010] (i) PSMGFR region of MUC1;
- [0011] (ii) PSMGFR peptide;
- [0012] (iii)a peptide having amino acid sequence of SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620);
- [0013] (iv)a peptide having amino acid sequence of SVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621);
- [0014] (v) a peptide having amino acid sequence of VQLTLAFREGTINVHDTVETQFNQY (SEQ ID NO:622); or
- [0015] (vi)a peptide having amino acid sequence of SNIKFRPGSVVVQLTLAFREGTIN (SEQ ID NO:623).
- [0016] The human or humanized antibody may be IgG1, IgG2, IgG3, IgG4 or IgM. The human or humanized antibody fragment or antibody-like protein may be scFv or scFv-Fc.
- [0017] The human or humanized antibody, antibody fragment or antibody-like protein as in above may comprise a heavy chain variable region and light chain variable region which is derived from mouse monoclonal MN-E6 antibody, and has at least 80%, 90% or 95% or 98% sequence identity to the mouse monoclonal MN-E6 antibody. The heavy chain variable region may have at least 90% or 95% or 98% sequence identity to SEQ ID NO:13 and the light chain variable region may have at least 90% or 95% or 98% sequence identity to SEQ ID NO:66.
- [0018] The human or humanized antibody, antibody fragment or antibody-like protein according to above may include complementarity determining regions (CDRs) in the heavy chain variable region and light chain variable region having at least 90% or 95% or 98% sequence identity to CDR1, CDR2 or CDR3 regions having sequence as follows:
- [0019] CDR1 heavy chain SEQ ID NO:17
- [0020] CDR1 light chain SEQ ID NO:70,
- [0021] CDR2 heavy chain SEQ ID NO:21
- [0022] CDR2 light chain SEQ ID NO:74,
- [0023] CDR3 heavy chain SEQ ID NO:25
- [0024] CDR3 light chain SEQ ID NO:78.
- [0025] The human or humanized antibody, antibody fragment or antibody-like protein described above may include a heavy chain variable region and light chain variable region which is derived from mouse monoclonal MN-C2 antibody, and has at least 80%, 90% or 95% or 98% sequence identity to the mouse monoclonal MN-C2 antibody. The heavy chain variable region may have at least 90% or 95% or 98% sequence identity to SEQ ID NO:119 and the light chain variable region

has at least 90% or 95% or 98% sequence identity to SEQ ID NO:169. The complementarity determining regions (CDRs) in the heavy chain variable region and light chain variable region may have at least 90% or 95% or 98% sequence identity to CDR1, CDR2 or CDR3 regions having sequence as follows:

[0026] CDR1 heavy chain SEQ ID NO:123

[0027] CDR1 light chain SEQ ID NO:173,

[0028] CDR2 heavy chain SEQ ID NO:127

[0029] CDR2 light chain SEQ ID NO:177,

[0030] CDR3 heavy chain SEQ ID NO:131

[0031] CDR3 light chain SEQ ID NO:181.

[0032] The human or humanized antibody, antibody fragment or antibody-like protein as in above may include a heavy chain variable region and light chain variable region which is derived from mouse monoclonal MN-C3 antibody, and may have at least 80%, 90% or 95% or 98% sequence identity to the mouse monoclonal MN-C3 antibody. The heavy chain variable region may have at least 90% or 95% or 98% sequence identity to SEQ ID NO:414 and the light chain variable region may have at least 90% or 95% or 98% sequence identity to SEQ ID NO:459. The complementarity determining regions (CDRs) in the heavy chain variable region and light chain variable region may have at least 90% or 95% or 98% sequence identity to CDR1, CDR2 or CDR3 regions having sequence as follows:

[0033] CDR1 heavy chain SEQ ID NO:418

[0034] CDR1 light chain SEQ ID NO:463,

[0035] CDR2 heavy chain SEQ ID NO:422

[0036] CDR2 light chain SEQ ID NO:467,

[0037] CDR3 heavy chain SEQ ID NO:426,

[0038] CDR3 light chain SEQ ID NO:471.

[0039] The human or humanized antibody, antibody fragment or antibody-like protein described above may include a heavy chain variable region and light chain variable region which is derived from mouse monoclonal MN-C8 antibody, and has at least 80%, 90% or 95% or 98% sequence identity to the mouse monoclonal MN-C8 antibody. The heavy chain variable region may have at least 90% or 95% or 98% sequence identity to SEQ ID NO:506 and the light chain variable region may have at least 90% or 95% or 98% sequence identity to SEQ ID NO:544. The complementarity determining regions (CDRs) in the heavy chain variable region and light chain variable region may

have at least 90% or 95% or 98% sequence identity to CDR1, CDR2 or CDR3 regions having sequence as follows:

[0040] CDR1 heavy chain SEQ ID NO:508

[0041] CDR1 light chain SEQ ID NO:546,

[0042] CDR2 heavy chain SEQ ID NO:510

[0043] CDR2 light chain SEQ ID NO:548,

[0044] CDR3 heavy chain SEQ ID NO:512,

[0045] CDR3 light chain SEQ ID NO:550.

[0046] In another aspect, the present invention is directed to an anti-MUC1* extracellular domain antibody comprised of sequences of a humanized MN-E6 represented by humanized IgG2 heavy chain, or humanized IgG1 heavy chain, paired with humanized Kappa light chain, or humanized Lambda light chain. The humanized IgG2 heavy chain may be SEQ ID NOS:53, humanized IgG1 heavy chain may be SEQ ID NO:57, humanized Kappa light chain may be SEQ ID NO:108, and humanized Lambda light chain may be SEQ ID NO:112, or a sequence having 90%, 95% or 98% sequence identity thereof.

[0047] In another aspect, the invention is directed to an anti-MUC1* extracellular domain antibody comprised of sequences of a humanized MN-C2 represented by humanized IgG1 heavy chain, humanized IgG2 heavy chain, paired with humanized Lambda light chain, and humanized Kappa light chain. The humanized IgG1 heavy chain MN-C2 may be SEQ ID NOS:159 or IgG2 heavy chain may be SEQ ID NOS:164 paired with Lambda light chain (SEQ ID NO:219) or Kappa light chain (SEQ ID NO:213), or a sequence having 90%, 95% or 98% sequence identity thereof.

[0048] In another aspect, the invention is directed to an anti-MUC1* extracellular domain antibody comprised of sequences of a humanized MN-C3 represented by humanized IgG1 heavy chain or humanized IgG2 heavy chain paired with humanized Lambda light chain or humanized Kappa light chain. The humanized MN-C3 IgG1 heavy chain may be SEQ ID NOS:454, IgG2 heavy chain may be SEQ ID NOS:456, Lambda light chain may be SEQ ID NO:501, and Kappa light chain may be SEQ ID NO:503, or a sequence having 90%, 95% or 98% sequence identity thereof.

[0049] In another aspect, the invention is directed to an anti-MUC1* extracellular domain antibody comprised of sequences of a humanized MN-C8 represented by humanized IgG1 heavy chain or humanized IgG2 heavy chain paired with humanized Lambda light chain or humanized Kappa light chain. The humanized MN-C8 IgG1 heavy chain may be SEQ ID NOS:540, IgG2 heavy chain may be SEQ ID NOS:542, Lambda light chain may be SEQ ID NO:580 and Kappa light chain may be SEQ ID NO:582, or a sequence having 90%, 95% or 98% sequence identity thereof.

[0050] In another aspect, the invention is directed to a human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein according to above, which inhibits the binding of NME protein to MUC1*. The NME may be NME1, NME6, NME7AB, NME7 or NME8.

[0051] In yet another aspect, the invention is directed to a single chain variable fragment (scFv) comprising a heavy and light chain variable regions connected via a linker, further comprising CDRs of antibodies that bind to MUC1* extracellular domain. The CDRs may be derived from MN-E6, MN-C2, MN-C3 or MN-C8 antibodies or humanized antibodies thereof. The scFv may be one that possesses the SEQ ID NOS:233, 235 and 237 (E6); SEQ ID NOS:239, 241, and 243 (C2); SEQ ID NOS:245, 247, and 249 (C3); or SEQ ID NOS:251, 253, and 255 (C8).

[0052] In still another aspect, the invention is directed to a chimeric antigen receptor (CAR) comprising a scFv or a humanized variable region that binds to the extracellular domain of a MUC1 that is devoid of tandem repeats, a linker molecule, a transmembrane domain and a cytoplasmic domain. The single chain antibody fragment may bind to

[0053] (i) PSMGFR region of MUC1,

[0054] (ii) PSMGFR peptide,

[0055] (iii) a peptide having amino acid sequence SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620);

[0056] (iv) a peptide having amino acid sequence of SVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621);

[0057] (v) a peptide having amino acid sequence of VQLTLAFREGTINVHDTVETQFNQY (SEQ ID NO:622); or

[0058] (vi) a peptide having amino acid sequence of SNIKFRPGSVVVQLTLAFREGTIN (SEQ ID NO:623).

[0059] In the CAR as describe above, portions of any of the variable regions set forth and described above, or combination thereof may be used in the extracellular domain, a transmembrane region and a cytoplasmic tail that comprises sequence motifs that signal immune system activation. The extracellular domain may be comprised of humanized single chain antibody fragments of an MN-E6 scFv, MN-C2 scFv, MN-C3 scFv or MN-C8 scFv.

[0060] In the CAR as described above, the extracellular domain include humanized single chain antibody fragments of an MN-E6 scFv set forth as SEQ ID NOS: 233, 235, or 237), MN-C2 scFv (SEQ ID NOS:239, 241, or 243), MN-C3 scFv (SEQ ID NOS: 245, 247, or 249) or MN-C8 scFv (SEQ ID NOS:251, 253, or 255).

[0061] In any of the CAR described above, the cytoplasmic tail may be comprised of one or more of signaling sequence motifs CD3-zeta, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICAM-1, LFA-1, ICOS, CD2, CD5, or CD7.

[0062] In any of the CAR described above, the sequence may be CARMN-E6 CD3z (SEQ ID NOS:295), CARMN-E6 CD28/CD3z (SEQ ID NOS:298); CARMN-E6 4-1BB/CD3z (SEQ ID NOS:301); CARMN-E6 OX40/CD3z (SEQ ID NOS:617); CARMN-E6 CD28/4-1BB/CD3z (SEQ ID NOS:304); CARMN-E6 CD28/OX40/CD3z (SEQ ID NOS:619); CAR MN-C2 CD3z (SEQ ID NOS:607); CAR MN-C2 CD28/CD3z (SEQ ID NOS:609); CAR MN-C2 4-1BB/CD3z (SEQ ID NOS:611); CAR MN-C2 OX40/CD3z (SEQ ID NOS:613); CAR MN-C2 CD28/4-1BB/CD3z (SEQ ID NOS:307); or CAR MN-C2 CD28/OX40/CD3z (SEQ ID NOS:615).

[0063] In another aspect, the CAR may have an extracellular domain unit that recognizes a peptide. The peptide may be PSMGFR (SEQ ID NO:2). The peptide may be a peptide derived from NME7. The peptide may be

[0064] NME7A peptide 1 (A domain): MLSRKEALDFHVDHQS (SEQ ID NO:7);

[0065] NME7A peptide 2 (A domain): SGVARTDASES (SEQ ID NO:8);

[0066] NME7B peptide 1 (B domain): DAGFEISAMQMFNMDRVNVE (SEQ ID NO:9);

[0067] NME7B peptide 2 (B domain): EVYKGVVTEYHDMVTE (SEQ ID NO:10); or

[0068] NME7B peptide 3 (B domain): AIFGKTKIQNAVHCTDLPEDGLLEVQYFF (SEQ ID NO:11).

[0069] In another aspect, the invention is directed a composition that includes at least two CARs with different extracellular domain units transfected into the same cell.

[0070] The at least two CARs may have one CAR that does not have a targeting recognition unit and the other CAR does have a targeting recognition unit. Or, one of the extracellular domain recognition units may bind to MUC1* extracellular domain. Or, one of the extracellular domain recognition units may bind PD-1. Or, one of the extracellular domain recognition units is an antibody fragment and the other is a peptide. Or, one is an anti-MUC1* scFv chosen from the group consisting of scFv of MN-E6 antibody, scFv of MN-C2 antibody, scFv of MN-C3 antibody or scFv of MN-C8 antibody and the other is a peptide derived from NME7 or chosen from the group consisting of

[0071] NME7A peptide 1 (A domain): MLSRKEALDFHVDHQS (SEQ ID NO:7);

[0072] NME7A peptide 2 (A domain): SGVARTDASES (SEQ ID NO:8);

[0073] NME7B peptide 1 (B domain): DAGFEISAMQMFNMDRVNVE (SEQ ID NO:9);

[0074] NME7B peptide 2 (B domain): EVYKGVVTEYHDMVTE (SEQ ID NO:10); and

[0075] NME7B peptide 3 (B domain): AIFGKTKIQNAVHCTDLPEDGLLEVQYFF (SEQ ID NO:11).

[0076] In another aspect, the invention is directed to a cell comprising a CAR with an extracellular domain that binds to MUC1* transfected or transduced cell. The cell that includes the CAR may be an immune system cell, preferably a T cell or dendritic cell or mast cell.

[0077] In another aspect, the invention is directed to an engineered antibody-like protein.

[0078] In another aspect, the invention is directed to a method of screening a library of antibodies or antibody fragments that are human, for those that bind to

[0079] (i) PSMGFR peptide;

[0080] (ii) a peptide having amino acid sequence SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620);

[0081] (iii) a peptide having amino acid sequence of SVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621);

[0082] (iv) a peptide having amino acid sequence of VQLTLAFREGTINVHDTVETQFNQY (SEQ ID NO:622);

[0083] (v) a peptide having amino acid sequence of SNIKFRPGSVVVQLTLAFREGTIN (SEQ ID NO:623);

[0084] (vi) NME7 protein; or

[0085] (vii) a peptide fragment of NME7 protein.

[0086] In another aspect, the invention is directed to a method for treating a disease in a subject comprising administering an antibody according to any claim above, to a person suffering from the disease, wherein the subject expresses MUC1 aberrantly. The disease may be cancer, such as breast cancer, lung cancer, colon cancer, gastric cancer.

[0087] In another aspect, the invention is directed to a method for treating a disease in a subject comprising administering an NME peptide, to a person suffering from the disease, wherein the subject expresses MUC1 aberrantly.

[0088] In another aspect, the invention is directed to a method of proliferating or expanding stem cell population comprising contacting the cells with the antibody according to any method or composition described above.

[0089] In another aspect, the invention is directed to a method of facilitating stem cell attachment to a surface comprising coating the surface with a humanized MN-C3 or MN-C8 antibody, antibody fragment or single chain antibody thereof and contacting stem cell to the surface.

[0090] In another aspect, the invention is directed to a method of delivering stem cell *in vitro* or *in vivo* comprising the steps of coating a surface with a humanized MN-C3 or MN-C8 antibody, antibody fragment or single chain antibody thereof, contacting the stem cell to the surface and delivering the stem cell to a specific location.

[0091] In another aspect, the invention is directed to a method of isolating stem cell comprising the steps of coating a surface with a humanized MN-C3 or MN-C8 antibody, antibody fragment or single chain antibody thereof, and contacting a mixed population of cells to the surface and isolating stem cell.

[0092] In another aspect, the invention is directed to a scFv comprising variable domain fragments derived from an antibody that binds to a extracellular domain of MUC1 isoform or cleavage product that is devoid of the tandem repeat domains. The variable domain fragments may be derived from mouse monoclonal antibody MN-E6 (SEQ ID NO:13 and 66) or from the humanized MN-E6 (SEQ ID NO: 39 and 94), or from MN-E6 scFv (SEQ ID NO: 233, 235 and 237). Or, the variable domain fragments may be derived from mouse monoclonal antibody MN-C2 (SEQ ID NO: 119 and 169) or from the humanized MN-C2 (SEQ ID NO: 145 and 195), or from MN-C2 scFv (SEQ ID NO: 239, 241 and 243). Or, the variable domain fragments may be derived from mouse monoclonal antibody MN-C3 (SEQ ID NO: 414 and 459) or from the humanized MN-C3 (SEQ ID NO: 440 and 487), or from MN-C3 scFv (SEQ ID NO: 245, 247 and 249). Or, the variable domain fragments may be derived from mouse monoclonal antibody MN-C8 (SEQ ID NO: 505 and 544) or from the humanized MN-C8 (SEQ ID NO: 526 and 566), or from MN-C8 scFv (SEQ ID NO: 251, 253, 255).

[0093] In another aspect, the invention is directed to a method for the treatment of a person diagnosed with, suspected of having or at risk of developing a MUC1 Or MUC1* positive cancer involving administering to the person an effective amount of the scFv described above.

[0094] In another aspect, the invention is directed to a scFv-Fc construct comprising the scFv as described above. The scFv-Fc may be dimerized. Or, the Fc component may be mutated so that scFv-Fc is monomeric. The mutation may include mutating or deleting hinge region on Fc, making F405Q, Y407R, T366W/L368W, and T364R/L368R mutation or combinations thereof on the Fc represented by SEQ ID NO: 281, 279, 285 and 287.

[0095] In another aspect, the invention is directed to a polypeptide comprising at least two different scFv sequences, wherein one of the scFv sequences is a sequence that binds to extracellular domain of MUC1 isoform or cleavage product that is devoid of the tandem repeat domains. The polypeptide may bind to

[0096] (i) PSMGFR region of MUC1;

[0097] (ii) PSMGFR peptide;

[0098] (iii) peptide having amino acid sequence of SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620);

[0099] (iv) a peptide having amino acid sequence of VQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621);

[00100] (v) a peptide having amino acid sequence of VQLTLAFREGTINVHDTVETQFNQY (SEQ ID NO:622); or

[00101] (vi) a peptide having amino acid sequence of SNIKFRPGSVVVQLTLAFREGTIN (SEQ ID NO:623).

[00102] The polypeptide may bind to a receptor on an immune cell, such as T cell, and in particular, CD3 on T-cell.

[00103] In another aspect, the invention is directed to a method of detecting presence of a cell that expresses MUC1* aberrantly, comprising contacting a sample of cells with the scFv-Fc described above and detecting for the presence of the binding of scFv-Fc to the cell. The cell may be cancer cell.

[00104] In another aspect, the invention is directed to a method for testing a subject's cancer for suitability of treatment with a composition comprising portions of the variable regions of MN-E6, MN-C2, MN-C3 or MN-C8, comprising the steps of contacting a bodily specimen from the patient with the corresponding MN-E6 scFv-Fc, MN-C3 scFv-Fc, MN-C3 scFv-Fc or MN-C8 scFv-Fc.

[00105] In another aspect, the invention is directed to a method of treating a subject suffering from a disease comprising, exposing T cells from the subject to MUC1* peptides wherein through various rounds of maturation, T cells develop MUC1* specific receptors, creating adapted T cells, and expanding and administering the adapted T cells to the donor patient who is diagnosed with, suspected of having, or is at risk of developing a MUC1* positive cancer.

[00106] These and other objects of the invention will be more fully understood from the following description of the invention, the referenced drawings attached hereto and the claims appended hereto.

BRIEF DESCRIPTION OF THE DRAWINGS

[00107] The present invention will become more fully understood from the detailed description given herein below, and the accompanying drawings which are given by way of illustration only, and thus are not limitative of the present invention, and wherein;

[00108] **Figure 1 A-D** show cell growth assay graphs of MUC1* positive cells treated with either bivalent 'bv' anti-MUC1* antibody, monovalent 'mv' or Fab, NM23-H1 dimers or NME7-AB. Bivalent anti-MUC1* antibodies stimulate growth of cancer cells whereas the monovalent Fab inhibits growth (A, B). Classic bell-shaped curve indicates ligand induced dimerization stimulates growth. Dimeric NM23-H1, aka NME1, stimulates growth of MUC1* positive cancer cells but siRNA to suppress MUC1 expression eliminate its effect (C). NME7-AB also stimulates the growth of MUC1* positive cells (D).

[00109] **Figure 2 A-F** show results of ELISA assays. MUC1* peptides PSMGFR, PSMGFR minus 10 amino acids from the N-terminus aka N-10, or PSMGFR minus 10 amino acids from the C-terminus, aka C-10 are immobilized on the plate and the following are assayed for binding: NME7-AB (A), MN-C2 monoclonal antibody (B), MN-E6 monoclonal antibody (C), or dimeric NME1 (D). These assays show that NME1, NME7-AB and monoclonal antibodies MN-C2 and MN-E6 all require the first membrane proximal 10 amino acids of the MUC1* extracellular domain to bind. MUC1* peptides PSMGFR minus 10 amino acids from the N-terminus aka N-10, or PSMGFR minus 10 amino acids from the C-terminus, aka C-10, are immobilized on the plate and the following are assayed for binding: MN-C3 (E) and MN-C8 (F).

[00110] **Figure 3 A-C** show results of competitive ELISA assays. The PSMGFR MUC1* peptide is immobilized on the plate and dimeric NM23-H1, aka NME1, is added either alone or after the MN-E6 antibody has been added (A). The same experiment was performed wherein NM23-H7, NME7-AB, is added alone or after MN-E6 has been added (B). Results show that MN-E6 competitively inhibits the binding of MUC1* activating ligands NME1 and NME7. In a similar experiment (C), PSMGFR or PSMGFR minus 10 amino acids from the N-terminus, aka N-10, is immobilized on the plate. Dimeric NM23-H1 is then added. Anti-MUC1* antibodies MN-E6, MN-C2, MN-C3 or MN-C8 are then tested for their ability to compete off the NM23-H1. Results show that although all three antibodies bind to the PSMGFR peptides, MN-E6 and MN-C2 competitively inhibit binding of the MUC1* activating ligands.

[00111] **Figure 4 A-F** shows FACS scans of anti-MUC1* antibodies binding specifically to MUC1* positive cancer cells and MUC1* transfected cells but not MUC1* or MUC1 negative cells. ZR-75-1, aka 1500, MUC1* positive breast cancer cells were stained with 1:2 or 1:10 dilutions of the 1.5 ug/ml humanized MN-C2. After two washes, cells were stained with secondary antibody, Anti-Penta-His antibody at conjugated to Alexa 488 (Qiagen) dilutions of 1:200 (A), 1:50 (B), or 1:10 (C) to detect the 6x His tag on the huMN-C2 scFv. Flow cytometric analysis revealed a concentration-dependent shift of a subset of cells, indicating specific binding, which is unseen in the

absence of the MN-C2 scFv (A-C). In another case, MN-E6 was used to stain MUC1 negative HCT-116 colon cancer cells transfected with the empty vector, single cell clone #8 (D), HCT-116 colon cancer cells transfected with MUC1* single cell clone #10 (E), or ZR-75-1, aka 1500, MUC1* positive breast cancer cells. As the FACS scans show, both MN-C2 and MN-E6 only stain MUC1* positive cells and not MUC1 or MUC1* negative cells.

[00112] Figure 5 shows a graph of an ELISA in which surface is coated with either the MUC1* PSMGFR peptide or a control peptide. Humanized MN-C2 scFv is then incubated with the surface, washed and detected according to standard methods. The ELISA shows that the huMN-C2 scFv binds to the MUC1* peptide with an EC-50 of about 333nM.

[00113] Figure 6 A-B shows graphs of cancer cell growth inhibition by MUC1* antibody variable region fragment humanized MN-C2 scFv. hMN-C2 scFv potently inhibited the growth of ZR-75-1, aka 1500, MUC1* positive breast cancer cells (A) and T47D MUC1* positive breast cancer cells (B) with approximately the same EC-50 as the *in vitro* ELISAs.

[00114] Figure 7 A-B shows graphs of tumor growth in immune compromised mice that have been implanted with human tumors then treated with anti-MUC1* antibody MN-E6 Fab or mock treatment. Female nu/nu mice implanted with 90-day estrogen pellets were implanted with 6 million T47D human breast cancer cells that had been mixed 50/50 with Matrigel. Mice bearing tumors that were at least 150 mm³ and had three successive increases in tumor volume were selected for treatment. Animals were injected sub cutaneously twice per week with 80 mg/kg MN-E6 Fab and an equal number of mice fitting the same selection criteria were injected with vehicle alone (A). Male NOD/SCID mice were implanted with 6 million DU-145 human prostate cancer cells that had been mixed 50/50 with Matrigel. Mice bearing tumors that were at least 150 mm³ and had three successive increases in tumor volume were selected for treatment. Animals were injected sub-cutaneously every 48 hours with 160 mg/kg MN-E6 Fab and an equal number of mice fitting the same selection criteria were injected with vehicle alone (B). Tumors were measured independently by two researchers twice per week and recorded. Statistics were blindly calculated by independent statistician, giving a P value of 0.0001 for each. Anti-MUC1* Fab inhibited breast cancer growth and prostate cancer growth. Treatment had no effect on weight, bone marrow cell type or number.

[00115] Figure 8 is a graph of an ELISA assay showing differing levels of expression of humanizedMN-E6 anti-MUC1* antibody depending on whether the light chain was kappa or lambda and whether the variable portion was fused to a human IgG1 or IgG2.

[00116] **Figure 9** is a graph of an ELISA assay comparing the binding of the parent mouse MN-E6 antibody to the humanized versions of the MN-E6 antibody to a surface presenting the PSMGFR peptide derived from the MUC1* extracellular domain.

[00117] **Figure 10** is a graph of an ELISA assay showing differing levels of expression of humanized MN-C2 anti-MUC1* antibody depending on whether the light chain was kappa or lambda and whether the variable portion was fused to a human IgG1 or IgG2.

[00118] **Figure 11** is a graph of an ELISA assay comparing the binding of the parent mouse MN-C2 antibody to the humanized versions of the MN-C2 antibody to a surface presenting the PSMGFR peptide derived from the MUC1* extracellular domain.

[00119] **Figure 12** is a graph of an ELISA assay showing binding of humanized single chain (scFv) MN-C2 and MN-E6 antibodies binding to a surface presenting the PSMGFR peptide derived from the MUC1* extracellular domain.

[00120] **Figure 13** A-C shows FPLC traces of the purification of MN-E6 scFv-Fc fusion protein that was grown in low IgG FBS over a Protein A affinity column. A) is the trace of the flow through. B) is the trace of the elution. C) shows the purified protein on a reducing or non-reducing gel.

[00121] **Figure 14** A-B shows photographs of SDS-PAGE characterization of purified MN-E6 scFv-Fc fusion proteins on a non-reducing gels, wherein the Fc portion that was fused to the MN-E6 was either wild type (wt) or mutated as follows: A) F405Q, Y407R, T394D; B) T366W/L368W, T364R/L368R, T366W/L368W or T364R/L368R. Fc mutants F405Q, Y407R, T366W/L368W, T364R/L368R, T366W/L368W and T364R/L368R all favored monomer over dimer formation. The reference construct amino acid sequence for the indicated mutations is SEQ ID NO:273.

[00122] **Figure 15** A-B shows FPLC traces of the purification of MN-E6 scFv-Fc Y407Q fusion protein that was grown in low IgG FBS over a Protein A affinity column. A) is the trace of the flow through. B) is the trace of the elution. The protein was further purified by size exclusion over an S200 column (C). (D) is a photograph of an SDS-PAGE gel showing which fractions had a predominance of monomer. The reference construct amino acid sequence for the indicated mutations is SEQ ID NO:273.

[00123] **Figure 16** shows a photograph of SDS-PAGE characterization of purified MN-E6 scFv-Fc-mutant fusion proteins on a non-reducing gel, wherein the Fc portion that was fused to the MN-E6 scFv was either wild type (wt) or mutated by elimination of the hinge region, 'DHinge', of the Fc or elimination of the hinge region of the Fc and also bearing the Y407R mutation. All the Fc mutants favored monomer over dimer formation. The reference construct amino acid sequence for the indicated mutations is SEQ ID NO:273.

[00124] Figure 17 A-C. A and B show photograph of non-reducing SDS-PAGE characterization of large scale expression and purification of MN-E6 scFv-Fc hingeless mutant, showing that it is a monomer. FPLC characterization and purification of MN-E6 scFv-Fc hingeless mutant is shown (C).

[00125] Figure 18 A-C shows photographs of the SDS-PAGE characterization of the purified MN-C3 scFv-Fc fusion protein on a non-reducing gel (A) or a reducing gel (B). The protein was purified by size exclusion. The FPLC trace is shown (C).

[00126] Figure 19 A-B shows photographs of Native gels of MN-C3 or MN-E6 Fabs, scFv, scFv-Fc, wherein the Fc portion is wild type or mutants that prefer or are exclusively monomers. Native gels show that the Y407R Fc mutation (A) and the double mutant Y407R and a deleted hinge (B) favor monomer over dimer the best. Note that proteins are loaded onto a gel at much higher concentrations than typical use concentrations. The dimer formation of other Fc mutants may only reflect the fact that loading concentration is very high.

[00127] Figure 20 shows a graph of an ELISA wherein the surface was immobilized with either PSMGFR peptide, PSMGFR minus 10 amino acids from the N-terminus or minus 10 amino acids from the C-terminus. The hu MN-E6 scFv-Fc bound to the PSMGFR peptide and to the PSMGFR N-10 peptide but not to the PSMGFR C-10 peptide. The parent MN-E6 antibody and the humanized MN-E6 require the C-terminal 10 amino acids of PSMGFR for binding.

[00128] Figure 21 A-B shows an ELISA graph of several anti-MUC1* scFv-Fc fusion proteins wherein the Fc region has been eliminated or mutated. Shown are hu MN-E6 scFv-Fc-wt, hu MN-E6 scFv-Fc-Y407R, hu MN-E6 scFv-Fc-hingeless, and hu MN-E6 scFv-Fc- Y407R-hingeless. All mutants bind to the PSMGFR peptide of the MUC1* extracellular domain (A). An ELISA graph of several anti-MUC1* scFv-Fc fusion proteins wherein the Fc region is either wild type or mutated. Shown are hu MN-E6 scFv-Fc-wt, hu MN-E6 scFv-Fc-hingeless, and hu MN-C3 scFv-Fc is shown (B). All bind to the PSMGFR peptide of the MUC1* extracellular domain.

[00129] Figure 22 A-C shows graphs of ELISAs wherein the assay plate surface was immobilized with either PSMGFR peptide, PSMGFR minus 10 amino acids from the N-terminus or minus 10 amino acids from the C-terminus. The MN-C3 antibody variants were then assayed for binding to the various MUC1* peptides. A) Purified mouse monoclonal MN-C3 antibody; B) Impure humanized MN-C3 antibody; and C) the humanized MN-C3 scFv-Fc. ELISAs show binding to the PSMGFR peptide as well as to certain deletion peptides.

[00130] Figure 23 shows a graph of an ELISA assay that quantifies the binding of humanized MN-E6 scFv-Fc-delta hinge, aka Dhinge or hingeless, and humanized MN-E6 scFv to the MUC1* peptide PSMGFR.

[00131] **Figure 24** shows photographs of immunofluorescence experiments in which humanized MN-C2 scFv or MN-E6 scFv specifically binds to MUC1* positive breast cancer cells in an identical concentration dependent manner. A-G: hu MN-C2 scFv binding to T47D breast cancer cells at concentrations indicated. H-N shows the fluorescently labeled scFv and DAPI. O-U: hu MN-E6 scFv binding to T47D breast cancer cells at concentrations indicated. V-B' shows the fluorescently labeled scFv and DAPI. C' is the secondary antibody control.

[00132] **Figure 25 A-L** shows photographs of 1500 MUC1* positive breast cancer cells that have been cultured in normal medium or in the presence of humanized MN-E6 scFv. A-D are bright field images taken at 4X magnification. E-H are calcein fluorescent images taken at 4X magnification. I-L are calcein fluorescent images taken at 10X magnification. A,E, I show control cells cultured in normal RPMI medium. B,F, J show control cells cultured in normal RPMI medium plus a volume of PBS equal to the volume of MN-E6 scFv in PBS that is added to experimental wells. C, G, K show cells cultured in normal RPMI medium plus 500 ug/mL MN-E6 scFv. D, H, L show cells cultured in normal RPMI medium plus 5 ug/mL MN-E6 scFv. The photographs show killing and/or growth inhibition of MUC1* positive cells by MN-E6 scFv at 5 ug/mL and an even greater effect at 500 ug/mL. Images were taken at 96 hours post addition of test molecule.

[00133] **Figure 26 A-L** shows photographs of 1500 MUC1* positive breast cancer cells that have been cultured in normal medium or in the presence of humanized MN-E6 scFv-Fc Dhinge, which is a hingeless or delta hinge mutant. A-F are bright field images taken at 20X magnification. G-L are calcein fluorescent images taken at 4X magnification. A, G show control cells cultured in normal RPMI medium. B, H show cells cultured in normal RPMI medium plus 100 ug/mL hMN-E6 scFv-Fc Dhinge. C, I show cells cultured in normal RPMI medium plus 50 ug/mL hMN-E6 scFv-Fc Dhinge. D, J show cells cultured in normal RPMI medium plus 5 ug/mL hMN-E6 scFv-Fc Dhinge. E, K show cells cultured in normal RPMI medium plus 0.5 ug/mL hMN-E6 scFv-Fc Dhinge. F, L show cells cultured in normal RPMI medium plus 500 ug/mL of MN-E6 Fab. The photographs show killing and/or growth inhibition of MUC1* positive cells by hMN-E6 scFv-Fc Dhinge 5 ug/mL, an even greater effect at 50 ug/mL and yet an even greater effect at 100 ug/mL. Comparing cell morphology to the control cells, cancer cells grown in MN-E6 Fab or in an effective amount of hMN-E6 scFv-Fc Dhinge, show rounding up of the cells which morphology change occurs before cell death. Images were taken at 96 hours post addition of test molecule.

[00134] **Figure 27** shows a graph of the image analysis of the fluorescent images of Figures 25 and 26. Image J was used to quantify the number of cells remaining after 96 hours treatment in humanized MN-E6scFv or MN-E6 scFv-Fc-delta hinge, aka Dhinge. The analysis software uses

pixel counting and pixel fluorescence intensity to quantify the number of cells in each photograph. Analysis was performed over the entire image 512X512 pixels, 8-bit image. For comparison, the inhibition of mouse monoclonal MN-E6 Fab is also analyzed.

[00135] **Figure 28 A-C** show schematics of CAR sequence components.

[00136] **Figure 29** is a graph of an experiment measuring IL-2 cytokine secretion by Jurkat T cells that were transduced with a panel of CARs, including MN-E6-CD8-3z, MN-E6-CD8-CD28-3z, MN-E6-CD8-41BB-3z, MN-E6-CD4-CD28-3z and MN-E6-CD4-CD28-41BB-3z, when the CAR T cells were exposed to K562-wt cells or K562 cells that had been transfected with MUC1*.

[00137] **Figure 30** is a graph of an experiment measuring IL-2 cytokine secretion by Jurkat T cells that were transduced with a panel of CARs, including MN-E6-CD8-CD28-3z, MN-E6-CD8-41BB-3z, MN-E6-CD4-CD28-3z and MN-E6-CD4-41BB-3z, when the CAR T cells were exposed to K562-wt cells or K562 cells that had been transfected with MUC1*.

[00138] **Figure 31** is a graph of an experiment measuring IL-2 cytokine secretion by primary human T cells, isolated from a blood sample, that were transduced with a panel of CARs, including MN-E6-CD8-CD28-3z, MN-E6-CD8-41BB-3z and MN-E6-CD4-41BB-3z, when the CAR T cells were exposed to K562-wt cells or K562 cells that had been transfected with MUC1*.

[00139] **Figure 32** is a graph of an experiment measuring interferon-gamma (IFN-g) cytokine secretion by primary human T cells, isolated from a blood sample, that were transduced with a panel of CARs, including MN-E6-CD8-CD28-3z and MN-E6-CD4-41BB-3z, when the CAR T cells were exposed to K562-wt cells or K562 cells that had been transfected with MUC1*.

[00140] **Figure 33** is a graph of an experiment measuring interferon-gamma (IFN-g) cytokine secretion by primary human T cells, isolated from a blood sample, that were transduced with a panel of CARs, including MN-E6-CD8-CD28-3z, MN-E6-CD8-41BB-3z and MN-E6-CD8-CD28-41BB-3z, when the CAR T cells were exposed to K562-wt cells, K562 cells that had been transfected with MUC1*, or MUC1* positive cancer cells of prostate cancer, breast cancer or pancreatic cancer.

[00141] **Figure 34** is a graph of an experiment measuring target cell death when primary human T cells, isolated from a blood sample, that were transduced with a panel of CARs, including MN-E6-CD8-CD28-3z, MN-E6-CD8-41BB-3z and MN-E6-CD4-41BB-3z, when the CAR T cells were exposed to K562-wt cells or K562 cells that had been transfected with MUC1*. The ratio of T cells to target cells was 1:1 and the cells were co-cultured for 24 hours.

[00142] **Figure 35 A-B** is a graph of FACS measuring a time course of target cell survival from Day 1 to Day 3. Primary human T cells, isolated from a blood sample, were transduced with a panel of CARs, including humanized MN-E6-CD8-3z, MN-E6-CD8-CD28-3z, MN-E6-CD8-41BB-3z and

MN-E6-CD8-CD28-41BB-3z. The CAR T cells were then exposed to K562-wt cells that naturally express low levels of MUC1*, or K562 cells that had been transfected with MUC1* high. The ratio of MUC1* targeting CAR T cells to target cells was either 1:1, 10:1, or 20:1. Surviving cells were detected and measured at Day 1 (A) or Day 3 (B).

[00143] **Figure 36** is a graph of FACS measurements of target cell survival at Day 3 of co-culture experiment. Primary human T cells were transduced with a panel of CARs, including humanized MN-E6-CD8-3z, MN-E6-CD8-CD28-3z, MN-E6-CD8-41BB-3z and MN-E6-CD8-CD28-41BB-3z. The CAR T cells were then exposed to MUC1* positive T47D breast cancer cells or MUC1* positive 1500 aka ZR-75-1 breast cancer cells. The ratio of MUC1* targeting CAR T cells to target cells was either 1:1 or 10:1. As can be seen from the graph, T cells transduced with a MUC1* targeting CAR have a much greater killing effect on MUC1* cancer cells than the untransduced control T cells. In addition, the killing effect is much greater when the ratio of T cells: target cells is increased.

[00144] **Figure 37** is a graph of FACS measurements of target cell survival at Day 1 of co-culture experiment. Primary human T cells were transduced with a panel of CARs, including humanized MN-E6-CD8-41BB-3z, MN-E6-CD4-41BB-3z, and MN-E6-CD8-CD28-41BB-3z. The CAR T cells were then exposed to the following MUC1* positive cancer cells: T47D breast cancer; capan2 pancreatic cancer; or DU-145 prostate cancer. The ratio of MUC1* targeting CAR T cells to target cells was 5:1. As can be seen from the graph, T cells transduced with a MUC1* targeting CAR have a much greater killing effect on MUC1* cancer cells than the untransduced control T cells. Note that the measurements were taken after 24 hours with only a 5:1 T cell to target cell ratio. Also note that MUC1* targeting CARs that have a CD4 extracellular domain-transmembrane-cytoplasmic tail work equally well as CD8 constructs.

[00145] **Figure 38** is a graph of FACS measurements of target cell survival at Day 3 of co-culture experiment. Primary human T cells were transduced with a panel of CARs, including humanized MN-E6-CD8-41BB-3z, MN-E6-CD4-41BB-3z, and MN-E6-CD8-CD28-41BB-3z. The CAR T cells were then exposed to the following MUC1* positive cancer cells: K562 leukemia cells transfected with MUC1*; T47D breast cancer; 1500 aka ZR-75-1 breast cancer cells; or CAPAN-2 pancreatic cancer cells. In addition to the untransduced T cell controls, the assay was performed on PC3 MUC1* negative prostate cancer cells. The ratio of MUC1* targeting CAR T cells to target cells was 1:1. As can be seen from the graph, T cells transduced with a MUC1* targeting CAR have a much greater killing effect on MUC1* cancer cells than the untransduced control T cells. In addition, the killing effect is specific for MUC1* positive cells. Note that MUC1* targeting CARs

that have a CD4 extracellular domain-transmembrane-cytoplasmic tail work equally well as CD8 constructs.

[00146] **Figure 39** is a graph of FACS measurements of CAR T cell expansion over 24 hours in co-culture with target cells at a ratio of 5:1 CAR T cells to target cells. The primary human T cells were transduced with a panel of CARs, including humanized MN-E6-CD8-41BB-3z, MN-E6-CD4-41BB-3z, and MN-E6-CD8-CD28-41BB-3z. The CAR T cells were co-cultured with MUC1* positive T47D breast cancer cells, MUC1* positive Capan pancreatic cancer cells, and MUC1-negative cells HCT-116 colon cancer cells and HEK-293 human embryonic kidney cells. As can be seen from the graph, the CAR T population is increased in the presence of MUC1* positive cells.

[00147] **Figure 40** shows a photograph of a Western blot of MUC1* targeting CARs. From 1 to 9 are:

1. E6scFv-Fc-8-41BB-CD3z (Human Fc as hinge region with CD8 TM);
2. E6scFv-FcH-8-41BB-CD3z (Human Fc hingeless as hinge region with CD8 TM)
3. E6scFv-Fc-4-41BB-CD3z (Human Fc as hinge region with CD4 TM)
4. E6scFv-FcH-4-41BB-CD3z (Human Fc as hingeless hinge region with CD4 TM)
5. E6scFv-IgD-8-41BB-CD3z (hinge region from human IgD with CD8 TM)
6. E6scFv-IgD-4-41BB-CD3z (hinge region from human IgD with CD4 TM)
7. E6scFv-X4-8-41BB-CD3z (Long flexible linker as hinge region with CD8 TM)
8. E6scFv-X4-4-41BB-CD3z (Long flexible linker as hinge region with CD4 TM)
9. E6scFv-8-4-41BB-CD3z (Hinge region from CD8 and CD4 a with CD4 TM).

[00148] **Figure 41** shows graphs of FACS scans of T47D breast cancer cells co-cultured with human T cells that were transduced with MN- E6scFv-Fc-8-41BB-CD3z, MN-E6scFv-FcH-8-41BB-CD3z, MN-E6scFv-Fc-4-41BB-CD3z, MN-E6scFv-IgD-8-41BB-CD3z, MN-E6scFv-X4-8-41BB-CD3z and MN-E6scFv-X4-4-41BB-CD3z. T cells and cancer cells were co-cultured at a 1:1 ratio for 48 hours. T cell counts were normalized to an average of all untransduced T cells and target cells were normalized to each specific cell type when co-cultured with untransduced T cells. The graph shows that when the CAR T cells are co-cultured with MUC1* positive cancer cells, the T cell population expands and the targeted cancer cell population decreases.

[00149] **Figure 42** shows graphs of FACS scans of T47D breast cancer cells, Capan-2 pancreatic cancer cells, K562-MUC1* transfected cells, and K562-wt cells that were co-cultured with human T cells that were transduced with MN- E6scFv-Fc-8-41BB-CD3z, MN-E6scFv-FcH-8-41BB-CD3z, MN-E6scFv-Fc-4-41BB-CD3z, MN-E6scFv-IgD-8-41BB-CD3z, MN-E6scFv-X4-8-41BB-CD3z and MN-E6scFv-X4-4-41BB-CD3z. T cells and cancer cells were co-cultured at a 1:1 ratio for 48

hours. T cell counts were normalized to an average of all untransduced T cells and target cells were normalized to each specific cell type when co-cultured with untransduced T cells. The graph shows that when the CAR T cells are co-cultured with MUC1* positive cancer cells, the T cell population expands and the targeted cancer cell population decreases.

[00150] Figure 43 A,B are photographs of breast cancer tissue arrays. A) was stained with VU4H5 which recognizes MUC1-FL (full length); B) was stained with mouse monoclonal antibody MN-C2 which recognizes cancerous MUC1*. Following automated staining (Clariant Diagnostics), the tissue staining was scored using Allred scoring method which combines an intensity score and a distribution score. C,D,E,F are color coded graphs showing the score calculated for MUC1 full-length staining for each patient's tissue. G,H,I,J are color coded graphs showing the score calculated for MUC1* staining for each patient's tissue.

[00151] Figure 44 A,B are photographs of breast cancer tissue arrays. A) was stained with VU4H5 which recognizes MUC1-FL (full length); B) was stained with mouse monoclonal antibody MN-C2 which recognizes cancerous MUC1*. Following automated staining (Clariant Diagnostics), the tissue staining was scored using Allred scoring method which combines an intensity score and a distribution score. C,D,E,F are color coded graphs showing the score calculated for MUC1 full-length staining for each patient's tissue. G,H,I,J are color coded graphs showing the score calculated for MUC1* staining for each patient's tissue.

[00152] Figure 45 shows photographs of normal breast and breast cancer tissues stained with humanized MN-E6-scFv-Fc biotinylated anti-MUC1* antibody at 2.5 ug/mL, then stained with a secondary streptavidin HRP antibody. A) is a normal breast tissue. B-D are breast cancer tissues from patients as denoted in the figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00153] Figure 46 shows photographs of normal breast and breast cancer tissues stained with humanized MN-E6-scFv-Fc biotinylated anti-MUC1* antibody at 2.5 ug/mL, then stained with a secondary streptavidin HRP antibody. A) is a normal breast tissue. B-C are breast cancer tissues from patients as denoted in the figure. D-F are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00154] Figure 47 shows photographs of breast cancer tissues stained with MN-E6 anti-MUC1* antibody at 10 ug/mL, then stained with a rabbit anti mouse secondary HRP antibody. A-D are breast cancer tissues from patient #300. E-H are breast cancer tissues from metastatic patient #291.

[00155] Figure 48 shows photographs of normal lung and lung cancer tissues stained with humanized MN-E6-scFv-Fc biotinylated anti-MUC1* antibody at 2.5 ug/mL, then stained with a

secondary streptavidin HRP antibody. A) is a normal lung tissue. B,C are lung cancer tissues from patients as denoted in the figure. D-F are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00156] Figure 49 shows photographs of normal lung and lung cancer tissues stained with humanized MN-E6-scFv-Fc biotinylated anti-MUC1* antibody at 2.5 ug/mL, then stained with a secondary streptavidin HRP antibody. A) is a normal lung tissue. B,C are lung cancer tissues from patients as denoted in the figure. D-F are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00157] Figure 50 shows photographs of normal lung and lung cancer tissues stained with humanized MN-E6-scFv-Fc biotinylated anti-MUC1* antibody at 25 ug/mL, then stained with a secondary streptavidin HRP antibody. A) is a normal lung tissue. B,C are lung cancer tissues from patients as denoted in the figure. D-F are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00158] Figure 51 shows photographs of normal lung and lung cancer tissues stained with humanized MN-E6-scFv-Fc biotinylated anti-MUC1* antibody at 25 ug/mL, then stained with a secondary streptavidin HRP antibody. A) is a normal lung tissue. B,C are lung cancer tissues from patients as denoted in the figure. D-F are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00159] Figure 52 shows photographs of normal small intestine and cancerous small intestine tissues stained with humanized MN-E6-scFv-Fc biotinylated anti-MUC1* antibody at 5 ug/mL, then stained with a secondary streptavidin HRP antibody. A) is a normal small intestine tissue. B) is small intestine cancer from patient as denoted in the figure. C,D are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00160] Figure 53 shows photographs of normal small intestine tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are normal small intestine tissue. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00161] Figure 54 shows photographs of cancerous small intestine tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are cancerous small intestine tissue from a patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00162] **Figure 55** shows photographs of cancerous small intestine tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are cancerous small intestine tissue from a patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00163] **Figure 56** shows photographs of normal colon tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are normal colon. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00164] **Figure 57** shows photographs of colon cancer tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are colon cancer tissue from a metastatic patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00165] **Figure 58** shows photographs of colon cancer tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are colon cancer tissue from a Grade 2 patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00166] **Figure 59** shows photographs of colon cancer tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are colon cancer tissue from a metastatic patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00167] **Figure 60** shows photographs of prostate cancer tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are prostate cancer tissue from a patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00168] **Figure 61** shows photographs of prostate cancer tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are prostate cancer tissue from a patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00169] **Figure 62** shows photographs of prostate cancer tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are prostate cancer tissue from a patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[00170] In the present application, “a” and “an” are used to refer to both single and a plurality of objects.

[00171] As used herein, “h” or “hu” placed before an antibody construct is short-hand for humanized.

[00172] As used herein, the term “antibody-like” means a molecule that may be engineered such that it contains portions of antibodies but is not an antibody that would naturally occur in nature. Examples include but are not limited to CAR (chimeric antigen receptor) T cell technology and the Ylanthia[®] technology. The CAR technology uses an antibody epitope fused to a portion of a T cell so that the body’s immune system is directed to attack a specific target protein or cell. The Ylanthia[®] technology consists of an “antibody-like” library that is a collection of synthetic human Fabs that are then screened for binding to peptide epitopes from target proteins. The selected Fab regions can then be engineered into a scaffold or framework so that they resemble antibodies.

[00173] As used herein, the antibodies MN-C2, MN-E6, MN-C3 and MN-C8, may also be referred to as C2, E6, C3 and C8, respectively.

[00174] As used herein, “PSMGFR” is abbreviation for Primary Sequence of the MUC1 Growth Factor Receptor which is identified by SEQ ID NO:2, and thus is not to be confused with a six amino acid sequence. “PSMGFR peptide” or “PSMGFR region” refers to a peptide or region that incorporates the Primary Sequence of the MUC1 Growth Factor Receptor (SEQ ID NO:2).

[00175] As used herein, the “MUC1*” extra cellular domain is defined primarily by the PSMGFR sequence (GTINVHDTVETQFNQYKTEAASRYNLTISDVSVSDVPFPFSAQSGA (SEQ ID NO:2)). Because the exact site of MUC1 cleavage depends on the enzyme that clips it, and that the cleavage enzyme varies depending on cell type, tissue type or the time in the evolution of the cell, the exact sequence of the MUC1* extra cellular domain may vary at the N-terminus.

[00176] Other clipped amino acid sequences may include SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620); or SVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621).

[00177] As used herein, the term “PSMGFR” is an acronym for Primary Sequence of MUC1 Growth Factor Receptor as set forth as GTINVHDTVETQFNQYKTEAASRYNLTISDVSVDVPPFSAQSGA (SEQ ID NO:2). In this regard, the “N-number” as in “N-10 PSMGFR”, “N-15 PSMGFR”, or “N-20 PSMGFR” refers to the number of amino acid residues that have been deleted at the N-terminal end of PSMGFR. Likewise “C-number” as in “C-10 PSMGFR”, “C-15 PSMGFR”, or “C-20 PSMGFR” refers to the number of amino acid residues that have been deleted at the C-terminal end of PSMGFR.

[00178] As used herein, the “extracellular domain of MUC1*” refers to the extracellular portion of a MUC1 protein that is devoid of the tandem repeat domain. In most cases, MUC1* is a cleavage product wherein the MUC1* portion consists of a short extracellular domain devoid of tandem repeats, a transmembrane domain and a cytoplasmic tail. The precise location of cleavage of MUC1 is not known perhaps because it appears that it can be cleaved by more than one enzyme. The extracellular domain of MUC1* will include most of the PSMGFR sequence but may have an additional 10-20 N-terminal amino acids.

[00179] As used herein “sequence identity” means homology in sequence of a particular polypeptide or nucleic acid to a reference sequence of nucleic acid or amino acid such that the function of the homologous peptide is the same as the reference peptide or nucleic acid. Such homology can be so close with the reference peptide such that at times the two sequences may be 90%, 95% or 98% identical yet possess the same function in binding or other biological activities.

[00180] MUC1* antibodies (anti-PSMGFR) for treatment or prevention of cancers

[00181] We discovered that a cleaved form of the MUC1 (SEQ ID NO:1) transmembrane protein is a growth factor receptor that drives the growth of over 75% of all human cancers. The cleaved form of MUC1, which we called MUC1* (pronounced muk 1 star), is a powerful growth factor receptor. Enzymatic cleavage releases the bulk of the MUC1 extracellular domain. It is the remaining portion comprising a truncated extracellular domain, transmembrane and cytoplasmic tail that is called MUC1*. Cleavage and release of the bulk of the extracellular domain of MUC1 un.masks a binding site for activating ligands dimeric NME1, NME6, NME8, NME7-AB or NME7. Cell growth assays show that it is ligand-induced dimerization of the MUC1* extracellular domain that promotes growth (**Figure 1 A-D**). MUC1* positive cells treated with either bivalent ‘bv’ anti-MUC1* antibody, monovalent ‘mv’ or Fab, NM23-H1 dimers or NME7-AB. Bivalent anti-MUC1* antibodies stimulate growth of cancer cells whereas the monovalent Fab inhibits growth. Classic bell-shaped curve indicates ligand induced dimerization stimulates growth. Dimeric NM23-H1, aka

NME1, stimulates growth of MUC1* positive cancer cells but siRNA to suppress MUC1 expression eliminate its effect (C). NME7-AB also stimulates the growth of MUC1* positive cells (D).

[00182] MUC1* is an ideal target for cancer drugs as it is aberrantly expressed on over 75% of all cancers and is likely overexpressed on an even higher percentage of metastatic cancers (Fessler SP, Wotkowicz MT, Mahanta SK and Bamdad C. (2009). MUC1* is a determinant of trastuzumab (Herceptin) resistance in breast cancer cells. *Breast Cancer Res Treat.* 118(1):113-124). After MUC1 cleavage most of its extracellular domain is shed from the cell surface. The remaining portion has a truncated extracellular domain that at least comprises the primary growth factor receptor sequence, PSMGFR (SEQ ID NO:2). Antibodies that bind to the PSMGFR sequence and especially those that competitively inhibit the binding of activating ligands such as NME proteins, including NME1, NME6, NME8 and NME7, are ideal therapeutics and can be used to treat or prevent MUC1 positive or MUC1* positive cancers, as stand-alone antibodies, antibody fragments or variable region fragments thereof incorporated into bispecific antibodies, or chimeric antigen receptors also called CARs. Therapeutics anti-MUC1* antibodies can be monoclonal, polyclonal, antibody mimics, engineered antibody-like molecules, full antibodies or antibody fragments. Examples of antibody fragments include but are not limited to Fabs, scFv, and scFv-Fc. Human or humanized antibodies are preferred for use in the treatment or prevention of cancers. In any of these antibody-like molecules, mutations can be introduced to prevent or minimize dimer formation. Anti-MUC1* antibodies that are monovalent or bispecific are preferred because MUC1* function is activated by ligand induced dimerization. Typical binding assays show that NME1 and NME7 bind to the PSMGFR peptide portion of MUC1* (Figure 2 A, D). Further, they show that these activating growth factors bind to the membrane proximal portion of MUC1*, as they do not bind to the PSMGFR peptide if the 10 C-terminal amino acids are missing. Similarly, anti-MUC1* antibodies MN-C2 and MN-E6 bind to the PSMGFR peptide if and only if the 10 C-terminal amino acids are present (Figure 2 B, C). Antibodies MN-C3 and MN-C8 bind to epitopes that are different from MN-C2 and MN-E6, as they do not depend on the presence of the 10 C-terminal amino acids of the PSMGFR peptide (Figure 2 E, F). Antibodies MN-C2, MN-E6, MN-C3 or MN-C8, or fragments derived from them, as stand-alone antibodies or incorporated into bispecific antibodies, BiTEs or chimeric antigen receptors also called CARs expressed by immune cells are all potent anti-cancer therapeutics.

[00183] Therapeutic anti-MUC1* antibodies for use as a stand alone antibody therapeutic or for integration into a BiTE or a CAR can be selected based on specific criteria. The parent antibody can be generated using typical methods for generating monoclonal antibodies in animals. Alternatively,

they can be selected by screening antibody and antibody fragment libraries for their ability to bind to a MUC1* peptide, which can be the PSMGFR peptide (SEQ ID NO:2), SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620); orSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621).

[00184] Resultant antibodies or antibody fragments generated or selected in this way can then be further selected by passing additional screens. For example, antibodies or antibody fragments become more preferred based on their ability to bind to MUC1* positive cancer cells or tissues but not to MUC1 negative cancer cells or tissues. Further, anti-MUC1* antibodies or antibody fragments may be de-selected as anti-cancer therapeutics if they bind to stem or progenitor cells. Anti-MUC1* antibodies or antibody fragments become more preferred if they have the ability to competitively inhibit the binding of activating ligands to MUC1*. Figure 3 A-C shows that MN-E6 and MN-C2 competitively inhibit the binding of activating ligands NME1 and NME7 to MUC1*. A process for selecting anti-MUC1* antibodies for use in treating a patient diagnosed with a MUC1 positive cancer, at risk of developing a MUC1 positive cancer or suspected of having a MUC1 positive cancer comprises one or more of the following steps of selecting antibodies or antibody fragments that 1) bind to the PSMGFR peptide; 2) bind to the N-10 PSMGFR peptide; 3) bind to cancer cells; 4) do not bind to stem or progenitor cells; and 5) competitively inhibited the binding of dimeric NME1 or NME7-AB to the PSMGFR peptide. For example, Figure 3 A-C show that monoclonals MN-E6 and MN-C2 satisfy all five criteria, while monoclonals MN-C3 and MN-C8 do not competitively inhibit the binding of activating ligands NME1 and NME7 (Figure M3 C). However, antibodies or antibody fragments derived from MN-C3 and MN-C8 are equally potent as anti-cancer agents when integrated into a BiTE or a CAR as in these methods, the killing effect of the immune cells is more important than the ability to inhibit the binding of activating ligands. In addition, toxic agents conjugated to MN-E6, MN-C2, MN-C3 or MN-C8 are potent anti-cancer therapeutics. Recall that the MUC1* growth factor receptor is activated by ligand-induced dimerization of its extracellular domain. Therefore the ideal antibody therapeutic should not dimerize the MUC1* extracellular domain. Preferably, suitable antibodies in this regard include monovalent antibodies such as those generated in llamas and camels, Fabs, scFv's, single domain antibodies (sdAb), scFv-Fc as long as the Fc portion is constructed such that it does not homo-dimerize.

[00185] FACS scans show that anti-MUC1* antibodies MN-C2, MN-E6, MN-C3 and MN-C8 specifically bind to MUC1* positive cancer cells and MUC1* transfected cells but not MUC1* or MUC1 negative cells. In one example, a humanized MN-C2 scFv is shown to bind to ZR-75-1, aka 1500, MUC1* positive breast cancer cells (Figure 4 A-C). MN-E6 was shown to bind to MUC1

negative HCT-116 colon cancer cells if and only if they were transfected with MUC1*. MN-E6 also bound to MUC1* positive cancer cells such as ZR-75-1, aka 1500, MUC1* positive breast cancer cells (Figure 4 D-F). Binding assays such as ELISAs, immunofluorescence, and the like all confirm that MN-C2 and MN-E6 bind to the PSMGFR peptide and to live MUC1 positive cancer cells. Humanized anti-MUC1* antibodies are selected based on their ability to also bind to the PSMGFR peptide or to MUC1 positive cancer cells. Figure 5 shows that humanized MN-C2 scFv binds with high affinity to the MUC1* peptide PSMGFR with an EC-50 of about 333nM. Humanized MN-C2 scFv, like Fabs, potentially inhibits the growth of MUC1* positive cancer cells as is shown in one example in Figure 6 A, B.

[00186] The Fabs of MN-E6 and MN-C2 or the comparable single chain variable regions derived from them potentially inhibit the growth of MUC1* positive cancers *in vitro* and *in vivo*. In several examples, the Fabs of Anti-MUC1* antibodies inhibited the growth of human MUC1* positive cancers *in vivo*. In one case, immune-compromised mice were implanted with human breast tumors then treated with MN-E6 Fab after tumor engraftment. Figure 7A shows that MN-E6 Fab potentially inhibited the growth of MUC1* positive breast cancers. Female nu/nu mice implanted with 90-day estrogen pellets were implanted with 6 million T47D human breast cancer cells that had been mixed 50/50 with Matrigel. Mice bearing tumors that were at least 150 mm³ and had three successive increases in tumor volume were selected for treatment. Animals were injected sub cutaneously twice per week with 80 mg/kg MN-E6 Fab and an equal number of mice fitting the same selection criteria were injected with vehicle alone (A). In another aspect, MN-E6 was shown to halt the growth of prostate cancer. Figure 7B shows that MN-E6 Fab potentially inhibited the growth of MUC1* positive prostate cancers. Male NOD/SCID mice were implanted with 6 million DU-145 human prostate cancer cells that had been mixed 50/50 with Matrigel. Mice bearing tumors that were at least 150 mm³ and had three successive increases in tumor volume were selected for treatment. Animals were injected sub-cutaneously every 48 hours with 160 mg/kg MN-E6 Fab and an equal number of mice fitting the same selection criteria were injected with vehicle alone (B). Tumors were measured independently by two researchers twice per week and recorded. Statistics were blindly calculated by independent statistician, giving a P value of 0.0001 for each. Anti-MUC1* Fab inhibited breast cancer growth and prostate cancer growth. Treatment had no effect on weight, bone marrow cell type or number. The MN-E6 Fab effectively inhibited the growth of the tumors, while the control group's tumors continued to grow until sacrifice. No adverse effects of treatment were observed or detected.

[00187] A recombinant MN-E6 was constructed that like the Fab is monomeric. In this case, MN-E6 was humanized. There are a number of methods known to those skilled in the art for humanizing antibodies. In addition to humanizing, libraries of human antibodies can be screened to identify other fully human antibodies that bind to the PSMGFR. A single chain of the humanized MN-E6 variable region, called an scFv, was genetically engineered such that it was connected to the Fc portion of the antibody (SEQ ID NO:256 and 257). Fc regions impart certain benefits to antibody fragments for use as therapeutics. The Fc portion of an antibody recruits complement, which in general means it can recruit other aspects of the immune system and thus amplify the anti-tumor response beyond just inhibiting the target. The addition of the Fc portion also increases the half-life of the antibody fragment (Czajkowsky DM, Hu J, Shao Z and Pleass RJ. (2012) Fc-fusion proteins: new developments and future perspectives. *EMBO Mol Med.* 4(10):1015-1028). However, the Fc portion of an antibody homo-dimerizes, which in the case of anti-MUC1* antibody based therapeutics is not optimal since ligand-induced dimerization of the MUC1* receptor stimulates growth. As can be seen in Figure 13 B, humanized MN-E6 scFv-Fc is a dimer, in part due to disulfide bonding. Therefore, mutations in the Fc region that resist dimer formation are preferred for anti-MUC1* anti-cancer therapeutics. Deletion of the hinge region (hingeless also called delta hinge or Dhinge in some figures and examples SEQ ID NO: 288 and 289) and other mutations in the Fc region that make the Fc-mutant resistant to dimerization were made. The following mutations were made in the CH3 domain to create a monomeric scFv-Fc fusion protein: Y407R (SEQ ID NO: 278 and 279), F405Q (SEQ ID NO: 280 and 281), T394D (SEQ ID NO: 282 and 283), T366W/L368W (SEQ ID NO: 284 and 285), T364R/L368R (SEQ ID NO: 286 and 287). Figure 14 shows photographs of SDS-PAGE characterization of purified MN-E6 scFv-Fc fusion proteins on a non-reducing gels, wherein the Fc portion that was fused to the MN-E6 was either wild type (wt) or mutated as follows: A) F405Q, Y407R, T394D; B) T366W/L368W, T364R/L368R, T366W/L368W or T364R/L368R. Fc mutants F405Q, Y407R, T366W/L368W, T364R/L368R, T366W/L368W and T364R/L368R all favored monomer over dimer formation. **Figure 15** shows FPLC traces of the purification of MN-E6 scFv-Fc Y407Q fusion protein that was grown in low IgG FBS over a Protein A affinity column. A) is the trace of the flow through. B) is the trace of the elution. The protein was further purified by size exclusion over an S200 column (C). (D) is a photograph of an SDS-PAGE gel showing which fractions had a predominance of monomer. **Figure 16** shows a photograph of SDS-PAGE characterization of purified MN-E6 scFv-Fc-mutant fusion proteins on a non-reducing gel, wherein the Fc portion that was fused to the MN-E6 scFv was either wild type (wt) or mutated by elimination of the hinge region, 'DHinge', of the Fc or elimination of the hinge region of the Fc and also bearing

the Y407R mutation. All the Fc mutants favored monomer over dimer formation. The reference construct amino acid sequence for the indicated mutation is SEQ ID NO:273. Other relevant sequences are SEQ ID NOS:289 and 279. **Figure 17 A-C.** A and B show photograph of non-reducing SDS-PAGE characterization of large scale expression and purification of MN-E6 scFv-Fc hingeless mutant, showing that it is a monomer. FPLC characterization and purification of MN-E6 scFv-Fc hingeless mutant is shown (C). **Figure 18 A-C** shows photographs of the SDS-PAGE characterization of the purified MN-C3 scFv-Fc fusion protein on a non-reducing gel (A) or a reducing gel (B). The protein was purified by size exclusion. The FPLC trace is shown (C). **Figure 19 A-B** shows photographs of Native gels of MN-C3 or MN-E6 Fabs, scFv, scFv-Fc, wherein the Fc portion is wild type or mutants that prefer or are exclusively monomers. Native gels show that the Y407R Fc mutation (A) and the double mutant Y407R and a deleted hinge (B) favor monomer over dimer the best. Note that proteins are loaded onto a gel at much higher concentrations than typical use concentrations. The dimer formation of other Fc mutants may only reflect the fact that loading concentration is very high.

[00188] Some mutations or deletions were so effective that, even when loaded onto a gel at high concentrations, they resist dimer formation (Figure 14 A, B). The Y407R mutation results in a nearly pure population of dimeric scFv-Fc (Figure 10). Similarly deletions of the hinge region of the Fc result in fusion proteins that are monomers rather than dimers. Combinations of mutations can result in even more effective resistance of dimer formation (Figures 16 and 17). These and other mutations and combinations thereof were introduced into CH2-CH3 (SEQ ID NO:274 and 275) and CH3 (SEQ ID NO:276 and 277) fusion proteins such as scFv or in the hingeless Fc-fusion proteins such as scFv and were shown to eliminate or minimize dimerization.

[00189] Like the parent mouse monoclonal antibodies, human or humanized antibodies as well as single chain constructs, scFv's, scFv-Fc fusions or scFv-Fc-mutants specifically bind to the synthetic MUC1* peptides (Figures 20-22). Figure E23 shows a graph of an ELISA assay that quantifies the binding of humanized MN-E6 scFv-Fc-delta hinge, aka Dhinge or hingeless, and humanized MN-E6 scFv to the MUC1* peptide PSMGFR.

[00190] The human or humanized anti-MUC1* antibody fragments described here specifically bind to MUC1 and MUC1* positive cancer cells. **Figure 24** shows photographs of immunofluorescence experiments in which humanized MN-C2 scFv or MN-E6 scFv specifically binds to MUC1* positive breast cancer cells in an identical concentration dependent manner. A-G: hu MN-C2 scFv binding to T47D breast cancer cells at concentrations indicated. H-N shows the fluorescently labeled scFv and DAPI. O-U: hu MN-E6 scFv binding to T47D breast cancer cells at

concentrations indicated. V-B' shows the fluorescently labeled scFv and DAPI. C' is the secondary antibody control.

[00191] In addition to binding to MUC1* positive cancer cells, the anti-MUC1* antibody variable region fragments, scFv's, scFv-Fc's and scFv-Fc-mutants inhibited growth of MUC1-positive cancer cells. **Figure 25 A-L** shows photographs of MUC1* positive breast cancer cells that have been cultured in normal medium or in the presence of humanized MN-E6 scFv. The photographs show killing and/or growth inhibition of MUC1* positive cells by MN-E6 scFv at 5 ug/mL and an even greater effect at 500 ug/mL. **Figure 26 A-L** shows photographs of MUC1* positive breast cancer cells that have been cultured in normal medium or in the presence of humanized MN-E6 scFv-Fc Dhinge, which is a hingeless or delta hinge mutant. The photographs show killing and/or growth inhibition of MUC1* positive cells by hMN-E6 scFv-Fc Dhinge 5 ug/mL, an even greater effect at 50 ug/mL and yet an even greater effect at 100 ug/mL. **Figure 27** shows a graph of the image analysis of the fluorescent images of Figures 25 and 26. Image J was used to quantify the number of cells remaining after 96 hours treatment in humanized MN-E6scFv or MN-E6 scFv-Fc-delta hinge, aka Dhinge. The analysis software uses pixel counting and pixel fluorescence intensity to quantify the number of cells in each photograph. Analysis was performed over the entire image 512X512 pixels, 8-bit image. For comparison, the inhibition of mouse monoclonal MN-E6 Fab is also analyzed.

[00192] These data show that a human or humanized MN-E6 antibody or antibody fragment, Fab, MN-E6 scFv or hu MN-E6 scFv-Fc_{mut} are effective anti-cancer agents that can be administered to a person diagnosed with a MUC1 or MUC1* positive cancer, suspected of having a MUC1 or MUC1* positive cancer or is at risk of developing a MUC1 or MUC1* positive cancer.

[00193] In these specific examples, the dimer resistant Fc that was fused onto an antibody fragment or scFv is hu MN-E7 scFv. However, any of these Fc region mutations or combinations thereof that eliminate or minimize dimerization can be fused onto variable region fragments or single chain constructs of MN-E6, MN-C2, MN-C3 or MN-C8 or other antibodies identified that selectively bind to MUC1* as it exists on cancer cells or tissues. In addition, the Fabs of these antibodies can be used as an anti-cancer therapeutic. In one aspect of the invention, a person diagnosed with, suspected of having or is at risk of developing a MUC1* or MUC1 positive cancer is treated with an effective amount of human or humanized MN-E6 scFv, MN-C2 scFv, MN-C3 scFv, or MN-C8 scFv. In another aspect of the invention, a person diagnosed with, suspected of having or is at risk of developing a MUC1* or MUC1 positive cancer is treated with an effective amount of human or humanized MN-E6 scFv-Fc_{Y407R}, MN-C2 scFv-Fc_{Y407R}, MN-C3 scFv-Fc_{Y407R},

or MN-C8 scFv-Fc_{Y407R}. In another aspect of the invention, a person diagnosed with, suspected of having or is at risk of developing a MUC1* or MUC1 positive cancer is treated with an effective amount of human or humanized MN-E6 scFv-Fc mutant_{Dhinge}, MN-C2 scFv-Fc mutant_{Dhinge}, MN-C3 scFv-Fc mutant_{Dhinge}, or MN-C8 scFv-Fc mutant_{Dhinge}. In yet another aspect of the invention, a person diagnosed with, suspected of having or is at risk of developing a MUC1* or MUC1 positive cancer is treated with an effective amount of human or humanized MN-E6 scFv-Fc mutant_{Y407R-Dhinge}, MN-C2 scFv-Fc mutant_{Y407R-Dhinge}, MN-C3 scFv-Fc mutant_{Y407R-Dhinge}, or MN-C8 scFv-Fc mutant_{Y407R-Dhinge}. One aspect of the invention is a method for treating a patient diagnosed with, suspected of having, or at risk of developing a MUC1 positive or MUC1* positive cancer, wherein the patient is administered an effective amount of a monomeric MN-E6 scFv, MN-C2 scFv, MN-C3 scFv, MN-C8 scFv, or MN-E6 scFv-Fc, MN-C2 scFv-Fc, MN-C3 scFv-Fc, MN-C8 scFv-Fc, wherein the Fc portion of the antibody-like protein has been mutated such that it resists dimer formation.

[00194] Humanizing

[00195] Humanized antibodies or antibody fragments or fully human antibodies that bind to the extracellular domain of -MUC1* are preferred for therapeutic use. The techniques described herein for humanizing antibodies are but a few of a variety of methods known to those skilled in the art. The invention is not meant to be limited by the technique used to humanize the antibody.

[00196] Humanization is the process of replacing the non-human regions of a therapeutic antibody (usually mouse monoclonal antibody) by human one without changing its binding specificity and affinity. The main goal of humanization is to reduce immunogenicity of the therapeutic monoclonal antibody when administered to human. Three distinct types of humanization are possible. First, a chimeric antibody is made by replacing the non-human constant region of the antibody by the human constant region. Such antibody will contain the mouse Fab region and will contain about 80-90% of human sequence. Second, a humanized antibody is made by grafting of the mouse CDR regions (responsible of the binding specificity) onto the variable region of a human antibody, replacing the human CDR (CDR-grafting method). Such antibody will contain about 90-95% of human sequence. Third and last, a full human antibody (100% human sequence) can be created by phage display, where a library of human antibodies is screened to select antigen specific human antibody or by immunizing transgenic mice expressing human antibody.

[00197] A general technique for humanizing an antibody is practiced approximately as follows. Monoclonal antibodies are generated in a host animal, typically in mice. Monoclonal antibodies are then screened for affinity and specificity of binding to the target. Once a monoclonal antibody that

has the desired effect and desired characteristics is identified, it is sequenced. The sequence of the animal-generated antibody is then aligned with the sequences of many human antibodies in order to find human antibodies with sequences that are the most homologous to the animal antibody. Biochemistry techniques are employed to paste together the human antibody sequences and the animal antibody sequences. Typically, the non-human CDRs are grafted into the human antibodies that have the highest homology to the non-human antibody. This process can generate many candidate humanized antibodies that need to be tested to identify which antibody or antibodies has the desired affinity and specificity.

[00198] Once a human antibody or a humanized antibody has been generated it can be further modified for use as an Fab fragment, as a full antibody, or as an antibody-like entity such as a single chain molecule containing the variable regions, such as scFv or an scFv-Fc. In some cases it is desirable to have Fc region of the antibody or antibody-like molecule mutated such that it does not dimerize.

[00199] In addition to methods that introduce human sequences into antibodies generated in non-human species, fully human antibodies can be obtained by screening human antibody libraries with a peptide fragment of an antigen. A fully human antibody that functions like MN-E6 or MN-C2 is generated by screening a human antibody library with a peptide having the sequence of the PSMGFR N-10 peptide. A fully human antibody that functions like MN-C3 or MN-C8 is generated by screening a human antibody library with a peptide having the sequence of the PSMGFR C-10 peptide.

[00200] Humanized anti-MUC1* antibodies were generated based on the sequences of the mouse monoclonal antibodies MN-E6, MN-C2, MN-C3 and MN-C8. In one aspect of the invention, a patient diagnosed with a MUC1* positive cancer is treated with an effective amount of humanized MN-E6, MN-C2, MN-C3 or MN-C8. In a preferred embodiment, a patient diagnosed with a MUC1* positive cancer is treated with an effective amount of humanized MN-E6 or MN-C2. In another aspect of the invention, a patient diagnosed with a MUC1* positive cancer is treated with an effective amount of humanized monovalent MN-E6, MN-C2, MN-C3 or MN-C8, wherein monovalent means the corresponding Fab fragment, the corresponding scFv or the corresponding scFv-Fc fusion. In a preferred embodiment, a patient diagnosed with a MUC1* positive cancer is treated with an effective amount of a humanized scFv or monomeric humanized scFv-Fc of MN-E6 or MN-C2. Since the MUC1* growth factor receptor is activated by ligand induced dimerization of its extracellular domain, and because the Fc portion of an antibody homo-dimerizes, it is preferable

that a construct that includes an Fc portion uses a mutated Fc region that prevents or minimizes dimerization.

[00201] Antibodies that bind to PSMGFR (SEQ ID NO:2) peptide of the extracellular domain of the MUC1* receptor are potent anti-cancer therapeutics that are effective for the treatment or prevention of MUC1* positive cancers. They have been shown to inhibit the binding of activating ligands dimeric NME1 (SEQ ID NOS: 3 and 4) and NME7 (SEQ ID NOS: 5 and 6) to the extracellular domain of MUC1*. Anti-MUC1* antibodies that bind to the PSMGFR sequence inhibit the growth of MUC1*-positive cancer cells, specifically if they inhibit ligand-induced receptor dimerization. Fabs of anti-MUC1* antibodies have been demonstrated to block tumor growth in animals. Thus, antibodies or antibody fragments that bind to the extracellular domain of MUC1* would be beneficial for the treatment of cancers wherein the cancerous tissues express MUC1*.

[00202] Antibodies that bind to PSMGFR region of MUC1* or bind to a synthetic PSMGFR peptide are preferred. We have identified several monoclonal antibodies that bind to the extracellular domain of MUC1*. Among this group are mouse monoclonal antibodies MN-E6, MN-C2, MN-C3 and MN-C8, the variable regions of which were sequenced and are given as for MN-E6 SEQ ID NOS: 12-13 and 65-66, for MN-C2 SEQ ID NOS: 118-119 and 168-169, for MN-C3 SEQ ID NOS: 413-414 and 458-459 and for MN-C8 SEQ ID NOS: 505-506 and 543-554. The CDRs of these antibodies make up the recognition units of the antibodies and are the most important parts of the mouse antibody that should be retained when grafting into a human antibody. The sequences of the CDRs for each mouse monoclonal are as follows, heavy chain sequence followed by light chain: MN-E6 CDR1 (SEQ ID NO:16-17 and 69-70) CDR2 (SEQ ID NO:20-21 and 73-74) CDR3 (SEQ ID NO: 24-25 and 77-78), MN-C2 CDR1 (SEQ ID NO:122-123 and 172-173) CDR2 (SEQ ID NO:126-127 and 176-177) CDR3 (SEQ ID NO:130-131 and 180-181), MN-C3 CDR1 (SEQ ID NO:417-418 and 462-463) CDR2 (SEQ ID NO:421-422 and 466-467) CDR3 (SEQ ID NO:425-426 and 470-471), MN-C8 CDR1 (SEQ ID NO:507-508 and 545-546) CDR2 (SEQ ID NO:509-510 and 547-548) CDR3 (SEQ ID NO:511-512 and 549-550). In some cases, portions of the framework regions that by modeling are thought to be important for the 3-dimensional structure of the CDRs, are also imported from the mouse sequence.

[00203] Monoclonal antibodies MN-E6 and MN-C2 have greater affinity for MUC1* as it appears on cancer cells. Monoclonal antibodies MN-C3 and MN-C8 have greater affinity for MUC1* as it appears on stem cells. By sequence alignment the following human antibodies were chosen as being sufficiently homologous to the mouse antibody that substitution of the mouse CDRs would result in an antibody that retained ability to recognize the target. Mouse MN-E6 heavy chain

variable region was homologous to human IGHV3-21*03 heavy chain variable region (SEQ ID NO: 26-27) and the light chain variable region was homologous to human IGKV3-11*02 light chain variable region (SEQ ID NO: 79-80). Mouse MN-C2 heavy chain variable region was homologous to human IGHV3-21*04 heavy chain variable region (SEQ ID NO: 132-133) and the light chain variable region was homologous to human IGKV7-3*01 light chain variable region (SEQ ID NO: 182-183). Mouse MN-C3 heavy chain variable region was homologous to human IGHV1-18*04 heavy chain variable region (SEQ ID NO: 427-428) and the light chain variable region was homologous to human IGKV2-29*03 light chain variable region (SEQ ID NO: 472-473). Mouse MN-C8 heavy chain variable region was homologous to human IGHV3-21*04 heavy chain variable region (SEQ ID NO: 513-514) and the light chain variable region was homologous to human Z00023 light chain variable region (SEQ ID NO: 551-552).

[00204] All four antibodies have been humanized, which process has resulted in several humanized forms of each antibody. CDRs derived from the variable regions of the mouse antibodies were biochemically grafted into a homologous human antibody variable region sequence. Humanized variable regions of MN-E6 (SEQ ID NOS: 38-39 and 93-94), MN-C2 (SEQ ID NOS: 144-145 and 194-195), MN-C3 (SEQ ID NOS: 439-440 and 486-487) and MN-C8 (SEQ ID NOS: 525-526 and 543-544) were generated by grafting the mouse CDRs into the variable region of a homologous human antibody. The humanized heavy chain variable constructs were then fused into constant regions of either human IgG1 heavy chain constant region (SEQ ID NOS: 58-59) or human IgG2 heavy chain constant region (SEQ ID NO: 54-55), which are then paired with either humanized light chain variable constructs fused to a human kappa chain (SEQ ID NO: 109-110) or human lambda chain (SEQ ID NO: 113-114) constant region. Other IgG isotypes could be used as constant region including IgG3 or IgG4.

[00205] Examples of humanized MN-E6 variable region into an IgG2 heavy chain (SEQ ID NOS: 52-53) and into an IgG1 heavy chain (SEQ ID NOS: 56-57), humanized MN-C2 variable into an IgG1 heavy chain (SEQ ID NOS: 158-159) or into an IgG2 heavy chain (SEQ ID NOS: 163-164) paired with either Lambda light chain (SEQ ID NO: 111-112 and 216-219) or Kappa chain (SEQ ID NO: 107-108 and 210-213) and , humanized MN-C3 (SEQ ID NOS: 455-456, 453-454 and 500-501, 502-503) and MN-C8 (SEQ ID NOS: 541-542, 539-540 and 579-580, 581-582) antibodies were generated. Which IgG constant region is fused to the humanized variable region depends on the desired effect since each isotype has its own characteristic activity. The isotype of the human constant region is selected on the basis of things such as whether antibody dependent cell cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC) is desired but can also depend

on the yield of antibody that is generated in cell-based protein expression systems. In a preferred embodiment, humanized anti-MUC1* antibodies or antibody fragments are administered to a person diagnosed with or at risk of developing a MUC1-positive cancer.

[00206] One method for testing and selecting the humanized anti-MUC1* antibodies that would be most useful for the treatment of persons with cancer or at risk of developing cancers is to test them for their ability to inhibit the binding of activating ligands to the MUC1* extracellular domain. Dimeric NME1 can bind to and dimerize the MUC1* extracellular domain and in so doing stimulates cancer cell growth. Antibodies and antibody fragments that compete with NME1 for binding to the MUC1* extracellular domain are therefore anti-cancer agents. NME7 is another activating ligand of MUC1*. In some cases, it is preferable to identify antibodies that block the binding of NME7, or an NME7 truncation or cleavage product, to the MUC1* extracellular domain. Antibodies and antibody fragments that compete with NME7 and NME7 variants for binding to the MUC1* extracellular domain are effective as anti-cancer therapeutics. These antibodies include but are not limited to MN-E6, MN-C2, MN-C3, MN-C8 as well as single chain versions, such as scFv, of these antibodies and humanized version thereof. Other NME proteins also bind to MUC1 or MUC1* including NME6 and NME8. Antibodies that compete with these proteins for binding to MUC1* may also be useful as therapeutics. In a preferred embodiment, humanized anti-MUC1* antibodies or antibody fragments are administered to a person diagnosed with or at risk of developing a MUC1-positive cancer. In a more preferred embodiment, single chain antibody fragments, or monomeric scFv-Fc fusions, derived from humanized sequences of MN-E6 and MN-C2 are administered to a person diagnosed with or at risk of developing a MUC1-positive cancer.

[00207] Single chain variable fragments, scFv, or other forms that result in a monovalent antibody or antibody-like protein are also useful. In some cases it is desired to prevent dimerization of the MUC1* extracellular domain. Single chain variable fragments, Fabs and other monovalent antibody-like proteins have been shown to be effective in binding to the extracellular domain of MUC1* and blocking MUC1* dimerization. These single chain variable fragments, Fabs and other monovalent antibody-like molecules effectively blocked cancer growth *in vitro* and in animals xenografted with human MUC1-positive cancer cells. Thus, humanized single chain variable fragments or monovalent anti-MUC1* antibodies or antibody-like molecules would be very effective as an anti-cancer therapeutic. Such humanized single chain antibodies, Fabs and other monovalent antibody-like molecules that bind to the MUC1* extracellular domain or to a PSMGFR peptide are therefore useful as anti-cancer therapeutics. Anti-MUC1* single chain variable fragments are generated by grafting non-human CDRs of antibodies, which bind to extracellular domain of MUC1* or bind to

PSMGFR peptide, into a framework of a homologous variable region human antibody. The resultant humanized heavy and light chain variable regions are then connected to each other via a suitable linker, wherein the linker should be flexible and of length that it allows heavy chain binding to light chain but discourages heavy chain of one molecule binding to the light chain of another. For example a linker of about 10-15 residues. Preferably, the linker includes [(Glycine)₄ (Serine)₁]₃ (SEQ ID NOS: 401-402), but is not limited to this sequence as other sequences are possible.

[00208] In one aspect, the humanized variable regions of MN-E6 (SEQ ID NOS: 38-39 and 93-94), MN-C2 (SEQ ID NOS: 144-145 and 194-195), MN-C3 (SEQ ID NOS: 439-440 and 486-487) and MN-C8 (SEQ ID NOS: 525-526 and 565-566) are biochemically grafted into a construct that connects heavy and light chains via a linker. Examples of humanized single chain anti-MUC1* antibodies comprising humanized sequences from the variable regions of MN-E6, MN-C2, MN-C3 and MN-C8 were generated. Several humanized MN-E6 single chain proteins were generated (SEQ ID NOS: 232-237). Several humanized MN-C2 single chain proteins were generated (SEQ ID NOS: 238-243). Several humanized MN-C3 single chain proteins were generated (SEQ ID NOS: 244-249). Several humanized MN-C8 single chain proteins were generated (SEQ ID NOS: 250-255). In a preferred embodiment, humanized anti-MUC1* antibody fragments, including variable fragments, scFv antibody fragments MN-E6 scFv, MN-C2 scFv, MN-C3 scFv, or MN-C8 scFv are administered to a person diagnosed with or at risk of developing a MUC1-positive cancer. In a more preferred embodiment, single chain antibody fragments, such as variable fragments derived from humanized sequences of MN-E6 and MN-C2, are administered to a person diagnosed with or at risk of developing a MUC1-positive cancer.

[00209] In another aspect, the humanized variable regions of MN-E6 (SEQ ID NOS: 38-39 and 93-94), MN-C2 (SEQ ID NOS: 144-145 and 194-195), MN-C3 (SEQ ID NOS: 439-440 and 486-487) and MN-C8 (SEQ ID NOS: 525-526 and 565-566) are biochemically grafted into a single chain variable fragment, scFv, that also contains an Fc portion of an antibody. Examples of humanized single chain variable fragment of MN-E6, MN-C2, MN-C3 and MN-C8 fused to a Fc region of an antibody were generated (SEQ ID NOS: 256-257, 260-261, 264-265 and 268-269). Inclusion of an Fc region serves several purposes. It increases the molecular weight of the antibody fragment, which slows degradation and increases half-life. An Fc region also recruits immune system complement to the tumor site. Additionally, the addition of an antibody Fc region makes the scFv a convenient diagnostic tool, as the secondary antibodies detect and label the Fc portion. However, the Fc portion homo-dimerizes. Thus an scFv-Fc would be bivalent and could dimerize and activate the MUC1* growth factor receptor. In order to get the benefits of having an Fc attached to an anti-MUC1* scFv,

without the drawback of inducing MUC1* dimerization, the Fc region was mutated to minimize or eliminate Fc homo-dimerization. The following mutations were made in the CH3 domain to create a monomeric scFv-Fc fusion protein: Y407R (SEQ ID NOS: 278 and 279), F405Q (SEQ ID NOS: 280 and 281), T394D (SEQ ID NOS: 282 and 283), T366W/L368W (SEQ ID NOS: 284 and 285), T364R/L368R (SEQ ID NOS: 286 and 285). Any combinations of those mutations can be tested and could be introduced into Fc (SEQ ID NOS: 272-273), CH2-CH3 (SEQ ID NOS: 274-275) or CH3 (SEQ ID NOS: 276-277) fusion proteins or in the hingeless Fc-fusion proteins (SEQ ID NOS: 288-289).

[00210] One aspect of the invention is a method for treating a patient diagnosed with, suspected of having, or at risk of developing a MUC1 positive or MUC1* positive cancer, wherein the patient is administered an effective amount of a monomeric MN-E6 scFv, MN-C2 scFv, MN-C3 scFv, MN-C8 scFv, or MN-E6 scFv-Fc, MN-C2 scFv-Fc, MN-C3 scFv-Fc, MN-C8 scFv-Fc, wherein the antibody variable fragment portions are human or have been humanized and wherein the Fc portion of the antibody-like protein has been mutated such that it resists dimer formation.

[00211] CAR T and cancer immuno therapy techniques

[00212] In another aspect of the invention, some or all of the single chain portions of anti-MUC1* antibody fragments are biochemically fused onto immune system molecules, using several different chimeric antigen receptor, 'CAR' strategies. The idea is to fuse the recognition portion of an antibody, typically as a single chain variable fragment, to an immune system molecule that has a transmembrane domain and a cytoplasmic tail that is able to transmit signals that activate the immune system. The recognition unit can be an antibody fragment, a single chain variable fragment, scFv, or a peptide. In one aspect, the recognition portion of the extracellular domain of the CAR is comprised of sequences from the humanized variable region of MN-E6 (SEQ ID NOS: 38-39 and 93-94), MN-C2 (SEQ ID NOS: 144-145 and 194-195), MN-C3 (SEQ ID NOS: 439-440 and 486-487) and MN-C8 (SEQ ID NOS: 525-526 and 565-566). In another aspect, it is comprised of sequences from a single chain variable fragment. Examples of single chain constructs are given. Several humanized MN-E6 single chain proteins, scFv, were generated (SEQ ID NOS: 232-237). Several humanized MN-C2 single chain proteins, scFv, were generated (SEQ ID NOS: 238-243). Several humanized MN-C3 single chain proteins, scFv, were generated (SEQ ID NOS: 244-249). Several humanized MN-C8 single chain proteins, scFv, were generated (SEQ ID NOS: 250-255). The transmembrane region of the CAR can be derived from CD8, CD4, antibody domains or other transmembrane region, including the transmembrane region of the proximal cytoplasmic co-stimulatory domain. The cytoplasmic tail of the CAR can be comprised of one or more motifs that

signal immune system activation. This group of cytoplasmic signaling motifs, sometimes referred to as, co-stimulatory cytoplasmic domains, includes but is not limited to CD3-zeta, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICAM-1, LFA-1, ICOS, CD2, CD5, CD7 and Fc receptor gamma domain. A minimal CAR may have the CD3-zeta or an Fc receptor gamma domain then one or two of the above domains in tandem on the cytoplasmic tail. In one aspect, the cytoplasmic tail comprises CD3-zeta, CD28, 4-1BB and/or OX40. Several examples of humanized MN-E6 CARs were generated: CAR MN-E6 CD3z (SEQ ID NOS: 294-295); CAR MN-E6 CD28/CD3z (SEQ ID NOS: 297-298); CAR MN-E6 4-1BB/CD3z (SEQ ID NOS: 300-301); CAR MN-E6 OX40/CD3z (SEQ ID NOS: 616-617); CAR MN-E6 CD28/OX40/CD3z (SEQ ID NOS: 618-619); CAR MN-E6 CD28/4-1BB/CD3z (SEQ ID NOS: 303-304). Several examples of humanized MN-C2 CARs were generated: CAR MN-C2 CD3z (SEQ ID NOS: 606-607); CAR MN-C2 CD28/CD3z (SEQ ID NOS: 608-609); CAR MN-C2 4-1BB/CD3z (SEQ ID NOS: 610-611); CAR MN-C2 OX40/CD3z (SEQ ID NOS: 612-613); CAR MN-C2 CD28/4-1BB/CD3z (SEQ ID NOS: 306-307); CAR MN-C2 CD28/OX40/CD3z (SEQ ID NOS: 614-615). Humanized MN-C3 CAR was generated: CAR MN-C3 4-1BB/CD3z (SEQ ID NOS: 600-601).

[00213] Several examples of humanized MN-E6 CARs with different hinge regions (SEQ ID NOS:345-360) were generated: CAR MN-E6-Fc/8/41BB/CD3z (SEQ ID NOS:310-311); CAR MN-E6 FcH/8/41BB/CD3z (SEQ ID NOS:315-316); CAR MN-E6 Fc/4/41BB/CD3z (SEQ ID NOS:318-319); CAR MN-E6 FcH/4/41BB/CD3z (SEQ ID NOS:321-322); CAR MN-E6 IgD/8/41BB/CD3z (SEQ ID NOS:323-324); CAR MN-E6 IgD/4/41BB/CD3z (SEQ ID NOS:327-328); CAR MN-E6 X4/8/41BB/CD3z (SEQ ID NOS:330-331); CAR MN-E6 X4/4/41BB/CD3z (SEQ ID NOS:333-334); CAR MN-E6 8+4/4/41BB/CD3z (SEQ ID NOS:336-337). In addition, several humanized MN-C3 single chain variable fragment and humanized MN-C8 single chain variable fragments were also generated.

[00214] The extracellular domain recognition unit of a MUC1* targeting CAR can comprise the variable regions of humanized MN-E6, MN-C2, MN-C3 or MN-C8 or other antibody that binds to the PSMGFR portion of MUC1* or a PSMGFR peptide. In one aspect, the extracellular domain recognition unit of a CAR is comprised essentially of a humanized MN-E6, MN-C2, MN-C3 or MN-C8 single chain variable fragment scFv. The transmembrane region of the CAR can be derived from CD8 (SEQ ID NOS:363-364), or can be the transmembrane domain of CD3-zeta, CD28, 41bb, OX40 or other transmembrane region (SEQ ID NOS:361-372) and the cytoplasmic domain of a CAR with antibody fragment targeting MUC1* extracellular domain can be comprised of one or more selected from the group comprising an immune system co-stimulatory cytoplasmic domain.

The group of immune system co-stimulatory domains includes but is not limited to CD3-zeta, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICAM-1, LFA-1, ICOS, CD2, CD5, CD7 and Fc receptor gamma domain (SEQ ID NOS:373-382). Alternatively, the recognition unit portion of a CAR can comprise a peptide wherein the peptide binds to the target. NME7 binds to and activates MUC1*. In one aspect of the invention, the recognition unit of a CAR is a peptide derived from NME7 (SEQ ID NOS: 5-6) or a peptide derived from NME7, including but not limited to NME7 peptide A1 (SEQ ID NO: 7), NME7 peptide A2 (SEQ ID NO: 8), NME7 peptide B1 (SEQ ID NO: 9), NME7 peptide B2 (SEQ ID NO: 10) and NME7 peptide B3 (SEQ ID NO: 11).

[00215] Some strategies for generating CARs include a portion of the molecule that dimerizes with itself. In some cases, dimerization of the target is not desirable. Therefore CARs can be constructed such that they heterodimerize. In one case the recognition unit of the first CAR binds to a first target while the recognition unit of the second CAR binds to a second target. Both recognition units can be antibody fragments, both can be peptides or one can be an antibody fragment and the other a peptide. A first target of the CAR can be the extracellular domain of MUC1*. The recognition unit of the CAR would be comprised of an antibody fragment that binds to MUC1* extracellular domain or to a PSMGFR peptide. Alternatively, the recognition unit of the CAR would be comprised of a peptide that binds to MUC1* extracellular domain, such peptides include peptides derived from an NME protein such as NME1 or NME7, more particularly NME7 derived peptides listed as SEQ ID NOS: 7-11. A second target of a heterodimeric CAR may be a peptide or antibody fragment that binds to NME7. Alternatively, a second target of a heterodimeric CAR may be a peptide or antibody fragment that binds to PD1 or other target on a MUC1*-presenting cell. A second target may be a peptide or antibody fragment that binds to NME1. Because it is desirable to prevent dimerization of MUC1 induced by a CAR, heterodimeric CARs can be constructed so that only the extracellular domain of one molecule has an extracellular recognition unit that binds to a target (SEQ ID NOS:584-587). The other molecule can have a truncated extracellular domain that is devoid of a target recognition unit or antibody fragment (SEQ ID NOS:588-599). The CARs described can be transfected or transduced into a cell of the immune system. In a preferred embodiment, a MUC1* targeting CAR is transfected or transduced into a T cell. In one aspect the T cell is a CD3+/CD28+ T cell. In another case it is a dendritic cell. In another case it is a B cell. In another case it is a mast cell. The recipient cell can be from a patient or from a donor. If from a donor, it can be engineered to remove molecules that would trigger rejection. Cells transfected or transduced with a CAR of the invention can be expanded *ex vivo* or *in vitro* then administered to a patient. Administrative routes are chosen from a group containing but not limited to bone marrow

transplant, intravenous injection, in situ injection or transplant. In a preferred embodiment, the MUC1* targeting CAR is administered to a person diagnosed with or at risk of developing a MUC1-positive cancer.

[00216] There are many possible anti-MUC1* CAR constructs that can be transduced into T cells or other immune cells for the treatment or prevention of MUC1* positive cancers. CARs are made up of modules and the identity of some of the modules is relatively unimportant, while the identity of other modules is critically important.

[00217] Our experiments demonstrate that the antibody recognition fragment at the outermost portion of the CAR is critically important because it targets the immune cell bearing the CAR to the tumor site. The intracellular signaling motifs are also very important but can be interchanged. Figure 28 shows a schematic of the components of CAR and the various sequences that may be included in a CAR. Referring to Figure 28,

[00218] **R1** is: nothing; or

[00219] a ligand or a fragment of a ligand of a cancer associated antigen; or

[00220] a ligand or a fragment of a ligand of MUC1 or MUC1*; or

[00221] an antibody or antibody fragment wherein the antibody or antibody fragment binds to MUC1 or MUC1*; or an antibody or antibody fragment wherein the antibody or antibody fragment binds to PSMGFR*, wherein the antibody may be human or humanized; or an antibody or antibody fragment of MN-E6, MN-C2, MN-C3 or MN-C8 or humanized MN-E6, MN-C2, MN-C3 or MN-C8; or a single chain variable fragment of an antibody, scFv, that binds to a cleaved MUC1 or MUC1*; or a scFv of MN-E6, MN-C2, MN-C3 or MN-C8, which may be humanized; or a peptide that binds to MUC1* or PSMGFR peptide; or is an antibody fragment, a scFv, or a peptide that binds the PSMGFR portion of MUC1*; or is comprised of sequence from the humanized variable region of MN-E6 (SEQ ID NOS: 38-39 and 93-94), MN-C2 (SEQ ID NOS: 144-145 and 194-195), MN-C3 (SEQ ID NOS: 439-440 and 486-487) and MN-C8 (SEQ ID NOS: 525-526 and 565-566). In one aspect R1 is a scFv that binds the PSMGFR portion of MUC1* comprised of sequence from humanized MN-E6 scFv (SEQ ID NOS: 232-237), humanized MN-C2 scFv (SEQ ID NOS: 238-243), humanized MN-C3 scFv (SEQ ID NOS: 244-249) or humanized MN-C8 scFv (SEQ ID NOS: 250-255). In another aspect R1 is a scFv that binds the PSMGFR portion of MUC1* comprised of sequence from humanized MN-E6 scFv (SEQ ID NOS: 232-237) or humanized MN-C2 scFv (SEQ ID NOS: 238-243). In one example R1 is a scFv that binds the PSMGFR portion of MUC1* comprised of sequence from humanized MN-E6 scFv (SEQ ID NOS: 232-237)

[00222] **R2** is a polypeptide flexible linker that connects the recognition portion to the transmembrane domain of the CAR. In one aspect, R2 can be a polypeptide linker of different length from 5 to 250 amino acids. In another aspect, R2 is a polypeptide linker of human origin. In one aspect R2 can be made of or a modification of the Fc region of a human immunoglobulin (IgG, IgA, IgE, IgM or IgD). In another aspect, R2 can be the hinge region or a modification of the hinge region of a human immunoglobulin (IgG, IgA, IgE, IgM or IgD). In one aspect, R2 can be the hinge region or a modification of the hinge region of a T-cell receptor (CD8a, CD28 or CD4). In one example, R2 is the hinge region of CD8a, the hinge region of human IgD or the Fc domain of human IgG1.

[00223] **R3** is a transmembrane domain. In one aspect, R3 can be a transmembrane domain or a modification of a transmembrane domain of any transmembrane human proteins. In another aspect, R3 can be a transmembrane domain or a modification of a transmembrane domain from human cell receptor. In one aspect, R3 can be a transmembrane domain or a modification of a transmembrane domain of a T-cell receptor (CD8a, CD4, CD28, CD3z, OX40 or 41-BB). In another aspect, R3 is a transmembrane domain from the first cytoplasmic co-stimulatory domain of the CAR. In one aspect, R3 can be a transmembrane domain or a modification of a transmembrane domain of a T-cell receptor extended with 1,2,3,4 or 5 amino acids of the cytoplasmic domain associated to the transmembrane domain. In another aspect, R3 can be a transmembrane domain or a modification of a transmembrane domain of a T-cell receptor extended with 1,2,3,4 or five amino acids of the cytoplasmic domain associated to the transmembrane domain followed by a cysteine for disulfide bond formation. In one example, R3 is the transmembrane domain of CD8a or CD4.

[00224] **R4** is a signaling domain from a T-cell receptor. In one aspect, R4 can be the cytoplasmic signaling domain of CD3-zeta, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICAM-1, LFA-1, ICOS, CD2, CD5, CD7 and Fc receptor gamma domain. In one example, R4 is the cytoplasmic domain of CD3-zeta. Several examples of humanized CAR with single signaling domain (CAR I) were regenerated: CAR MN-E6 CD3z (SEQ ID NOS: 294-295); CAR MN-C2 CD3z (SEQ ID NOS: 606-607)

[00225] **R5** is a co-stimulatory domain from a T-cell receptor. In one aspect, R5 can be the cytoplasmic signaling domain of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICAM-1, LFA-1, ICOS, CD2, CD5, CD7 and Fc receptor gamma domain. R5 will be different from R4 and R6. In one example, R5 is the cytoplasmic domain of CD28, 4-1BB or OX40. Several examples of humanized CAR with two signaling domain (CAR II) were regenerated: CAR MN-E6 CD28/CD3z (SEQ ID NOS: 297-298); CAR MN-E6 4-1BB/CD3z (SEQ ID NOS: 300-301); CAR MN-E6 OX40/CD3z (SEQ ID NOS: 616-617); CAR MN-C2 CD28/CD3z (SEQ ID NOS: 608-609); CAR MN-C2 4-

1BB/CD3z (SEQ ID NOS: 610-611); CAR MN-C2 OX40/CD3z (SEQ ID NOS: 612-613); MN-C3 4-1BB/CD3z (SEQ ID NOS: 600-601); CAR MN-E6-Fc/8/41BB/CD3z (SEQ ID NOS:310-311); CAR MN-E6 FcH/8/41BB/CD3z (SEQ ID NOS:315-316); CAR MN-E6 Fc/4/41BB/CD3z (SEQ ID NOS:318-319); CAR MN-E6 FcH/4/41BB/CD3z (SEQ ID NOS:321-322); CAR MN-E6 IgD/8/41BB/CD3z (SEQ ID NOS:323-324); CAR MN-E6 IgD/4/41BB/CD3z (SEQ ID NOS:327-328); CAR MN-E6 X4/8/41BB/CD3z (SEQ ID NOS:330-331); CAR MN-E6 X4/4/41BB/CD3z (SEQ ID NOS:333-334); CAR MN-E6 8+4/4/41BB/CD3z (SEQ ID NOS:336-337).

[00226] R6 is a co-stimulatory domain from a T-cell receptor. In one aspect, R6 can be the cytoplasmic signaling domain of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICAM-1, LFA-1, ICOS, CD2, CD5, CD7 and Fc receptor gamma domain. R6 will be different from R4 and R5. In one example, R5 is the cytoplasmic domain of CD28. Several examples of humanized CAR with two signaling domain (CAR III) were regenerated: CAR MN-E6 CD28/OX40/CD3z (SEQ ID NOS: 618-619); CAR MN-E6 CD28/4-1BB/CD3z (SEQ ID NOS: 303-304); CAR MN-C2 CD28/4-1BB/CD3z (SEQ ID NOS: 306-307); CAR MN-C2 CD28/OX40/CD3z (SEQ ID NOS: 614-615)

[00227] We and others (Pulè MA, Straathof KC, Dotti G, Heslop HE, Rooney CM and Brenner MK. (2005) A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells. *Mol Ther.* 12(5):933-941; Hombach AA, Heiders J, Foppe M, Chmielewski M and Abken H. (2012) OX40 costimulation by a chimeric antigen receptor abrogates CD28 and IL-2 induced IL-10 secretion by redirected CD4(+) T cells. *Oncoimmunology.* 1(4):458-466; Kowolik CM, Topp MS, Gonzalez S, Pfeiffer T, Olivares S, Gonzalez N, Smith DD, Forman SJ, Jensen MC and Cooper LJ. (2006) CD28 costimulation provided through a CD19-specific chimeric antigen receptor enhances in vivo persistence and antitumor efficacy of adoptively transferred T cells. *Cancer Res.* 66(22):10995-11004; Loskog A, Giandomenico V, Rossig C, Pule M, Dotti G and Brenner MK. (2006) Addition of the CD28 signaling domain to chimeric T-cell receptors enhances chimeric T-cell resistance to T regulatory cells. *Leukemia.* 20(10):1819-1828; Milone MC, Fish JD, Carpenito C, Carroll RG, Binder GK, Teachey D, Samanta M, Lakhali M, Gloss B, Danet-Desnoyers G, Campana D, Riley JL, Grupp SA and June CH. (2009) Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. *Mol Ther.* 17(8):1453-1464; Song DG, Ye Q, Carpenito C, Poussin M, Wang LP, Ji C, Figini M, June CH, Coukos G, Powell DJ Jr. (2011) In vivo persistence, tumor localization, and antitumor activity of CAR-engineered T cells is enhanced by costimulatory signaling through CD137 (4-1BB). *Cancer Res.* 71(13):4617-4627) have shown that intracellular signaling modules, such as CD3-zeta (SEQ ID NOS: 373-376), CD28 (SEQ ID NOS: 377-378) and

41BB (SEQ ID NOS: 379-380), alone or in combinations stimulate immune cell expansion, cytokine secretion and immune cell mediated killing of the targeted tumor cells. Less important is the identity of the short extracellular piece that presents the antibody fragment, the transmembrane domain, and the short cytoplasmic tail that comes before the intracellular signaling motifs.

[00228] The identity of the recognition antibody fragment that targets the CAR to a tumor is critically important. For the treatment of MUC1 positive or MUC1* positive cancers, that antibody recognition fragment must bind to the extracellular domain of portion of MUC1 that remains after cleavage and shedding of the bulk of the extracellular domain, which contains the tandem repeat domains. In one aspect of the invention, the portion that remains comprises the PSMGFR sequence. In another aspect of the invention, the portion of MUC1 that remains after cleavage and shedding contains the PSMGFR sequence plus nine (9) more amino acids extended at the N-terminus. In another aspect of the invention, the portion of MUC1 that remains after cleavage and shedding contains the PSMGFR sequence plus twenty one (21) more amino acids extended at the N-terminus. In one aspect the antibody recognition fragment binds to a PSMGFR peptide. In another aspect of the invention, the antibody recognition fragment binds to a peptide comprising the sequence SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620); or SVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621) As a demonstration, a single chain antibody fragment that included the variable domain of the monoclonal anti-MUC1* antibodies called MN-E6 or MN-C2 were engineered into a panel of CARs. The MUC1* targeting CARs were then transduced, separately or in combinations, into immune cells. When challenged with surfaces presenting a MUC1* peptide, an antigen presenting cell transfected with MUC1*, or MUC1* positive cancer cells, the immune cells that were transduced with MUC1* targeting CARs elicited immune responses, including cytokine release, killing of the targeted cells and expansion of the immune cells. In one case, human jurkat cells were transduced with MUC1*-targeting CARs and upon exposure to a surface presenting the PSMGFR peptide, K562 antigen presenting cells that had been transfected with MUC1* or MUC1* positive cancer cells, the jurkat cells secreted IL-2. In another case, purified human T cells were transduced with MUC1*-targeting CARs and upon exposure to a surface presenting the PSMGFR peptide, K562 antigen presenting cells that had been transfected with MUC1* or MUC1* positive cancer cells, the T cells secreted IL-2, interferon gamma, and killed the targeted antigen presenting cells and cancer cells, while the T cells expanded. As demonstrated, CARs that comprise an antibody fragment, wherein the antibody fragment is able to bind to the PSMGFR peptide, a transmembrane domain and a cytoplasmic tail bearing co-stimulatory domains, elicit an immune system anti-tumor cell response when said CARs are

transduced into immune cells, which include T cells. Therefore, other antibodies, antibody fragments or antibody mimics that are able to bind to the PSMGFR peptide will perform similarly and can be used to treat or prevent cancers. Those skilled in the art will recognize that there are a number of technologies available for transfecting or transducing cells with CARs and the invention is not limited by the method used for making the immune cell express a MUC1*-targeting CAR. For example, retroviruses, adeno viruses, lenti viruses and the like can be used. Similarly, the identity of molecules that make up the non-targeting, portions of the CAR such as the extracellular domain, transmembrane domain and membrane proximal portion of the cytoplasmic domain, are not essential to the function of a MUC1*-targeting CAR. For example, the extracellular domain, transmembrane domain and membrane proximal portion of the cytoplasmic domain can be comprised of portions of CD8, CD4, CD28, or generic antibody domains such as Fc, CH2CH3, or CH3. Further, the non-targeting portions of a CAR can be a composite of portions of one or more of these molecules or other family members.

[00229] One aspect of the invention is a method for treating a patient diagnosed with, suspected of having, or at risk of developing a MUC1 positive or MUC1* positive cancer, wherein the patient is administered an effective amount of immune cells that have been transduced with a MUC1* targeting CAR. In another aspect of the invention, the immune cells are T cells isolated from a patient, which are then transduced with CARs wherein the targeting head of the CAR binds to MUC1*, and after expansion of transduced T cells, the CAR T cells are administered in an effective amount to the patient. In yet another aspect of the invention, the immune cells are T cells isolated from a patient, which are then transduced with CARs wherein the targeting head of the CAR comprises portions of huMN-E6, huMN-C2, huMN-C3 or huMN-C8, and after optional expansion of transduced T cells, the CAR T cells are administered in an effective amount to the patient.

[00230] Specifics of CARs made and tested

[00231] Many MUC1* targeting CARs were generated wherein the targeting antibody fragment at the distal end of the CAR was either MN-E6, MN-C2, MN-C3 or MN-C8. The DNA of each CAR was sequenced to verify that cloning was correctly done. Each construct was then shuffled into an expression plasmid, transfected into cells and then verified that the construct had successfully inserted by Western blot. Surface expression was verified by FACS. The MUC1* targeting CARs were then virally transduced into immune cells. In one aspect they were transduced into Jurkat cells. In another aspect they were transduced into primary human T cells that were purified from blood. A series of functional assays were performed and verified that the CARs were functional. Functional assays showed that both Jurkat cells and primary T cells transduced with MUC1* targeting CAR

secreted the cytokine IL-2 when challenged with cells presenting MUC1*. **Figure 29** is a graph of an experiment measuring IL-2 cytokine secretion by Jurkat cells that were transduced with a panel of CARs, including MN-E6 CD8/CD3z, MN-E6 CD8/CD28/CD3z, MN-E6 CD8/41BB/CD3z, MN-E6 CD4/CD28/CD3z and MN-E6 CD4/CD28/41BB/CD3z. IL-2 was secreted only when the CAR Jurkat cells were exposed to K562-wt cells or K562 cells that had been transfected with MUC1*. It should be noted that the parent K562-wt cells express very low levels of MUC1*. Another group of CARs transfected into Jurkat cells was similarly tested for cytokine secretion. **Figure 30** shows IL-2 secretion by Jurkat T cells that were transduced with MN-E6 CD8/CD28/CD3z, MN-E6 CD8/41BB/CD3z, MN-E6 CD4/CD28/CD3z or MN-E6 CD4/41BB/CD3z, when the CAR T cells were exposed to K562-wt cells or K562 cells that had been transfected with MUC1*. Similarly, **Figure 31** shows IL-2 cytokine secretion by primary human T cells that were transduced with MN-E6 CD8/CD28/CD3z, MN-E6 CD8/41BB/CD3z or MN-E6 CD4/41BB/CD3z. Cytokine secretion only occurred when the MUC1* targeting CAR T cells were exposed to K562-wt cells or K562 cells that had been transfected with MUC1*. Another cytokine that is secreted by activated T cells when they see a target cell is interferon-gamma (IFN-g). **Figure 32** shows that interferon-gamma was secreted by primary human T cells that were transduced with a panel of CARs, including MN-E6 CD8/CD28/CD3z and MN-E6 CD4/41BB/CD3z, when the CAR T cells were exposed to K562-wt cells or K562 cells that had been transfected with MUC1*. Interferon-gamma was similarly secreted by primary human T cells that were transduced with a panel of CARs, including MN-E6 CD8/CD28/CD3z, MN-E6 CD8/41BB/CD3z and MN-E6 CD8/CD28/41BB/CD3z, when the MUC1* targeting CAR T cells were exposed to K562-wt cells, K562 cells that had been transfected with MUC1*, or MUC1* positive cancer cells of prostate cancer (DU145), breast cancer (1500) or pancreatic cancer (Capan) (**Figure 33**).

[00232] Another measure of function of CAR T cells is whether or not they induce killing of the targeted cells. T cells transfected with a variety of CARs comprising antibody fragments that bind to the PSMGFR sequence of MUC1* killed MUC1* expressing cells in co-culture assays. In one assay, target MUC1* expressing cells are incubated with calcein. When they are mixed with CAR T cells wherein the CAR comprises an antibody fragment such as MN-E6, MN-C2, MN-C3 or MN-C8 the CAR T cells kill the MUC1* presenting cells which causes the target cells to lyse and releases calcein into the supernatant. **Figure 34** is a graph of an experiment measuring target cell death when primary human T cells, isolated from a blood sample, that were transduced with a panel of CARs, including MN-E6 CD8/CD28/CD3z, MN-E6 CD8/41BB/CD3z and MN-E6 CD4/41BB/CD3z, when the CAR T cells were exposed to K562-wt cells or K562 cells that had been transfected with

MUC1*. The ratio of T cells to target cells was 1:1 and the cells were co-cultured for 24 hours. **Figure 35 A-B** is a graph of FACS measuring a time course of target cell survival from Day 1 to Day 3. Primary human T cells, isolated from a blood sample, were transduced with a panel of CARs, including humanized MN-E6-CD8-3z, MN-E6-CD8-CD28-3z, MN-E6-CD8-41BB-3z and MN-E6-CD8-CD28-41BB-3z. The CAR T cells were then exposed to K562-wt cells that naturally express low levels of MUC1*, or K562 cells that had been transfected with MUC1* high. The ratio of MUC1* targeting CAR T cells to target cells was either 1:1, 10:1, or 20:1. Surviving cells were detected and measured at Day 1 (A) or Day 3 (B).

[00233] **Figure 36** is a graph of FACS measurements of target cell survival at Day 3 of co-culture experiment. Primary human T cells were transduced with a panel of CARs, including humanized MN-E6-CD8-3z, MN-E6-CD8-CD28-3z, MN-E6-CD8-41BB-3z and MN-E6-CD8-CD28-41BB-3z. The CAR T cells were then exposed to MUC1* positive T47D breast cancer cells or MUC1* positive 1500 aka ZR-75-1 breast cancer cells. The ratio of MUC1* targeting CAR T cells to target cells was either 1:1 or 10:1. As can be seen from the graph, T cells transduced with a MUC1* targeting CAR have a much greater killing effect on MUC1* cancer cells than the untransduced control T cells. In addition, the killing effect is much greater when the ratio of T cells: target cells is increased. **Figure 37** is a graph of FACS measurements of target cell survival at Day 1 of co-culture experiment. Primary human T cells were transduced with a panel of CARs, including humanized MN-E6-CD8-41BB-3z, MN-E6-CD4-41BB-3z, and MN-E6-CD8-CD28-41BB-3z. The CAR T cells were then exposed to the following MUC1* positive cancer cells: T47D breast cancer; capan2 pancreatic cancer; or DU-145 prostate cancer. The ratio of MUC1* targeting CAR T cells to target cells was 5:1. As can be seen from the graph, T cells transduced with a MUC1* targeting CAR have a much greater killing effect on MUC1* cancer cells than the untransduced control T cells. Note that the measurements were taken after 24 hours with only a 5:1 T cell to target cell ratio. Also note that MUC1* targeting CARs that have a CD4 extracellular domain-transmembrane-cytoplasmic tail work equally well as CD8 constructs. **Figure 38** is a graph of FACS measurements of target cell survival at Day 3 of co-culture experiment. Primary human T cells were transduced with a panel of CARs, including humanized MN-E6-CD8-41BB-3z, MN-E6-CD4-41BB-3z, and MN-E6-CD8-CD28-41BB-3z. The CAR T cells were then exposed to the following MUC1* positive cancer cells: K562 leukemia cells transfected with MUC1*; T47D breast cancer; 1500 aka ZR-75-1 breast cancer cells ; or CAPAN-2 pancreatic cancer cells. In addition to the untransduced T cell controls, the assay was performed on PC3 MUC1* negative prostate cancer cells. The ratio of MUC1* targeting CAR T cells to target cells was 1:1. As can be seen from the graph, T cells transduced with a MUC1*

targeting CAR have a much greater killing effect on MUC1* cancer cells than the untransduced control T cells. In addition, the killing effect is specific for MUC1* positive cells. Note that MUC1* targeting CARs that have a CD4 extracellular domain-transmembrane-cytoplasmic tail work equally well as CD8 constructs. **Figure 39** is a graph of FACS measurements of CAR T cell expansion over 24 hours in co-culture with target cells at a ratio of 5:1 CAR T cells to target cells. The primary human T cells were transduced with a panel of CARs, including humanized MN-E6-CD8-41BB-3z, MN-E6-CD4-41BB-3z, and MN-E6-CD8-CD28-41BB-3z. The CAR T cells were co-cultured with MUC1* positive T47D breast cancer cells, MUC1* positive Capan pancreatic cancer cells, and MUC1-negative cells HCT-116 colon cancer cells and HEK-293 human embryonic kidney cells. As can be seen from the graph, the CAR T population is increased in the presence of MUC1* positive cells. **Figure 40** shows a photograph of a Western blot of MUC1* targeting CARs. From 1 to 9 are: 1. MN-E6scFv-Fc-8-41BB-CD3z (Human Fc as hinge region with CD8 TM); 2. MN-E6scFv-FcH-8-41BB-CD3z (Human Fc hingeless as hinge region with CD8 TM); 3. MN-E6scFv-Fc-4-41BB-CD3z (Human Fc as hinge region with CD4 TM); 4. MN-E6scFv-FcH-4-41BB-CD3z (Human Fc as hingeless hinge region with CD4 TM); 5. MN-E6scFv-IgD-8-41BB-CD3z (hinge region from human IgD with CD8 TM); 6. MN-E6scFv-IgD-4-41BB-CD3z (hinge region from human IgD with CD4 TM); 7. MN-E6scFv-X4-8-41BB-CD3z (Long flexible linker as hinge region with CD8 TM); 8. MN-E6scFv-X4-4-41BB-CD3z (Long flexible linker as hinge region with CD4 TM); 9. MN-E6scFv-8-4-41BB-CD3z (Hinge region from CD8 and CD4 a with CD4 TM).

[00234] **Figure 41** shows graphs of FACS scans of T47D breast cancer cells co-cultured with human T cells that were transduced with MN- E6scFv-Fc-8-41BB-CD3z, MN-E6scFv-FcH-8-41BB-CD3z (hingeless), MN-E6scFv-Fc-4-41BB-CD3z, MN-E6scFv-IgD-8-41BB-CD3z, MN-E6scFv-X4-8-41BB-CD3z and MN-E6scFv-X4-4-41BB-CD3z. T cells and cancer cells were co-cultured at a 1:1 ratio for 48 hours. T cell counts were normalized to an average of all untransduced T cells and target cells were normalized to each specific cell type when co-cultured with untransduced T cells. The graph shows that when the CAR T cells are co-cultured with MUC1* positive cancer cells, the T cell population expands and the targeted cancer cell population decreases.

[00235] **Figure 42** shows graphs of FACS scans of T47D breast cancer cells, Capan-2 pancreatic cancer cells, K562-MUC1* transfected cells, and K562-wt cells that were co-cultured with human T cells that were transduced with MN- E6scFv-Fc-8-41BB-CD3z, MN-E6scFv-FcH-8-41BB-CD3z, MN-E6scFv-Fc-4-41BB-CD3z, MN-E6scFv-IgD-8-41BB-CD3z, MN-E6scFv-X4-8-41BB-CD3z and MN-E6scFv-X4-4-41BB-CD3z. T cells and cancer cells were co-cultured at a 1:1 ratio for 48 hours. T cell counts were normalized to an average of all untransduced T cells and target cells were

normalized to each specific cell type when co-cultured with untransduced T cells. The graph shows that when the CAR T cells are co-cultured with MUC1* positive cancer cells, the T cell population expands and the targeted cancer cell population decreases.

[00236] As these experiments demonstrate, the critical portion of a CAR is the antibody fragment that directs the immune cell to the tumor cell. As we will show in the following section, MN-E6 and MN-C2 are specific for the form of MUC1* that is expressed on tumor cells. The next most important part of a CAR is the cytoplasmic tail bearing immune system co-stimulatory domains. The identity of these domains modulates the degree of immune response but in no way effect the specificity. As shown, the identity of the transmembrane portion of a CAR is the least important. It appears that as long as the transmembrane portion has some flexibility and is long enough to allow the antibody fragment to reach its cognate receptor on the tumor cell, it will suffice. This is demonstrated in Figures 40-42. CARs comprising the MN-E6 targeting antibody fragment, and intracellular co-stimulatory domains 41BB and CD3-zeta but having a variety of different extracellular, transmembrane and short cytoplasmic tail all worked in that they specifically killed the targeted cells while stimulating the expansion of the host T cells. These CARs with variable mid-sections are: MN-E6scFv-Fc-8-41BB-CD3z (Human Fc as hinge region with CD8 TM); 2: MN-E6scFv-FcH-8-41BB-CD3z (Human Fc hingeless as hinge region with CD8 TM); 3: MN-E6scFv-Fc-4-41BB-CD3z (Human Fc as hinge region with CD4 TM); 4: MN-E6scFv-FcH-4-41BB-CD3z (Human Fc as hingeless hinge region with CD4 TM); 5: MN-E6scFv-IgD-8-41BB-CD3z (hinge region from human IgD with CD8 TM); 6: MN-E6scFv-IgD-4-41BB-CD3z (hinge region from human IgD with CD4 TM); 7: MN-E6scFv-X4-8-41BB-CD3z (Long flexible linker as hinge region with CD8 TM); 8: MN-E6scFv-X4-4-41BB-CD3z (Long flexible linker as hinge region with CD4 TM); 9: MN-E6scFv-8-4-41BB-CD3z (Hinge region from CD8 and CD4 a with CD4 TM).

[00237] One aspect of the invention is a method for treating a patient diagnosed with, suspected of having, or at risk of developing a MUC1 positive or MUC1* positive cancer, wherein the patient is administered an effective amount of immune cells that have been transduced with a MUC1* targeting CAR, wherein the CAR is chosen from among the group consisting of MN-E6-CD8-3z; MN-E6-CD4-3z; MN-E6-CD8-CD28-3z; MN-E6-CD4-CD28-3z; MN-E6-CD8-41BB-3z; MN-E6-CD4-41BB-3z; MN-E6-CD8-CD28-41BB-3z; MN-E6-CD4-CD28-41BB-3z; MN-E6scFv-Fc-8-41BB-CD3z; MN-E6scFv-FcH-8-41BB-CD3z; MN-E6scFv-Fc-4-41BB-CD3z; MN-E6scFv-FcH-4-41BB-CD3z; MN-E6scFv-IgD-8-41BB-CD3z; MN-E6scFv-IgD-4-41BB-CD3z; MN-E6scFv-X4-8-41BB-CD3z; MN-E6scFv-X4-4-41BB-CD3z; MN-E6scFv-8-4-41BB-CD3z, or any of the aforementioned CARs wherein the MN-E6 is replaced by MN-C2, MN-C3 or MN-C8. Another

aspect of the invention is a method for treating a patient diagnosed with, suspected of having, or at risk of developing a cancer, wherein the patient is administered an effective amount of immune cells that have been transduced with one of the aforementioned CARs wherein the MN-E6 is replaced by a peptide comprising antibody variable domain fragments that are specific for a cancer antigen. In any of the above methods, the immune cell may be a T cell and may further be isolated from the patient to be treated.

[00238] Specificity of anti-MUC1* targeting antibodies

[00239] The most accurate way of demonstrating antibody specificity is testing the antibody on normal human tissue specimens compared to cancerous tissue specimens. MN-C2 and MN-E6 were shown to specifically bind to MUC1 or MUC1* positive cancer cells. Several breast tumor arrays were assayed using several anti-MUC1 or MUC1* antibodies. Essentially the studies involving serial sections of breast cancer tissue specimens from over 1,200 different breast cancer patients showed that very little full-length MUC1 remains on breast cancer tissues. The vast majority of the MUC1 expressed is MUC1* and is stained by MN-C2. The analysis was performed by Clariant Diagnostics and tissue staining was scored using the Allred method. For example, Figure 43 shows serial sections of breast cancer tissue arrays that were stained with either VU4H5, a commercially available anti-MUC1 antibody that binds to the tandem repeats, or MN-C2 that binds to MUC1*. **Figure 43** and 44 are photographs of breast cancer tissue arrays stained with either VU4H5 which recognizes MUC1-FL (full length) or MN-C2 which recognizes cancerous MUC1*. Tissue staining was scored using Allred scoring method which combines an intensity score and a distribution score. Below the photographs of the tissue arrays are color-coded graphs displaying the results. As can be seen, the arrays stained with VU4H5 are very light and many tissues do not stain at all despite the published reports that MUC1 is aberrantly expressed on over 96% of all breast cancers as evidenced by nucleic acid based diagnostics. In contrast, the arrays stained with MN-C2 are very dark (red versus yellow or white in graph). Additionally, many tissues did not stain at all with anti-full-length MUC1 but stained very dark with MN-C2, (see green boxes in graph). Similarly, we stained normal or cancerous breast tissues with humanized MN-E6 scFv-Fc. The antibody fragment was biotinylated so it could be visualized by a secondary streptavidin based secondary. As can be seen in Figure 45, hMN-E6 scFv-Fc does not stain normal breast tissue but stains cancerous breast tissue. Further, the intensity and homogeneity of staining increases with tumor grade and/or metastatic grade of the patient (Figures 45 and 46). Similarly, hMN-E6 scFv-Fc did not stain normal lung tissue but did stain lung cancer tissue (Figures 47 – 51) and the intensity and distribution of staining increased as tumor grade or metastatic grade increased. **Figure 52** shows photographs of normal

small intestine and cancerous small intestine tissues stained with humanized MN-E6-scFv-Fc biotinylated anti-MUC1* antibody at 5 ug/mL, then stained with a secondary streptavidin HRP antibody. A) is a normal small intestine tissue. B) is small intestine cancer from patient as denoted in the figure. C,D are photographs of the corresponding serial sections that were stained with the secondary antibody alone. **Figure 53** shows photographs of normal small intestine tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are normal small intestine tissue. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone. **Figure 54** shows photographs of cancerous small intestine tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are cancerous small intestine tissue from a patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone. **Figure 55** shows photographs of cancerous small intestine tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are cancerous small intestine tissue from a patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone. **Figure 56** shows photographs of normal colon tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are normal colon. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone. **Figure 57** shows photographs of colon cancer tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are colon cancer tissue from a metastatic patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone. **Figure 58** shows photographs of colon cancer tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are colon cancer tissue from a Grade 2 patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone. **Figure 59** shows photographs of colon cancer tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are colon cancer tissue from a metastatic patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone. **Figure 60** shows photographs of prostate cancer tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP

antibody. A-D are prostate cancer tissue from a patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone. **Figure 61** shows photographs of prostate cancer tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are prostate cancer tissue from a patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone. **Figure 62** shows photographs of prostate cancer tissues stained with humanized MN-E6-scFv-Fc anti-MUC1* antibody at 50 ug/mL, then stained with a secondary goat-anti-human HRP antibody. A-D are prostate cancer tissue from a patient as denoted in figure. E-H are photographs of the corresponding serial sections that were stained with the secondary antibody alone.

[00240] One aspect of the invention is a method for treating a patient diagnosed with, suspected of having, or at risk of developing a MUC1 positive or MUC1* positive cancer, wherein a specimen is obtained from the patient's cancer and is tested for reactivity with an antibody that binds to PSMGFR SEQ ID NO:2, SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620) or SVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621). The patient is then treated with an scFv, scFv-Fc or CAR T that comprises antibody variable fragments from the antibody that reacted with their cancer specimen. Another aspect of the invention is a method for treating a patient diagnosed with, suspected of having, or at risk of developing a MUC1 positive or MUC1* positive cancer, wherein a specimen is obtained from the patient's cancer and is tested for reactivity with MN-E6-scFv, MN-C2-scFv, MN-C3-scFv or MN-C8-scFv; the patient is then treated with the scFv, scFv-Fc-mut or CAR T that comprises portions of the antibody that reacted with their cancer specimen.

[00241] BiTEs

[00242] Divalent (or bivalent) single-chain variable fragments (di-scFvs, bi-scFvs) can be engineered by linking two scFvs. This can be done by producing a single peptide chain with two V_H and two V_L regions, yielding tandem scFvs. Another possibility is the creation of scFvs with linker peptides that are too short for the two variable regions to fold together (about five amino acids), forcing scFvs to dimerize. This type is known as diabodies. Diabodies have been shown to have dissociation constants up to 40-fold lower than corresponding scFvs, meaning that they have a much higher affinity to their target. Consequently, diabody drugs could be dosed much lower than other therapeutic antibodies and are capable of highly specific targeting of tumors *in vivo*. Still shorter linkers (one or two amino acids) lead to the formation of trimers, so-

called triabodies or tribodies. Tetrabodies have also been produced. They exhibit an even higher affinity to their targets than diabodies.

[00243] All of these formats can be composed from variable fragments with specificity for two different antigens, in which case they are types of bispecific antibodies. The furthest developed of these are bispecific tandem di-scFvs, known as bi-specific T-cell engagers (BiTE antibody constructs). BiTEs are fusion proteins consisting of two scFvs of different antibodies, on a single peptide chain of about 55 kilodaltons. One of the scFvs may bind to T cells such as via the CD3 receptor, and the other to a tumor cell via a tumor specific molecule, such as aberrantly expressed MUC1*.

[00244] Another aspect of the invention is a method for treating a patient diagnosed with, suspected of having, or at risk of developing a MUC1 positive or MUC1* positive cancer, wherein the patient is administered an effective amount of a BiTE wherein one antibody variable fragment of the BiTE binds to a T cell surface antigen and the other antibody variable fragment of the BiTE binds to PSMGFR SEQ ID NO:2, SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620) or SVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621). In one case, the antibody variable fragment of the BiTE that binds to MUC1* comprises portions of huMN-E6, huMN-C2, huMN-C3, or huMN-C8.

[00245] In another aspect of the invention, MUC1* peptides including PSMGFR SEQ ID NO:2, most or all of SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620) or SVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621) are used in adoptive T cell approaches. In this case, a patient's T cells are exposed to the MUC1* peptides and through various rounds of maturation, the T cells develop MUC1* specific receptors. The adapted T cells are then expanded and administered to the donor patient who is diagnosed with, suspected of having, or is at risk of developing a MUC1* positive cancer.

[00246] Other MUC1 cleavage sites

[00247] However, MUC1 is cleaved to the growth factor receptor form, MUC1*, on some healthy cells in addition to cancer cells. For example, MUC1 is cleaved to MUC1* on healthy stem and progenitor cells. A large percentage of bone marrow cells are MUC1* positive. Portions of the intestine are MUC1* positive.

[00248] The inventors have discovered that MUC1 can be cleaved at different positions that are relatively close to each other but the location of cleavage changes the fold of the remaining portion of the extracellular domain. As a result, monoclonal antibodies can be identified that bind to MUC1*

cleaved at a first position but do not bind to MUC1* that has been cleaved at a second position. This discovery is disclosed in WO2014/028668, filed August 14, 2013, the contents of which are incorporated by reference herein its entirety. We identified a set of anti-MUC1* monoclonal antibodies that bind to a MUC1* as it appears on cancer cells but do not bind to MUC1* as it appears on stem and progenitor cells. Conversely, we identified a second set of monoclonal antibodies that bind to stem and progenitor cells but do not bind to cancer cells. One method used to identify stem specific antibodies is as follows: supernatants from monoclonal hybridomas were separately adsorbed onto 2 multi-well plates. Stem cells, which are non-adherent cells, were put into one plate and cancer cells which are adherent were put into an identical plate. After an incubation period, the plates were rinsed and inverted. If the non-adherent stem cells stuck to the plate, then the monoclonal in that particular well recognizes stem cells and will not recognize cancer cells. Antibodies that did not capture stem cells or antibodies that captured cancer cells were identified as cancer specific stem cells. FACS analysis has confirmed this method works. Antibodies MN-E6 and MN-C2 are examples of cancer-specific antibodies. Antibodies MN-C3 and MN-C8 are examples of stem-specific antibodies. Although both sets of antibodies are able to bind to a peptide having the PSMGFR sequence, FACS analysis shows that the anti-MUC1* polyclonal antibody and MN-C3 bind to MUC1* positive bone marrow cells but MN-E6 does not. The MUC1* polyclonal antibody was generated by immunizing a rabbit with the PSMGFR peptide. Similarly, MN-C3 binds to stem cells of the intestinal crypts but MN-E6 does not. Conversely, MN-E6 antibody binds to cancerous tissue while the stem-specific MN-C3 does not. Competition ELISA experiments indicate that the C-terminal 10 amino acids of the PSMGFR peptide are required for MN-E6 and MN-C2 binding, but not for MN-C3 and MN-C8. Therefore, another method for identifying antibodies that are cancer specific is to immunize with a peptide having the sequence of the PSMGFR peptide minus the 10 N-terminal amino acids or use that peptide to screen for antibodies or antibody fragments that will be cancer specific. Antibodies that bind to a peptide with a sequence of PSMGFR peptide minus the N-terminal 10 amino acids but do not bind to a peptide with a sequence of PSMGFR peptide minus the C-terminal 10 amino acids are cancer specific antibodies for use in the treatment or prevention of cancers.

[00249] The extracellular domain of MUC1 is also cleaved on stem cells and some progenitor cells, where activation of cleaved MUC1 by ligands NME1 in dimer form or NME7 promotes growth and pluripotency and inhibits differentiation. The transmembrane portion of MUC1 that remains after cleavage is called MUC1* and the extracellular domain is comprised essentially of the Primary Sequence of MUC1 Growth Factor Receptor (PSMGFR) sequence. However, the exact site

of cleavage can vary depending on cell type, tissue type, or which cleavage enzyme a particular person expresses or overexpresses. In addition to the cleavage site that we previously identified which leaves the transmembrane portion of MUC1* comprising most or all of the PSMGFR SEQ ID NO:2, other cleavage sites result in an extended MUC1* comprised of most or all of SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620); or SVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621). The site of MUC1 cleavage affects how the remaining extracellular domain folds. We have identified monoclonal antibodies that bind to cleaved MUC1* on cancer cells but do not bind to cleaved MUC1* as it exists on healthy stem and progenitor cells.

[00250] Whereas an anti-MUC1* antibody or antibody-like molecule may be most effective if it competitively inhibits the binding of NME1, NME6, NME8 or NME7 or NME7-AB to MUC1*, for example an antibody that binds to the PSMGFR sequence especially if said antibody is unable to bind to a PSMGFR peptide if the 10 C-terminal amino acids are missing, antibodies or antibody-like molecules that carry a payload need not competitively inhibit the binding of MUC1* ligands to be effective as anti-cancer agents. For example antibodies or antibody-like molecules that are conjugated to a toxin could be effective at killing target cancer cells without necessarily inhibiting binding of the activating ligands. For example, antibodies or antibody-like molecules such as CARs or BiTEs which recruit the patient's immune system to the tumor can be effective as anti-cancer agents even if the antibody fragment targets a portion of MUC1* such that antibody fragment binding does not competitively inhibit the binding of NME1, NME6, NME8, NME7-AB or NME7. In a preferred embodiment the antibody fragment incorporated into a CAR, an adaptive T cell receptor or a BiTE does competitively inhibit the binding of NME1, NME6, NME8, NME7-AB or NME7 to MUC1*.

[00251] Antibodies that are able to bind to the extracellular domain of the remaining transmembrane portion block the interaction between the MUC1* extracellular domain and activating ligands and in this way can be used as therapeutic agents, for example for the treatment of cancers. Anti-MUC1* antibodies are also useful for the growth, delivery, identification or isolation of stem cells both *in vitro* and *in vivo*.

[00252] General Strategy for using antibodies, antibody fragments and CARs that target the extracellular domain of MUC1*

[00253] Monoclonal antibodies MN-C3 and MN-C8 have a greater binding affinity for stem cells than cancer cells. Humanized antibodies and antibody fragments containing sequences derived from

the variable regions of MN-C3 and MN-C8 can be used as an adhesion surface coating for human stem cells.

[00254] Alternatively, humanized antibodies and antibody fragments containing sequences derived from the variable regions of MN-C3 and MN-C8 can be used to deliver stem cells to a specific location such as for in situ human therapeutics. In one case, a substrate coated with humanized MN-C3 or MN-C8 derived antibodies or antibody fragments is loaded with stem cells then inserted into a patient. In another case, a substrate coated with humanized MN-C3 or MN-C8 derived antibodies or antibody fragments is inserted into a patient in order to recruit the patient's own stem cells to a specific area for therapy. Human therapies in which antibodies that bind to human stem cells will be of therapeutic use include spinal cord repair. Substrates coated with humanized MN-C3 or MN-C8 derived antibodies or antibody fragments are also used to identify or isolate human antibodies. Humanized MN-C3 or MN-C8 derived antibodies can also be used to stimulate the growth of stem cells.

[00255] Sequence Listing Free Text

[00256] As regards the use of nucleotide symbols other than a, g, c, t, they follow the convention set forth in WIPO Standard ST.25, Appendix 2, Table 1, wherein k represents t or g; n represents a, c, t or g; m represents a or c; r represents a or g; s represents c or g; w represents a or t and y represents c or t.

MUC1 Receptor

(Mucin 1 precursor, Genbank Accession number: P15941)

MTPGTQSPFFLLLLLLTVLTVVTGSGHASSTPGGEKETSATQRSSVPSSTEKNAVSMTSSVLSSHSPGSGSSTT
 QGQDVT LAPATEPASGSAATWGQDVT SVPVTRPALGSTTPPAHDVTSAPDNKPAPGSTAPPAHGVTSAPDTRP
 APGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTS
 SAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTA
 PPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTR
 PAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGV
 TSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGST
 APPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDT
 RPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAH
 VTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGS
 TAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPD
 TRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAH
 GVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDNRPALG
 STAPPVHNVTASGSASGSASTLVHNGTSARATTTASKSTPFSIPSHSDTPTTLASHSTKTDASSTHHSSV
 PPLTSSNHSTSPQLSTGVSFLLSFHISNLQFNSSLED PSTDYYQELQRDISEMFLQIYKQGGFLGLSNIKFR
 PGSVVVQLTLAFREGTINVHDVETQFNQYKTEAASRYNLTISDVSVDVPFPFSAQSGAGVPGWGIALLVLC
 VLVALAIVYLIALAVCQCRKNYGLDIFPARDTYHPMSEYPTYHTHGRYVPPSSSTRSPYEKVSAGNGGSSL
 SYTNPAVAAAANL (SEQ ID NO:1)

PSMGFR

GTINVHDVETQFNQYKTEAASRYNLTISDVSVDVPFPFSAQSGA (SEQ ID NO: 2)

Human NME1

(DNA)

atggccaactgtgagcgtaccttcattgcgatcaaaccagatgggggtccagcgggggtcttgtgggagagatta
tcaagcgtttttgagcagaaaaggattccgccttggttggtctgaaattcatgcaagcttccgaagatcttctcaa
ggaacactacgttgacctgaaggaccgtccattctttgcccggcctgggtgaaatacatgcactcagggccggta
gttgccatgggtctgggaggggctgaatgtggtgaagacggggccgagtcagtcggtgggagaccaaccctgcag
actccaagcctgggacctccgtggagacttctgcatacaagttggcaggaacattatacatggcagtgattc
tgtggagagtgcagagaaggagatcggttgggtttcacccctgaggaactggtagattacacgagctgtgct
cagaactggatctatgaatga (SEQ ID NO:3)

(amino acids)

MANCERTFIAIKPDGVQRGLVGEIIKRFEQKGFRLVGLKFMQASEDLLKEHYVDLKDPRFFAGLVKYMHS GPV
VAMVWEGLNVVKTGRVMLGETNPADSKPGTIRGDFCIQVGRNIIHGSDSVESAEKEIGLWFHPEELVDYTSCA
QNWIIYE- (SEQ ID NO:4)

Human NME7

(DNA)

atgaatcatagtgaagattcggttttcattgcagagtggatgatccaaatgcttcacttcttcgacggttatg
agctttttattttaccaggggatggatctggtgaaatgcatgatgtaagaatcatcgacacctttttaagcg
gaccaaataatgataacctgcacttggaagatttatttataggcaacaaagtgaatgtcttttctcgacaactg
gtattaattgactatggggatcaatatacagctcgccagctgggcagtaggaaagaaaaaacgctagccctaa
ttaaacagatgcaatatcaaaggctggagaaataattgaaataataaacaagctggatttactataaccaa
actcaaaatgatgatgctttcaaggaaagaagcattggattttcatgtagatcaccagtcagagacctttttc
aatgagctgatccagtttattacaactggctcctattattgccatggagattttaagagatgatgctatatgtg
aatggaaaagactgctgggacctgcaaactctggagtggcagcacagatgcttctgaaagcattagagccct
ctttggaacagatggcataagaaatgcagcgcagtgccctgattcttttgcttctgcccagagaaatggag
ttgttttttcttcaagtggaggttggtgggcccggcaaacactgctaaatttactaattgtacctgttgcatg
ttaaaccccatgctgtcagtgaggactgttgggaaagatcctgatggtatccgagatgcaggttttgaaat
ctcagctatgcagatgttcaatatggatcgggttaattgttgaggaattctatgaagtttataaaggagtagtg
accgaatatcatgacatggtgacagaaatgtattctggcccttggtgtagcaatggagattcaacagaataatg
ctacaaagacatttctgagaattttgtggacctgctgatcctgaaattgcccggcattttacgccctggaaactct
cagagcaatcttttggtaaaactaagatccagaatgctgttccactgtactgatctgccagaggatggcctatta
gaggttcaatacttcttcaagatcttggataattag (SEQ ID NO:5)

(amino acids)

MNHSERFVFIAEWYDPNASLLRRYELLFYPGDGSVEMHDVKNHRTFLKRTKYDNLHLEDLFIGNKVNVFSRQL
VLIDYGDQYTARQLGSRKEKTLALIKPDAISKAGEIIEIINKAGFTITKLKMMMLSRKEALDFHVDHQSRPFF
NELIQFITTGPIIAMEILRDDAICEWKRLGLPANSVARTDASESIRALFGTDGIRNAAHGPDSFASAAREME
LFFPSSGGCGPANTAKFTNCTCCIVKPHAVSEGLLGKILMAIRDAGFEISAMQMFNMDRVNVVEEFYEVYKGVV
TEYHDMVTEMYSGPCVAMEIQNNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNAVHCTDLPEDGLL
EVQYFFKILDN- (SEQ ID NO:6)

NME7 peptides**NME7A peptide 1** (A domain): MLSRKEALDFHVDHQS (SEQ ID NO:7)**NME7A peptide 2** (A domain): SGVARTDASES (SEQ ID NO:8)**NME7B peptide 1** (B domain): DAGFEISAMQMFNMDRVNVE (SEQ ID NO:9)**NME7B peptide 2** (B domain): EVYKGVVTEYHDMVTE (SEQ ID NO:10)**NME7B peptide 3** (B domain): AIFGKTKIQNAVHCTDLPEDGLLEVQYFF (SEQ ID NO:11)**Mouse E6 Heavy chain variable region sequence:**

(DNA)

gaggtgaaggtgggtggagtctgggggagacttagtgaagcctggaggggtccctgaaactctcctgtgtagtct
ctggattcactttcagtagatatggcatgtcttgggttcgccagactccaggcaagaggctggagtgggtcgc
aaccattagtgggtggcgggtacttacatctactatccagacagtggtgaagggggcgattccaccatctccagagac

aatgccaagaacaccctgtacctgcaaagtgcagctctgaagtctgaggacacagccatgtatcactgtacaa
gggataactacggttaggaactacgactacggtatggactactggggtcaaggaacctcagtcaccgtctcctc
a (SEQ ID NO:12)

(amino acids)

EVKVVESGGDLVKPGGSLKLSCVVSGFTFSRYGMSWVRQTPGKRLEWVATISGGGTYYYPDSVKGRFTISR
NAKNTLYLQMSSLKSEDTAMYHCTRDNYGRNYDYGMDYWGQGTSTVSS (SEQ ID NO:13)

Mouse E6 heavy chain variable framework region 1 (FWR1) sequence:

(DNA)

gaggtgaaggtggtggagctctgggggagacttagtgaagcctggagggtccctgaaactctcctgtgtagtct
ct (SEQ ID NO:14)

(amino acids)

EVKVVESGGDLVKPGGSLKLSCVVSGFTFS (SEQ ID NO:15)

Mouse E6 heavy chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

ggattcactttcagtagatatggcatgtct (SEQ ID NO:16)

(amino acids)

RYGMS (SEQ ID NO:17)

Mouse E6 heavy chain variable framework region 2 (FWR2) sequence:

(DNA)

tgggttcgccagactccaggcaagaggtggagtggtcgca (SEQ ID NO:18)

(amino acids)

WVRQTPGKRLEWVA (SEQ ID NO:19)

Mouse E6 heavy chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

accattagtgggtggcggtacttacatctactatccagacagtgtgaagggg (SEQ ID NO:20)

(amino acids)

TISGGGTYYYPDSVKG (SEQ ID NO:21)

Mouse E6 heavy chain variable framework region 3 (FWR3) acid sequence:

(DNA)

cgattcaccatctccagagacaatgccaagaacaccctgtacctgcaaagtgcagctctgaagtctgaggaca
cagccatgtatcactgtacaagg (SEQ ID NO:22)

(amino acids)

RFTISRDNKNTLYLQMSSLKSEDTAMYHCTR (SEQ ID NO:23)

Mouse E6 heavy chain variable complementarity determining regions 3 (CDR3) sequence:

(DNA)

gataactacggttaggaactacgactacggtatggactac (SEQ ID NO:24)

(amino acids)

DNYGRNYDYGMDY (SEQ ID NO:25)

IGHV3-21*03 heavy chain variable region sequence:

(DNA)

gaggtgcagctggtggagtcctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcct
ctggattcaccttcagtagctatagcatgaactgggtccgccaggtccagggaaggggctggagtgggtctc
atccattagtagtagtagtagttacatatatactacgcagactcagtgaagggccgattcaccatctccagagac
aacgccaagaactcactgtatctgcaaataaacagcctgagagccgaggacacggctgtgtattactgtgcga
ga (SEQ ID NO:26)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKLEWVSSISSSSSYIYYADSVKGRFTISR
NAKNSLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:27)

IGHV3-21*01 heavy chain variable framework region 1 (FWR1) sequence:

(DNA)

gaggtgcagctggtggagtcctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcct
ctggattcaccttcagt (SEQ ID NO:28)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFS (SEQ ID NO:29)

IGHV3-21*01 heavy chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

agctatagcatgaac (SEQ ID NO:30)

(amino acids)

YSYMN (SEQ ID NO:31)

IGHV3-21*01 heavy chain variable framework region 2 (FWR2) sequence:

(DNA)

tgggtccgccaggtccagggaaggggctggagtgggtctca (SEQ ID NO:32)

(amino acids)

WVRQAPGKLEWVS (SEQ ID NO:33)

IGHV3-21*01 heavy chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

tccattagtagtagtagtagttacatatatactacgcagactcagtgaagggc (SEQ ID NO:34)

(amino acids)

SISSSSSYIYYADSVKG (SEQ ID NO:35)

IGHV3-21*01 heavy chain variable framework region 3 (FWR3) sequence:

(DNA)

cgattcaccatctccagagacaacgccaagaactcactgtatctgcaaataaacagcctgagagccgaggaca
cggctgtgtattactgtgcgaga (SEQ ID NO:36)

(amino acids)

RFTISRDNKNSLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:37)

Humanized E6 heavy chain variable region sequence:

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcct
ctggattcaccttcagtaggtatggcatgagctgggtccgccaggtccaggggaagaggctggagtgggtctc
aaccattagtggcgaggacacctacatatactaccagactcagtgaagggccgattcaccatctccagagac
aacgccaagaacaccctgtatctgcaaataaacagcctgagagccgaggacacggctgtgtattactgtacca
gagataactatggccgcaactatgattatggcatggattattggggccagggcaccctggtgaccgtgagcag
c (SEQ ID NO:38)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLEWVSTISGGGTYYYPDSVKGRFTISR
NAKNTLYLQMNSLRAEDTAVYYCTRDN YGRNYDYGMDYWGQGLVTVSS (SEQ ID NO:39)

Humanized E6 heavy chain variable framework region 1 (FWR1) acid sequence:

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcct
ctggattcaccttcagt (SEQ ID NO:40)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFS (SEQ ID NO:41)

Humanized E6 heavy chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

aggtatggcatgagc (SEQ ID NO:42)

(amino acids)

RYGMS (SEQ ID NO:43)

Humanized E6 heavy chain variable framework region 2 (FWR2) acid sequence:

(DNA)

tgggtccgccaggtccaggggaagaggctggagtgggtctca (SEQ ID NO:44)

(amino acids)

WVRQAPGKRLEWVS (SEQ ID NO:45)

Humanized E6 heavy chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

accattagtggcgaggacacctacatatactaccagactcagtgaagggc (SEQ ID NO:46)

(amino acids)

TISGGGTYYYPDSVKG (SEQ ID NO:47)

Humanized E6 heavy chain variable framework region 3 (FWR3) acid sequence:

(DNA)

cgattcaccatctccagagacaacgccaagaacaccctgtatctgcaaataaacagcctgagagccgaggaca
cggctgtgtattactgtaccaga (SEQ ID NO:48)

(amino acids)

RFTISRDNKNTLYLQMNSLRAEDTAVYYCTR (SEQ ID NO:49)

Humanized E6 heavy chain variable complementarity determining regions 3 (CDR3) sequence:

(DNA)

gataactatggccgcaactatgattatggcatggattat (SEQ ID NO:50)

(amino acids)

DNYGRNYDYGMDY (SEQ ID NO:51)

Humanized E6 IgG2 heavy chain synthesized by Genescript:

(DNA)

gaattctaagcttggggccaccatggaactggggctccgctggggttttccttgttgctattttagaaggtgtcc
 agtgtgaggtgcagctggtggagtctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgc
 agcctctggattcaccttcagtaggtatggcatgagctgggtccgccaggctccagggaagaggctggagtgg
 gtctcaaccattagtggcggaggcacctacataactaccagactcagtgaaggggccgattcaccatctcca
 gagacaacgccaagaacacccctgtatctgcaaataaacagcctgagagccgaggacacggctgtgtattactg
 taccagagataactatggccgcaactatgattatggcatggattattggggccaggggcaccctggtgaccgtg
 agcagcgctccaccaagggcccatcggtcttccccctggcgccctgctccaggagcacctccgagagcacag
 ccgcccctgggctgctggtcaaggactacttccccgaaccgggtgacggtgtcgtggaactcaggcgctctgac
 cagcggcgtgcacaccttcccagctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtg
 ccctccagcaacttcggcaccacagacctacacctgcaacgtagatcacaagcccagcaacaccaaggtggaca
 agacagttgagcgcaaatgttgtgtcgagtgccaccgtgccagcaccacctgtggcaggaccgtcagtcctt
 cctcttccccccaaaacccaaggacacctcatgatctccggaccctgaggtcacgtgctggtggtggac
 gtgagccacgaagacccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaa
 agccacgggaggagcagttcaacagcacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggct
 gaacggcaaggagtacaagtgaaggtctccaacaaaggcctcccagcccccatcgagaaaaccatctccaaa
 accaaagggcagccccgagaaccacaggtgtacacctgcccccatcccgaggagatgaccaagaaccagg
 tcagcctgacctgctggtcaaaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggcagcc
 ggagaacaactacaagaccacacctcccatgctggactccgacggctccttcttctctacagcaagetcaac
 gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcaggtctgcacaaccact
 acacgcagaagagcctctccctgtctccgggtaaatagtaagtttaaactctaga (SEQ ID NO:52)

(amino acids)

EF*AWATMELGLRWVFLVAILEGVQCEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLEW
 VSTISGGGTYYIYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWGQGLVTV
 SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVVTV
 PSSNFGTQTYTCNVDHKPSNTKVDKTKVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVD
 VSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISK
 TKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLT
 VDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK**V*TLX (SEQ ID NO:53)

Human IgG2 heavy chain constant region sequence:

(DNA)

gcctccaccaagggcccatcggtcttccccctggcgccctgctccaggagcacctccgagagcacagccgccc
 tgggctgctggtcaaggactacttccccgaaccgggtgacggtgtcgtggaactcaggcgctctgaccagcgg
 cgtgcacaccttcccagctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgcccctcc
 agcaacttcggcaccacagacctacacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagacag
 ttgagcgcgaatgttgtgtcgagtgccaccgtgccagcaccacctgtggcaggaccgtcagtccttccctctt
 ccccccaaaaacccaaggacacctcatgatctccggaccctgaggtcacgtgctggtggtggacgtgagc
 cacgaagacccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccac
 gggaggagcagttcaacagcacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacgg
 caaggagtacaagtgaaggtctccaacaaaggcctcccagcccccatcgagaaaaccatctccaaaacccaaa
 gggcagccccgagaaccacaggtgtacacctgcccccatcccgaggagagatgaccaagaaccaggtcagcc
 tgacctgctggtcaaaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggcagccggagaa

caactacaagaccacacctcccatgctggactccgacggctccttcttctctacagcaagctcacggtggac
aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatag (SEQ ID NO:54)

(amino acids)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS
SNFGTQTYTCNVDHKPSNTKVDKTVKCCVECPPEPPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVDVS
HEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPMLDSDGSFFLYSKLTVD
KSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:55)

Humanized E6 IgG1 heavy chain sequence:

(DNA)

gaggtgcagctggtggagctctgggggaggcctgggtcaagcctgggggggtccctgagactctcctgtgcagcct
ctggattcaccttcagtaggtatggcatgagctgggtccgccaggtccagggaagaggtggtgggtctc
aaccattagtggcggaggcacctacatatactaccagactcagtgaaggggcgtattcacctatctccagagac
aacgccaagaacccactgtatctgcaaatgaacagcctgagagccgaggacacggctgtgtattactgtccca
gagataactatggccgcaactatgattatggcatggattattggggccagggaaccctgggtgaccgtgagcag
cgctagcaccaaggggcccatcggtcttccccctggcaccctcctccaagagcacctctgggggcacagcgcc
ctgggtgctgctgggtcaaggactacttccccgaaccgggtgacgggtgtcgtggaactcaggcgccctgaccagcg
gcgtgcacaccttcccggtgtctctacagtctcaggactctactccctcagcagcgtgggtgacagtgcctc
cagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaa
gttgagcccaaattctgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgt
cagtcttctcttccccccaaaacccaaggacaccctcatgatctccgggaccctgaggtcacatgcgtggt
ggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcc
aagacaaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcacctcctgcaccagg
actggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagcccccatcgagaaaaccat
ctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaag
aaccaggtcagcctgacctgcctggtcaaaggttctatcccagcgacatcgccgtggagtgaggagcaatg
ggcagccgggagaacaactacaagaccagcctcccggtgctggactccgacggctccttcttctctacagcaa
gtcacccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctctgcac
aaccactacacgcagaagagcctctccctgtctccgggtaaatgataa (SEQ ID NO:56)

(amino acids)

EVQLVESGGGLVKPGLSLRLSCAASGFTFSRYGMSWVRQAPGKRLEWVSTISGGGTYYIYPDSVKGRFTISR
NAKNPLYLQMNSLRAEDTAVYYCPRDNYGRNYDYGMDYWGGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK
VEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH
NHYTQKSLSLSPGK** (SEQ ID NO:57)

Human IgG1 heavy chain constant region sequence:

(DNA)

gctagcaccaaggggcccatcggtcttccccctggcaccctcctccaagagcacctctgggggcacagcgcc
tggtgctgctggtcaaggactacttccccgaaccgggtgacgggtgtcgtggaactcaggcgccctgaccagcg
cgtgcacaccttcccggtgtctctacagtctcaggactctactccctcagcagcgtgggtgacagtgcctcc
agcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaag
ttgagcccaaattctgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtc
agtcttctcttccccccaaaacccaaggacaccctcatgatctccgggaccctgaggtcacatgcgtggtg
gtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcc
agacaaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcacctcctgcaccagga
ctggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagcccccatcgagaaaaccatc

tccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaaga
accaggtcagcctgacctgcttgggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgg
gcagccgggagaacaactacaagaccacgcctcccgtgctggactccgacgggtccttcttctctacagcaag
ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgagggtctgcaca
accactacacgcagaagagcctctccctgtctccgggtaaatgataa (SEQ ID NO:58)

(amino acids)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS
SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK
LTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK** (SEQ ID NO:59)

Human IgG1 heavy chain constant region gBLOCK#1 sequence:

(DNA)

atggcatggattattggggccagggcaccctgggtgacctgagcagcgctagcaccaagggcccatcggtctt
ccccctggcaccctcctccaagagcacctctgggggcacagcgccctgggctgcttgggtcaaggactacttc
cccgaaccgggtgacgggtgctggtggaactcaggcgccctgaccagcggtgacacacctcccggctgtcctac
agtctcaggactctactccctcagcagcggtgacagtgcctccagcagcttgggcaccagacctacat
ctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagccaaatcttgtgacaaaact
cacacatgcccacggtgcccagcacctgaactcctggggggacggtcagtcctcctcttccccccaaaaccca
aggacaccctcatgatctccggaccctgaggtcacatgctgtggtgggtggacgtgagccacgaagaccctga
ggtaagttcaactggtacgtggacggcgtggaggtgcataatgccaaag (SEQ ID NO:60)

Human IgG1 heavy chain constant region gBLOCK#2 sequence:

(DNA)

tacgtggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaacagcacgtaccgtg
tggtcagcgtcctcaccgtcctgcaccaggaactggctgaatggcaaggagtacaagtgcagggtctccaacaa
agccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacc
ctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatccca
gagacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccgtgctgga
ctccgacgggtccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttc
tcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaat
gataagtttaaaccgctgatcagcctcgactgtgccttctagtgtg (SEQ ID NO:61)

E6 heavy chain variable region overlapping sequence:

(DNA)

atggcatggattattggggccagggcaccct (SEQ ID NO:62)

IgG1 heavy chain constant region overlapping region sequence:

(DNA)

tacgtggacggcgtggaggtgcataatgccaaag (SEQ ID NO:63)

pCDNA3.1 V5 and pSECTag2 overlapping sequence:

(DNA)

ccgctgatcagcctcgactgtgccttctagtgtg (SEQ ID NO:64)

Mouse E6 Light Chain variable region sequence:

(DNA)

caaattgttctcaccagctctccagcaatcatgtctgcatctccaggggaggaggtcaccctaacct
gcagtgccacctcaagtgttaagttacatacactggttccagcagaggccaggcacttctcccaaact
ctggatttatagcacatccaacctggcttctggagtcctgttcgcttcagtggtgagtgatggatggg

acctcttactctctcacaatcagccgaatggaggctgaagatgctgccacttattactgccagcaaa
ggagtagttccccattcacgttcggctcggggacaaagttggaaataaaa (SEQ ID NO:65)
(amino acids)
QIVLTQSPAIMASAPGEEVTLTCSATSSVSYIHWFQQRPGTSPKLWIYSTSNLASGVPVRFSGSGYGTSYSLT
ISRMEAEDAATYYCQQRSSSPFTFGSGTKLEIK (SEQ ID NO:66)

Mouse E6 light chain variable framework region 1 (FWR1) sequence:

(DNA)
caaattgttctcaccagtcctccagcaatcatgtctgcatctccagggaggaggtcacccctaacctgc
(SEQ ID NO:67)

(amino acids)
QIVLTQSPAIMASAPGEEVTLTC (SEQ ID NO:68)

Mouse E6 light chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)
AGTGCCACCTCAAGTGTAAGTTACATACAC (SEQ ID NO:69)

(amino acids)
SATSSVSYIH (SEQ ID NO:70)

Mouse E6 light chain variable framework region 2 (FWR2) sequence:

(DNA)
tggttccagcagaggccaggcacttctcccaaactctggatttat (SEQ ID NO:71)

(amino acids)
WFQQRPGTSPKLWIY (SEQ ID NO:72)

Mouse E6 light chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)
agcacatccaacctggcttct (SEQ ID NO:73)

(amino acids)
STSNLAS (SEQ ID NO:74)

Mouse E6 light chain variable framework region 3 (FWR3) sequence:

(DNA)
ggagtccctgttcgcttcagtggcagtgatgggacctcttactctctcacaatcagccgaatggaggctg
aagatgctgccacttattactgc (SEQ ID NO:75)

(amino acids)
GVPVRFSGSGYGTSYSLTISRMEAEDAATYYC (SEQ ID NO:76)

Mouse E6 light chain variable complementarity determining regions 3 (CDR3) sequence:

(DNA)
cagcaaaggagtagttccccattcacg (SEQ ID NO:77)

(amino acids)
QQRSSSPFT (SEQ ID NO:78)

IGKV3-11*02 light chain variable region sequence:

(DNA)

gaaattgtgttgacacagtctccagccaccctgtctttgtctccaggggaaagagccaccctctcctgcaggg
ccagtcagagtgttagcagctacttagcctggtagccaacagaaacctggccaggctcccaggctcctcatcta
tgatgcacccaacagggccactggcatcccagccaggttcagtggcagtggtctgggagagacttcactctc
accatcagcagcctagagcctgaagatTTTgcagtttattactgtcagcagcgtagcaactggcctcc (SEQ
ID NO:79)

(amino acids)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPA
RFSGSGSGRDFLTITISLEPEDFAVYYCQQRSNWPP (SEQ ID NO:80)

IGKV3-11*02 light chain variable framework region 1 (FWR1) acid sequence:

(DNA)

gaaattgtgttgacacagtctccagccaccctgtctttgtctccaggggaaagagccaccctctcctgc
(SEQ ID NO:81)

(amino acids)

EIVLTQSPATLSLSPGERATLSC (SEQ ID NO:82)

IGKV3-11*02 light chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

agggccagtcagagtgttagcagctacttagcc (SEQ ID NO:83)

(amino acids)

RASQSVSSYLA (SEQ ID NO:84)

IGKV3-11*02 light chain variable framework region 2 (FWR2) sequence:

(DNA)

tggtaccaacagaaacctggccaggctcccaggctcctcatctat (SEQ ID NO:85)

(amino acids)

WYQQKPGQAPRLLIY (SEQ ID NO:86)

IGKV3-11*02 light chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

gatgcacccaacagggccact (SEQ ID NO:87)

(amino acids)

DASNRAT (SEQ ID NO:88)

IGKV3-11*02 light chain variable framework region 3 (FWR3) sequence:

(DNA)

ggcatcccagccaggttcagtggcagtggtctgggagagacttcactctcaccatcagcagcctagagcctg
aagatTTTgcagtttattactgt (SEQ ID NO:89)

(amino acids)

GIPARFSGSGSGTDFLTITISLEPEDFAVYYC (SEQ ID NO:90)

IGKV3-11*02 light chain variable complementarity determining regions3 (CDR3) sequence:

(DNA)

cagcagcgtagcaactggcctcc (SEQ ID NO:91)

(amino acids)
QQRSNWPP (SEQ ID NO:92)

Humanized E6 light chain variable region sequence:

(DNA)
gaaattgtgttgacacagtcctccagccaccctgtctttgtctccaggggaaagagccaccctcacctgcagcgc
ccaccagcagtggttagctacatccactggtagccaacagaggcctggccagagccccagggtcctcatctatag
cacctccaacctggccagcggcatcccagccagggttcagtggcagtggtctgggagcgactacactctcacc
atcagcagcctagagcctgaagattttgcagtttattactgtcagcagcgtagcagctccccctttcacctttg
gcagcggcaccaaagtggaaattaaa (SEQ ID NO:93)

(amino acids)
EIVLTQSPATLSLSPGERATLTCSATSSVSYIHWHYQQRPGQSPRLLIYSTSNLASGIPARFSGSGSGSDYTLT
ISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIK (SEQ ID NO:94)

Humanized E6 light chain variable framework region 1 (FWR1) acid sequence:

(DNA)
gaaattgtgttgacacagtcctccagccaccctgtctttgtctccaggggaaagagccaccctcacctgc
(SEQ ID NO:95)

(amino acids)
EIVLTQSPATLSLSPGERATLTC (SEQ ID NO:96)

Humanized E6 light chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)
agcgccaccagcagtggttagctacatccac (SEQ ID NO:97)

(amino acids)
SATSSVSYIH (SEQ ID NO:98)

Humanized E6 heavy light variable framework region 2 (FWR2) acid sequence:

(DNA)
tggtaccaacagaggcctggccagagccccagggtcctcatctat (SEQ ID NO:99)

(amino acids)
WYQQRPGQSPRLLIY (SEQ ID NO:100)

Humanized E6 light chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)
agcacctccaacctggccagc (SEQ ID NO:101)

(amino acids)
STSNLAS (SEQ ID NO:102)

Humanized E6 light chain variable framework region 3 (FWR3) acid sequence:

(DNA)
ggcatcccagccagggttcagtggcagtggtctgggagcgactacactctcaccatcagcagcctagagcctg
aagattttgcagtttattactgt (SEQ ID NO:103)

(amino acids)

GIPARFSGSGSGSDYTLTISSLEPEDFAVYYC (SEQ ID NO:104)

Humanized E6 light chain variable complementarity determining regions 3 (CDR3) sequence:

(DNA)

cagcagcgtagcagctccccctttcacc (SEQ ID NO:105)

(amino acids)

QQRSSSPFT (SEQ ID NO:106)

Humanized E6 Kappa light chain synthesized by Genescript:

(DNA)

gaattctaagcttggggccaccatggaagccccagcgcagcttctcttcctcctgctactctggctcccagata
ccactggagaaattgtgttgacacagtctccagccaccctgtctttgtctccaggggaaagagccaccctcac
ctgcagcgcaccagcagtggttagctacatccactggtagccaacagaggcctggccagagccccagggtcctc
atctatagcacctccaacctggccagcggcatcccagccagggttcagtggcagtggtctgggagcgactaca
ctctcaccatcagcagcctagagcctgaagattttgcagtttattactgtcagcagcgtagcagctccccctt
cacctttggcagcggcaccaaagtggaaattaaaaggacgggtggctgcaccatctgtcttcattcttcccgcca
tctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggcca
aagtacagtggaaggtggataaacgcctccaatcgggtaactcccaggagagtggtcacagagcaggacagcaa
ggacagcacctacagcctcagcagcacctgacgctgagcaaagcagactacgagaaacacaaagtctacgcc
tgcaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacagggggagagtggttagtaagttt
aaactctaga (SEQ ID NO:107)

(amino acids)

EF*AWATMEAPAQLLFLLLWLPD TTGEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWHYQQRPGQSPRL
IYSTSNLASGIPARFSGSGSGSDYTLTISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYA
CEVTHQGLSPVTKSFNRGEC**V*TLX (SEQ ID NO:108)

Human Kappa light chain constant region sequence:

(DNA)

aggacgggtggctgcaccatctgtcttcattcttcccgccatctgatgagcagttgaaatctggaactgcctctg
ttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataaacgcctccaatc
gggtaactcccaggagagtggtcacagagcaggacagcaaggacagcacctacagcctcagcagcacctgacg
ctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccg
tcacaaagagcttcaacagggggagagtggttag (SEQ ID NO:109)

(amino acids)

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLT
LSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO:110)

Humanized E6 lambda light chain sequence:

(DNA)

gaaattgtgttgacacagtctccagccaccctgtctttgtctccaggggaaagagccaccctcacctgcagcg
ccaccagcagtggttagctacatccactggtagccaacagaggcctggccagagccccagggtcctcatctatag
cacctccaacctggccagcggcatcccagccagggttcagtggcagtggtctgggagcgactacactctcacc
atcagcagcctagagcctgaagattttgcagtttattactgtcagcagcgtagcagctccccctttcacctttg
gcagcggcaccaaagtggaaattaaaggctcagcccaaggctgccccctcggtcactctgttcccgccctcctc
tgaggagcttcaagccaacaaggccacactgggtgtgtctcataagtgaattctacccgggagccgtgacagtg
gcctggaaggcagatagcagccccgtcaaggcgggagtgagagaccaccacaccctccaaacaaagcaacaaca
agtacgcggccagcagctatctgagcctgacgcctgagcagtggaagtcacacagaagctacagctgccaggt

cacgcatgaagggagcacccgtggagaagacagtgggccctacagaatgttcataagtaa (SEQ ID NO:111)

(amino acids)

EIVLTQSPATLSLSPGERATLTCSATSSVSIIHWYQQRPGQSPRLIIYSTSNLASGIPARFSGSGSGSDYTLT
ISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV
AWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS** (SEQ
ID NO:112)

Humanized lambda light chain constant region sequence:

(DNA)

ggtcagcccaaggetgccccctcggtcactctgttcccgccctcctctgaggagcttcaagccaacaaggcca
cactgggtgtgtctcataagtgaacttctaccggggagccgtgacagtgccctggaaggcagatagcagccccgt
caaggcgggagtgaggaccaccacacccctccaaacaagaacaagaagtagcgggccagcagctatctgagc
ctgacgcctgagcagtggaagtcccacagaagctacagctgccaggtcacgcatgaagggagcacccgtggaga
agacagtgggccctacagaatgttcataagtaa (SEQ ID NO:113)

(amino acids)

GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLS
LTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS** (SEQ ID NO:114)

Human lambda light chain constant region gBLOCK#3 sequence:

(DNA)

agcgccaccagcagtggttagctacatccactggtagccaacagaggcctggccagagccccaggctcctcatct
atagcacctccaacctggccagcggcatcccagccaggttcagtgccagtggtctgggagcgactacactct
caccatcagcagcctagagcctgaagattttgcagtttattactgtcagcagcgtagcagctccccctttcacc
tttggcagcggcaccaaagtggaaattaaaggtagcccaaggctgccccctcggtcactctgttcccgccct
cctctgaggagcttcaagccaacaaggccacactgggtgtgtctcataagtgaacttctaccggggagccgtgac
agtgccctggaaggcagatagcagccccgtcaaggcgggagtgaggaccaccacacccctccaaacaagaac
aacaagtagcgggccagcagctatctgagcctgacgcctgagcagtggaagtcccacagaagctacagctgcc
aggtagcagcatgaagggagcacccgtggagaagacagtgggccctacagaatgttcataagtaagtttaaacccg
ctgatcagcctcgactgtgccttctagttg (SEQ ID NO:115)

E6 light chain variable region overlapping sequence:

(DNA)

agcgccaccagcagtggttagctacatccact (SEQ ID NO:116)

pCDNA3.1 V5 and pSECTag2 overlapping sequence:

(DNA)

ccgctgatcagcctcgactgtgccttctagttg (SEQ ID NO:117)

Mouse C2 heavy chain variable region sequence:

(DNA)

gaggtagcagctggaggagtcagggggaggccttagtgaagcctggagggtccctgaaactctcctgtgcagcct
ctggattcactttcagtggtatgccatgtcttgggttcgccagactccggagaagaggctggagtgggtcgc
aaccattagtagtggtggtacttatactactatccagacagtgtagggggcgattcaccatctccagagac
aatgccaagaacacctgtacctgcaaatgagcagctctgaggctctgaggacacggccatgtattactgtgcaa
gacttgggggggataattactacgaatacttcgatgtctggggcgcaggggaccacgggtcacccgtctcctccgc
caaaacgacacccccatctgtctat (SEQ ID NO:118)

(amino acids)

EVQLEESGGGLVLPKPGSLKLSAASGFTFSGYAMSWVRQTPEKRLQWVATISSGGTYIYYPDSVKGRFTISR
NAKNTLYLQMSSLRSEDAMYYCARLGGDNYYEYFDVWGAGTTTVSSAKTTPPSVY (SEQ ID NO:119)

Mouse C2 heavy chain variable framework region 1 (FWR1) sequence:

(DNA)

gaggtccagctggaggagtcagggggaggccttagtgaagcctggaggggccctgaaactctcctgtgcagcct
ctggattcactttcagt (SEQ ID NO:120)

(amino acids)

EVQLEESGGGLVKPGGSLKLSAASGFTFS (SEQ ID NO:121)

Mouse C2 heavy chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

ggctatgccatgtct (SEQ ID NO:122)

(amino acids)

GYAMS (SEQ ID NO:123)

Mouse C2 heavy chain variable framework region 2 (FWR2) sequence:

(DNA)

tgggttcgccagactccggagaagaggctggagtggtcgca (SEQ ID NO:124)

(amino acids)

WVRQTPEKRLEWVA (SEQ ID NO:125)

Mouse C2 heavy chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

accattagtagtggtggtacttatatctactatccagacagtggtgaagggg (SEQ ID NO:126)

(amino acids)

TISSGGTYIYPDSVKG (SEQ ID NO:127)

Mouse C2 heavy chain variable framework region 3 (FWR3) sequence:

(DNA)

cgattcaccatctccagagacaatgccaagaacaccctgtacctgcaaagagcagtcctgaggtctgaggaca
cggccatgtattactgtgcaaga (SEQ ID NO:128)

(amino acids)

RFTISRDNKNTLYLQMSSLRSEDAMYYCAR (SEQ ID NO:129)

Mouse C2 heavy chain variable complementarity determining regions 3 (CDR3) sequence:

(DNA)

cttgggggggataattactacgaatacttcgatgtc (SEQ ID NO:130)

(amino acids)

LGGDNYEYFDV (SEQ ID NO:131)

IGHV3-21*04 heavy chain variable region sequence:

(DNA)

gaggtgcagctggtggagtcctgggggaggcctggtcaagcctgggggggccctgagactctcctgtgcagcct
ctggattcaccttcagtagctatagcatgaactgggtccgccaggctccagggaaggggctggagtggtctc
atccattagtagtagtagttacatatactacgcagactcagtgaaaggccgattcaccatctccagagac
aacgccaagaactcactgtatctgcaaataaacagcctgagagccgaggacacggccgtgtattactgtgcga
ga (SEQ ID NO:132)

(amino acids)

EVQLVESGGGLVQPKGSLRLSCAASGFTFSYSMNWVRQAPGKGLEWVSSISSSSSYIYYADSVKGRFTISRDN
NAKNSLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:133)

IGHV3-21*04 heavy chain variable framework region 1 (FWR1) sequence:

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggccctgagactctcctgtgcagcct
ctggattcaccttcagt (SEQ ID NO:134)

(amino acids)

EVQLVESGGGLVQPKGSLRLSCAASGFTFS (SEQ ID NO:135)

IGHV3-21*04 heavy chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

agctatagcatgaac (SEQ ID NO:136)

(amino acids)

YSYMN (SEQ ID NO:137)

IGHV3-21*04 heavy chain variable framework region 2 (FWR2) sequence:

(DNA)

gggtccgccaggctccagggaaggggctggagtggtctca (SEQ ID NO:138)

(amino acids)

WVRQAPGKGLEWVS (SEQ ID NO:139)

IGHV3-21*04 heavy chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

tccattagtagtagtagtagttacatatatactacgcagactcagtgaagggc (SEQ ID NO:140)

(amino acids)

SISSSSSYIYYADSVKG (SEQ ID NO:141)

IGHV3-21*04 heavy chain variable framework region 3 (FWR3) sequence:

(DNA)

cgattcaccatctccagagacaacgccagaactcactgtatctgcaaataaacagcctgagagccgaggaca
cggcctgtattactgtgcgaga (SEQ ID NO: 142)

(amino acids)

RFTISRDNKNSLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:143)

Humanized C2 heavy chain variable region sequence:

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggccctgagactctcctgtgcagcct
ctggattcaccttcagtggctatgccatgagctgggtccgccaggctccagggaaggggctggagtggtctc
aaccattagtagtggcgaacctacatatatactacccgactcagtgaagggccgattcaccatctccagagac
aacgccagaactcactgtatctgcaaataaacagcctgagagccgaggacacggcctgtattactgtgcga
gacttgggggggataaattactacgaatacttcgatgtctggggcaaagggaccacggtcaccgtctcctcc
(SEQ ID NO:144)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDSVKGRFTISRDN
NAKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYFDVWGKGTITVTVSS (SEQ ID NO:145)

Humanized C2 heavy chain variable framework region 1 (FWR1) sequence:

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggccctgagactctcctgtgcagcct
ctggattcaccttcagt (SEQ ID NO:146)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFS (SEQ ID NO:147)

Humanized C2 heavy chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

ggctatgccatgagc (SEQ ID NO:148)

(amino acids)

GYAMS (SEQ ID NO:149)

Humanized C2 heavy chain variable framework region 2 (FWR2) sequence:

(DNA)

tgggtccgccaggctccaggaaggggctggagtggtctcaa (SEQ ID NO:150)

(amino acids)

WVRQAPGKGLEWVS (SEQ ID NO:151)

Humanized C2 heavy chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

accattagtagtgggcggaacctacatatactaccccgactcagtgaagggc (SEQ ID NO:152)

(amino acids)

TISSGGTYIYYPDSVKG (SEQ ID NO:153)

Humanized C2 heavy chain variable framework region 3 (FWR3) sequence:

(DNA)

cgattcaccatctccagagacaacgccagaactcactgtatctgcaaataaacagcctgagagccgaggaca
cggcctgtattactgtgcgaga (SEQ ID NO:154)

(amino acids)

RFTISRDNKNSLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:155)

Humanized C2 heavy chain variable complementarity determining regions 3 (CDR3) sequence:

(DNA)

cttgggggggataattactacgaatacttcgatgtc (SEQ ID NO:156)

(amino acids)

LGGDNYEYFDV (SEQ ID NO:157)

Humanized C2 IgG1 heavy chain sequence

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggccctgagactctcctgtgcagcct
ctggattcaccttcagtggctatgccatgagctgggtccgccaggctccaggaaggggctggagtgggtctc
aaccattagtagtgggcggaacctacatatactaccccgactcagtgaagggccgattcaccatctccagagac

aacgccaagaactcactgtatctgcaaataaacagcctgagagccgaggacacggccgtgtattactgtgcga
gacttgggggggataattactacgaatacttcgatgtctggggcaaagggaccacggtcaccgtctcctccgc
tagcaccaagggcccatcgggtcttccccctggcaccctcctccaagagcacctctgggggcacagcgccctg
ggctgcctgggtcaaggactacttccccgaaccgggtgacgggtgtcgtggaactcaggcgccctgaccagcgcg
tgcacaccttccccggctgtcctacagtcctcaggactctactcctcagcagcgtggtgacagtgcctccag
cagcttgggcaccagacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagt
gagcccaaatcttgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcag
tcttctcttccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtgggtgg
ggacgtgagccacgaagaccctgaggtcaagttcaactggtagcgtggacggcgtggaggtgcataatgccag
aaaagccgcgggaggagcagtaaacagcacgtaccgtgtggtagcgtcctcaccgtcctgcaccaggact
ggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagcccccatcgagaaaaccatctc
caaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgaggaggagatgaccaagaac
caggtcagcctgacctgcctgggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggc
agccggagaaactacaagaccacgcctcccggtgctggactccgacggctccttcttctctacagcaagct
caccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaatgataa (SEQ ID NO:157)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDSVKGRFTISR
NAKNSLYLQMNSLRAEDTAVYYCARLGGDNYYEYFDVWGKTTTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHN
HYTQKSLSLSPGK** (SEQ ID NO:158)

Humanized C2 gBLOCK#4 sequence:

(DNA)

actcactataggggagacccaagctggctagttaagcttggggccaccatggagacagacacactcctgctatgg
gtactgctgctctgggttccaggttcactgggtgacgaggtgcagctgggtggagtctgggggaggcctgggtca
agcctgggggggtccctgagactctcctgtgcagcctctggattcaccttcagtggctatgccatgagctgggt
ccgccaggtccaggggaaggggtggagtgggtctcaaccattagtagtggcgaacctacatatactacccc
gactcagtgaagggccgattcaccatctccagagacaacgccaagaactcactgtatctgcaaataaacagcc
tgagagccgaggacacggccgtgtattactgtgcgagacttgggggggataattactacgaatacttcgatgt
ctggggcaaagggaccacgggtcacctgctcctccgctagcaccgaagggcccatcgggtcttccccctggcacc
tctccaagagcacctctgggggcacagcgccctgggtgcctgggtcaaggactacttccccgaaccgggtga
cgggtgtcgtggaactcaggcgccctgaccagc (SEQ ID NO:160)

pCDNA3.1 V5 overlapping sequence:

(DNA)

actcactataggggagacccaagctggctagtt (SEQ ID NO:161)

Human IgG1 constant region overlapping sequence:

(DNA)

gacgggtgtcgtggaactcaggcgccctgaccagc (SEQ ID NO:162)

Humanized C2 IgG2 heavy chain sequence

(DNA)

gaggtgcagctgggtggagtctgggggaggcctgggtcaagcctgggggggtccctgagactctcctgtgcagcct
ctggattcaccttcagtggctatgccatgagctgggtccgccaggtccaggggaaggggctggagtgggtctc
aaccattagtagtggcgaacctacatatactaccccgactcagtgaagggccgattcaccatctccagagac
aacgccaagaactcactgtatctgcaaataaacagcctgagagccgaggacacggccgtgtattactgtgcga
gacttgggggggataattactacgaatacttcgatgtctggggcaaagggaccacgggtcaccgtctcctccgc
ctccaccaagggcccatcgggtcttccccctggcgccctgtccaggagcacctccgagagcacagccgcctg

ggctgcctgggtcaaggactacttccccgaaccgggtgacgggtgtcgtggaactcaggcgctctgaccagcggtg
 tgcacaccttcccagctgtcctacagtcctcaggactctactccctcagcagcgtgggtgacctgacctccag
 caacttcggcaccacagacctacacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagacagtt
 gagcgcaaatggtgtgtcaggtgcccaccgtgcccagcaccacctgtggcaggacctgagtccttctctcc
 cccaaaacccaaggacacctcatgatctcccgacccctgaggtcacgtgctgggtggtagctgagcca
 cgaagaccccaggtccagttcaactggtacgtggacggcggtggaggtgcataatgccaagacaaagccacgg
 gaggagcagttcaacagcacgttcctgtgtgggtcagcgtcctcaccgttgtgcaccaggactggctgaacggca
 aggagtacaagtgaagggtctccaacaaaggcctcccagcccccatcgagaaaaccatctccaaaaccaaagg
 gcagccccgagaaccacaggtgtacacctgcccccatcccgggaggagatgaccaagaaccaggtcagcctg
 acctgcctgggtcaaaggcttctaccccagcgacatcgccgtggagtgaggagcaatgggcagccgggagaaca
 actacaagaccacacctcccatgctggactccgacgggtccttcttctctacagcaagctcaccgtggacaa
 gagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcag
 aagagcctctccctgtctccgggtaaatagtaa (SEQ ID NO:163)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDSVKGRFTISR
 NAKNSLYLQMNSLRAEDTAVYYCARLGGDNYYEYFDVWGKGTITVTVSSASTKGPSVFPLAPCSRSTSESTAAL
 GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSNFGTQTYTCNVDPKPSNTKVDKTV
 ERKCCVECPPEPVPVAGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKTKPR
 EEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSL
 TCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQ
 KSLSLSPGK** (SEQ ID NO:164)

Humanized C2 gBLOCK#5 sequence:

(DNA)

tgctctgggttccaggttccactggtgacgcggcccagccggccgaggtgcagctggtggagtctgggggagg
 cctggtcaagcctggggggtccctgagactctcctgtgcagcctctggattcaccttcagtggtctatgccatg
 agctgggtccgccaggtccagggaaggggtgagtggtctcaaccattagtagtggcgggaacctacatat
 actaccccgactcagtggaagggccgattcacctctccagagacaacgccaaagaactcactgtatctgcaaat
 gaacagcctgagagccgaggacacggcctgtattactgtgcgagacttgggggggataattactacgaatac
 ttcatgtctggggcaagggaacacgggtcaccgtctcctccgcctccaccaagggcccatcggtcttcccc
 tggcgccctgtccaggagcacctccgagagcacagccgcctgggtgacctggtcaaggactacttccccga
 accggtgacgggtgtcgtggaactcaggcgctctgacca (SEQ ID NO:165)

pSEC Tag2 overlapping sequence:

(DNA)

tgctctgggttccaggttccactggtgacgc (SEQ ID NO:166)

Human IgG2 constant region overlapping sequence:

(DNA)

gacgggtgtcgtggaactcaggcgctctgacca (SEQ ID NO:167)

Mouse C2 light chain variable region sequence:

(DNA)

gacattgtgatcacacagtctacagcttcccttaggtgtatctctggggcagagggccaccatctcatgcaggg
 ccagcaaaagtgtcagttacatctggctatagttatatgcactggtaccaacagagaccaggacagccacccaa
 actcctcatctatcttgcacccaacctagaatctgggggtccctgccaggttcagtggcagtggtctgggaca
 gacttcacctcaacatccatcctgtggaggaggaggtgctgcaacctattactgtcagcacagtagggagc
 ttccgttcacgttcggaggggggaccaagctggagataaaacgggctgatgctgcaccaactgtatcc (SEQ
 ID NO:168)

(amino acids)

DIVITQSTASLGVS LGQRATISCRASKSVSTSGYSYMHYQQRPGQPPKLLIYLASNLESGV PARFSGSGSGT
 DFTLNIHPVEEEDAATYYCQHSREL PFTFGGGTKLEIKRADAAPT VS (SEQ ID NO:169)

Mouse C2 light chain variable framework region 1 (FWR1) sequence:

(DNA)

gacattgtgatcacacagtctacagcttccttaggtgtatctctgtgggcagagggccaccatctcatgc
(SEQ ID NO:170)

(amino acids)

DIVITQSTASLGVS LGQRATISC (SEQ ID NO:171)

Mouse C2 light chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

agggccagcaaaagtgtcagttacatctggctatagttatatgcac (SEQ ID NO:172)

(amino acids)

RASKSVSTSGYSYMH (SEQ ID NO:173)

Mouse C2 light chain variable framework region 2 (FWR2) sequence:

(DNA)

tggtaccaacagagaccaggacagccacccaaactcctcatctat (SEQ ID NO:174)

(amino acids)

WYQQRPGQPPKLLIY (SEQ ID NO:175)

Mouse C2 light chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

cttgcattccaacctagaatc (SEQ ID NO:176)

(amino acids)

LASNLES (SEQ ID NO:177)

Mouse C2 light chain variable framework region 3 (FWR3) sequence:

(DNA)

tgggggtccctgccaggttcagtggtcagtggtctgtggacagacttcaccctcaacatccatcctgtggaggag
gaggatgctgcaacctattactgt (SEQ ID NO:178)

(amino acids)

GVPARFSGSGSGTDFTLNIHPVEEEDAATYYC (SEQ ID NO:179)

Mouse C2 light chain variable complementarity determining regions 3 (CDR3) sequence:

(DNA)

cagcacagtagggagcttccgttcacg (SEQ ID NO:180)

(amino acids)

QHSRELPFT (SEQ ID NO:181)

IGKV7-3*01 light chain variable region sequence:

(DNA)

gacattgtgctgaaccagttctccagctccttggcgtgtctccaggacagagggccaccatcacctgcagag
ccagtgagagtgtcagtttcttgggaataaaacttaattcactgggtatcagcagaaaccaggacaacctcctaa
actcctgatttaccagcatccaataaagacactgggggtcccagccaggttcagcggcagtggtgtctgggacc

gatttcaccctcacaattaatcctgtggaagctaatactgataactgcaaattattactgtctgcagagtaagaatt
ttcctcccaca (SEQ ID NO:182)

(amino acid)

DIVLTQSPASLAVSPGQRATITCRASESVSFLGINLIHWYQQKPGQPPKLLIYQASNKDTGVPARFSGSGSGT
DFTLTINPVEANDTANYYCLQSKNFPPT (SEQ ID NO:183)

IGKV7-3*01 light chain variable framework region 1 (FWR1) sequence:

(DNA)

gacattgtgctgacccagtcctccagcctccttgcccggtgtctccaggacagagggccaccatcacctgc
(SEQ ID NO:184)

(amino acids)

DIVLTQSPASLAVSPGQRATITC (SEQ ID NO:185)

IGKV7-3*01 light chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

agagccagtgagagtggtcagtttcttggaataaacttaattcac (SEQ ID NO:186)

(amino acids)

RASESVSFLGINLIH (SEQ ID NO:187)

IGKV7-3*01 light chain variable framework region 2 (FWR2) sequence:

(DNA)

tggtatcagcagaaaccaggacaacctcctaaactcctgatttac (SEQ ID NO:188)

(amino acids)

WYQQKPGQPPKLLIY (SEQ ID NO:189)

IGKV7-3*01 light chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

caagcatccaataaagacact (SEQ ID NO:190)

(amino acids)

QASNKDT (SEQ ID NO:191)

IGKV7-3*01 light chain variable framework region 3 (FWR3) sequence:

(DNA)

gggggtcccagccaggttcagcggcagtggtctgggaccgatttcaccctcacaattaatcctgtggaagcta
atgataactgcaaattattactgt (SEQ ID NO:192)

(amino acids)

GVPARFSGSGSGTDFTLTINPVEANDTANYYC (SEQ ID NO:193)

Humanized C2 light chain variable region sequence:

(DNA)

gacattgtgctgacccagtcctccagcctccttgcccggtgtctccaggacagagggccaccatcacctgcagag
ccagtaagagtgtcagtagcagcggatactcctacatgcactggtatcagcagaaaccaggacaacctcctaa
actcctgatttacctggcatccaatctggagagcgggggtcccagccaggttcagcggcagtggtctgggacc
gatttcaccctcacaattaatcctgtggaagctaatactgataactgcaaattattactgtcagcacagtagggagc
tgcctttcacattcggcggagggaaggtggagatcaaacgaact (SEQ ID NO:194)

(amino acids)

DIVLTQSPASLAVSPGQRATITCRASKSVSTSGYSYMHWYQQKPGQPPKLLIYLASNLESGVPARFSGSGSGT
DFTLTINPVEANDTANYYCQHSRELPFTFGGGTKVEIKRT (SEQ ID NO:195)

Humanized C2 light chain variable framework region 1 (FWR1) acid sequence:

(DNA)

gacattgtgctgacccagtcctccagcctccttgccgtgtctccaggacagagggccaccatcacctgc
(SEQ ID NO:196)

(amino acids)

DIVLTQSPASLAVSPGQRATITC (SEQ ID NO:197)

Humanized C2 light chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

agagccagtaagagtgtcagtaccagcggatactcctacatgcac (SEQ ID NO:198)

(amino acids)

RASKSVSTSGYSYMH (SEQ ID NO:199)

Humanized C2 heavy light variable framework region 2 (FWR2) acid sequence:

(DNA)

tggtatcagcagaaaccaggacaacctcctaaactcctgatttac (SEQ ID NO:200)

(amino acids)

WYQQKPGQPPKLLIY (SEQ ID NO:201)

Humanized C2 light chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

ctggcatccaatctggagagc (SEQ ID NO:202)

(amino acids)

LASNLES (SEQ ID NO:203)

Humanized C2 light chain variable framework region 3 (FWR3) acid sequence:

(DNA)

ggggtcccagccaggttcagcggcagtggtctgggaccgatttcaccctcacaattaatcctgtggaagcta
atgatactgcaaattattactgt (SEQ ID NO:204)

(amino acids)

GVPARFSGSGSGTDFTLTINPVEANDTANYYC (SEQ ID NO:205)

Humanized C2 light chain variable complementarity determining regions 3 (CDR3) sequence:

(DNA)

cagcacagtagggagctgcctttcaca (SEQ ID NO:206)

(amino acids)

QHSRELPFT (SEQ ID NO:207)

Humanized C2 light chain variable complementarity determining regions 3 (CDR3) sequence:

(DNA)

ctgcagagtaagaattttcctcccaca (SEQ ID NO:208)

(amino acids)

LQSKNFPPT (SEQ ID NO:209)

Humanized C2 gBLOCK#6 sequence (Kappa light chain in pCDNA3.1 V5):

(DNA)

actcactatagggagacccaagctggctagttaagcttggggccaccatggagacagacacactcctgctatgg
gtactgctgctctgggttccagggttccactgggtgacgacattgtgctgaccagtcctccagcctccttggccg
tgtctccaggacagagggccaccatcacctgcagagccagtaagagtgtcagtaccagcggatactcctacat
gcactggtatcagcagaaaccaggacaacctcctaaactcctgatttacctggcatccaatctggagagcggg
gtcccagccagggttcagcggcagtggtctgtgggaccgatttcaccctcacaattaatcctgtggaagctaag
atactgcaaattattactgtcagcacagtagggagctgcctttcacattcggcggagggaccaaggtggagat
caaacgaactacggtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaact
gcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtgggaaggtggataacgccc
tccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcac
cctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtacccatcagggcctgagc
tcgcccgtcacaaagagcttcaacaggggagagtgttagtaagtttaaaccgcgtgatcagcctcgactgtgc
cttctagttg (SEQ ID NO:210)

pCDNA3.1 V5 5' overlapping sequence:

(DNA)

actcactatagggagacccaagctggctagtt (SEQ ID NO:211)

pCDNA3.1 V5 3' overlapping sequence:

(DNA)

ccgctgatcagcctcgactgtgccttctagttg (SEQ ID NO:212)

Humanized C2 gBLOCK#7 sequence (Kappa light chain in pSEC Tag2):

(DNA)

tgctctgggttccagggttccactgggtgacgcggcccagccggccgacattgtgctgaccagtcctccagcctc
cttggcctgtctccaggacagagggccaccatcacctgcagagccagtaagagtgtcagtaccagcggatac
tctacatgcactggtatcagcagaaaccaggacaacctcctaaactcctgatttacctggcatccaatctgg
agagcggggtcccagccaggttcagcggcagtggtctgtgggaccgatttcaccctcacaattaatcctgtgga
agctaataactgcaaaattattactgtcagcacagtagggagctgcctttcacattcggcggagggaccaag
gtggagatcaaacgaactacggtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaat
ctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtgggaaggtgga
taacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctc
agcagcacctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtacccatcagg
gcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttagtaagtttaaaccgcgtgatcagcctc
gactgtgccttctagttg (SEQ ID NO:213)

pSEC Tag2 5' overlapping sequence:

(DNA)

tgctctgggttccagggttccactgggtgacgc (SEQ ID NO:214)

pSEC Tag2 3' overlapping sequence:

(DNA)

ccgctgatcagcctcgactgtgccttctagttg (SEQ ID NO:215)

Humanized C2 gBLOCK#8 sequence (lambda light chain in pCDNA3.1 V5):

(DNA)

actcactatagggagacccaagctggctagttaagcttggggccaccatggagacagacacactcctgctatgg
gtactgctgctctgggttccaggttccactggtgacgacattgtgctgacccagtcctccagcctccttggccg
tgtctccaggacagagggccaccatcacctgcagagccagtaagagtgtcagtaccagcggatactcctacat
gcactggtatcagcagaaaccaggacaacctcctaaactcctgatttacctggcatccaatctggagagcggg
gtcccagccaggttcagcggcagtggtctgggaccgatttcaccctcacaattaatcctgtggaagctaag
atactgcaaattattactgtcagcacagtagggagctgcctttcacattcggcggagggaccaaggtggagat
caaacgaactggtcagcccaaggctgccccctcggtcactctgttcccgcctcctctgaggagcttcaagcc
aacaaggccacactggtgtgtctcataagtgacttctacccgggagccgtgacagtggcctggaaggcagata
gcagccccgtcaaggcgggagtggagaccaccacacctccaaacaagcaacaacaagtacgcggccagcag
ctatctgagcctgacgcctgagcagtggaagtcccacagaagctacagctgccaggtcacgcgatgaagggagc
accgtggagaagacagtggtccctacagaatgttcatagtaagtttaaacccgctgatcagcctcgactgtgc
cttctagttg (SEQ ID NO:216)

pCDNA3.1 V5 5' overlapping sequence:

(DNA)

actcactatagggagacccaagctggctagtt (SEQ ID NO:217)

pCDNA3.1 V5 3' overlapping sequence:

(DNA)

ccgctgatcagcctcgactgtgccttctagttg (SEQ ID NO:218)

Humanized C2 gBLOCK#9 sequence (lambda light chain in pSEC Tag2):

(DNA)

tgctctgggttccaggttccactggtgacgcggcccagccggccgacattgtgctgacccagtcctccagcctc
cttggccgtgtctccaggacagagggccaccatcacctgcagagccagtaagagtgtcagtaccagcggatac
tctacatgcactggtatcagcagaaaccaggacaacctcctaaactcctgatttacctggcatccaatctgg
agagcgggggtcccagccaggttcagcggcagtggtctgggaccgatttcaccctcacaattaatcctgtgga
agctaattgatactgcaaattattactgtcagcacagtagggagctgcctttcacattcggcggagggaccaag
gtggagatcaaacgaactggtcagcccaaggctgccccctcggtcactctgttcccgcctcctctgaggagc
ttcaagccaacaaggccacactggtgtgtctcataagtgacttctacccgggagccgtgacagtggcctggaa
ggcagatagcagccccgtcaaggcgggagtggagaccaccacacctccaaacaagcaacaacaagtacgcg
gccagcagctatctgagcctgacgcctgagcagtggaagtcccacagaagctacagctgccaggtcacgcgatg
aaggagcaccgtggagaagacagtggtccctacagaatgttcatagtaagtttaaacccgctgatcagcctc
gactgtgccttctagttg (SEQ ID NO:219)

pSEC Tag2 5' overlapping sequence:

(DNA)

tgctctgggttccaggttccactggtgacgc (SEQ ID NO:220)

pSEC Tag2 3' overlapping sequence:

(DNA)

ccgctgatcagcctcgactgtgccttctagttg (SEQ ID NO:221)

Murine Ig kappa chain leader sequence

(DNA)

atggagacagacacactcctgctatgggtactgctgctctgggttccaggttccactggtgac (SEQ ID NO:222)

(amino acids)

METDTLLLWVLLLWVPGSTGD (SEQ ID NO:223)

Interleukin-2 (IL-2) leader sequence

(DNA)

atgtacaggatgcaactcctgtcttgcattgcactaagtcttgcacttgtcacaacagt (SEQ ID NO:224)

(amino acids)
MYRMQLLSIALSLALVTNS (SEQ ID NO:225)

CD33 leader sequence

(DNA)
atgcctcttctgcttctgcttcctctgctttgggctggagctcttgc (SEQ ID NO:226)

(amino acids)
MPLLLLLLPLLWAGALA (SEQ ID NO:227)

IGHV3-21*03 leader sequence

(DNA)
atggaaactggggctccgctgggttttccttgctattttagaaggtgtccagtgt (SEQ ID NO:228)

(amino acids)
MELGLRWVFLVAILEGVQC (SEQ ID NO:229)

IGHV3-11*02 leader sequence

(DNA)
atggaagccccagcgcagcttctcttcctcctgctactctggctcccagataccactgga (SEQ ID NO:230)

(amino acids)
MEAPAQLLFLLLLWLPDTTG (SEQ ID NO:231)

Humanized E6 single chain GS3

(DNA)
gagggtgcagctggtggagtctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtg
cagcctctggattcaccttcagtaggtatggcatgagctgggtccgccagggtccagggaagaggct
ggagtgggtctcaaccattagtggcggaggcacctacataactaccagactcagtgaagggccga
ttcaccatctccagagacaacgccaaagaacacctgtatctgcaaataaacagcctgagagccgagg
acacggctgtgtattactgtaccagagataactatggccgcaactatgattatggcatggattattg
gggccagggcaccctggtgaccgtgagcagcggcggtggcggtatccggcggtggcggtatccggcggt
ggcggtatccgaaattgtgttgacacagtctccagccaccctgtctttgtctccaggggaaagagcca
ccctcacctgcagcgcaccagcagtggttagctacatccactggtaccaacagaggcctggccagag
ccccaggctcctcatctatagcacctccaacctggccagcggcatcccagccagggttcagtggcagt
gggtctgggagcgactacactctcaccatcagcagcctagagcctgaagattttgcagtttattact
gtcagcagcgtagcagctcccctttcacctttggcagcggcaccaaagtggaaattaaa (SEQ ID NO:232)

(amino acids)
EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLEWVSTISGGGTYYIYPDSVKGRFTISR
NAKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWGQGLVTVSSGGGGSGGGGSGGGGSEIVLTQSPA
TSLSPGERATLTCSATSSVSYIHQYQQRPGQSPRLLIYSTSNLASGIPARFSGSGSGSDYTLTISSLEPEDF
AVYYCQQRSSSPFTFGSGTKVEIK (SEQ ID NO:233)

Humanized E6 single chain IgG1noC

(DNA)
gagggtgcagctggtggagtctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcct
ctggattcaccttcagtaggtatggcatgagctgggtccgccagggtccagggaagaggctggagtgggtctc
aaccattagtggcgaggacacctacataactaccagactcagtgaagggccgattccaccatctccagagac

aacgccaagaacaccctgtatctgcaaataaacagcctgagagccgaggacacggctgtgtattactgtacca
gagataactatggccgcaactatgattatggcatggattattggggccagggcaccctgggtgaccgtgagcag
cgataaaaaccataactaaaccgccccaaaccggcgccggaactgctgggtgggtcctgggtaccgtgaaattgtg
ttgacacagtctccagccaccctgtctttgtctccaggggaaagagccaccctcacctgcagcgccaccagca
gtgttagctacatccactgggtaccaacagaggcctggccagagccccagggtcctcatctatagcacctccaa
cctggccagcgccatcccagccaggttcagtggcagtggtctgggagcgactacactctcaccatcagcagc
ctagagcctgaagattttgcagtttattactgtcagcagcgtagcagctcccctttcacctttggcagcggca
ccaaagtggaaattaaa (SEQ ID NO:234)

(amino acids)

EVQLVESGGGLVQPKGSLRLSCAASGFTFSRYGMSWVRQAPGKRLEWVSTISGGGTYYIYPDSVKGRFTISR
NAKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWGQGLTVTVSSDKTHTKPPKPAPELLGGPGTGEIV
LTQSPATLSLSPGERATLTCSATSSVSYIHQYQQRPGQSPRLLIYSTSNLASGIPARFSGSGSGSDYTLTISS
LEPEDFAVYYCQQRSSSPFTFGSGTKVEIK (SEQ ID NO:235)

Humanized E6 single chain X4 (linker is IgG1 and IgG2 modified hinge region)

(DNA)

gaggtgcagctgggtggagtctgggggaggcctgggtcaagcctgggggggtccctgagactctcctgtgcagcct
ctggattcaccttcagtaggtatggcatgagctgggtccgccaggctccagggaagaggctggagtgggtctc
aaccattagtagtgccggaggcacctacatatactaccagactcagtgaaggggccgattcaccatctccagagac
aacgccaagaacaccctgtatctgcaaataaacagcctgagagccgaggacacggctgtgtattactgtacca
gagataactatggccgcaactatgattatggcatggattattggggccagggcaccctgggtgaccgtgagcag
cgataaaaaccataactaaaccgccccaaaccggcgccggaactgctgggtgggtcctgggtaccgtactgggtgt
ccgactattaaacctccgaaacctccgaaacctgctccgaacctgctgggtgggtccggaaattgtgttgacac
agtctccagccaccctgtctttgtctccaggggaaagagccaccctcacctgcagcgccaccagcagtggttag
ctacatccactgggtaccaacagaggcctggccagagccccagggtcctcatctatagcacctccaacctggcc
agcgccatcccagccaggttcagtggcagtggtctgggagcgactacactctcaccatcagcagcctagagc
ctgaagattttgcagtttattactgtcagcagcgtagcagctcccctttcacctttggcagcggccaccaagt
ggaaattaaa (SEQ ID NO:236)

(amino acids)

EVQLVESGGGLVQPKGSLRLSCAASGFTFSRYGMSWVRQAPGKRLEWVSTISGGGTYYIYPDSVKGRFTISR
NAKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWGQGLTVTVSSDKTHTKPPKPAPELLGGPGTGTGG
PTIKPPKPPKPPNLLGGPEIVLTQSPATLSLSPGERATLTCSATSSVSYIHQYQQRPGQSPRLLIYSTSNLA
SGIPARFSGSGSGSDYTLTISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIK (SEQ ID NO:237)

Humanized C2 single chain GS3

(DNA)

gaggtgcagctgggtggagtctgggggaggcctgggtcaagcctgggggggtccctgagactctcctgtgcagcct
ctggattcaccttcagtggtatgccatgagctgggtccgccaggctccagggaaggggctggagtgggtctc
aaccattagtagtgccggaacctacatatactaccagactcagtgaaggggccgattcaccatctccagagac
aacgccaagaactcactgtatctgcaaataaacagcctgagagccgaggacacggccgtgtattactgtgcga
gacttggggggggataaattactacgaatacttcgatgtctggggcaaagggaccaggtcacctgtcctccgg
cggtggcggtatccggcggtggcggtatccggcggtggcggtatccgacattgtgctgaccagctctccagcctcc
ttggccgtgtctccagacagagggccaccatcacctgcagagccagtaagagtgctcagtagcagcggtatcc
cctacatgcactgggtatcagcagaaaccaggacaacctcctaaactcctgatttacctggcatccaatctgga
gagcgggggtcccagccaggttcagcggcagtggtctgggacagatttcacctcacaattaatcctgtggaa
gctaataactgatactgcaaattattactgtcagcagcagtagggagctgcctttcacattcggcgaggggaccaag
tgagatcaaacgaact (SEQ ID NO:238)

(amino acids)

EVQLVESGGGLVQPKGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYYIYPDSVKGRFTISR
NAKNSLYLQMNSLRAEDTAVYYCARLGGDNYYEYFDVWGKGTITVTVSSGGGGSGGGSGGGGSDIVLTQSPAS

LAVSPGQRATITCRASKSVSTSGYSYMHYQKPGQPPKLLIYLASNLESGVPARFSGSGSGTDFTLTINPVE
ANDTANYYCQHSRELPFTFGGGTKVEIKRT (SEQ ID NO:239)

Humanized C2 single chain IgG (no Cysteine)

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcct
ctggattcaccttcagtggtatgccatgagctgggtccgccaggctccagggaaggggctggagtggtctc
aaccattagtagtggcggaacctacatataactacccgactcagtgaagggccgattcaccatctccagagac
aacgccaagaactcactgtatctgcaaataaacagcctgagagccgaggacacggccgtgtattactgtgcga
gacttggggggggataaattactacgaataacttcgatgtctggggcaaagggaccacggtcaccgtctcctccga
taaaaccataactaaaccgcaaaaaccggcgccggaactgctgggtggtcctggtaccggtgacattgtgctg
accagctctccagcctccttgccgtgtctccaggacagagggccaccatcacctgcagagccagtaagagtg
tcagtaccagcggatactcctacatgcactggtatcagcagaaaccaggacaacctcctaaactcctgattta
cctggcatccaatctggagagcgggggtcccagccagggttcagcggcagtggtctgggaccgatttcaccctc
acaattaatcctgtggaagctaatactgatactgcaaattattactgtcagcacagtagggagctgcctttcacat
tcggcgaggaggaccaaggtggagatcaaacgaact (SEQ ID NO:240)

(amino acids)

EVQLVESGGGLVLPKPGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDSVKGRFTISR
NAKNSLYLQMNSLRAEDTAVYYCARLGGDNYYEYFDVWGKGTITVTVSSDKTHTKPPKPAPELLGGPGTGDIVL
TQSPASLAVSPGQRATITCRASKSVSTSGYSYMHYQKPGQPPKLLIYLASNLESGVPARFSGSGSGTDFTL
TINPVEANDTANYYCQHSRELPFTFGGGTKVEIKRT (SEQ ID NO:241)

Humanized C2 single chain X4 (linker is IgG1 and IgG2 modified hinge region)

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcct
ctggattcaccttcagtggtatgccatgagctgggtccgccaggctccagggaaggggctggagtggtctc
aaccattagtagtggcggaacctacatataactacccgactcagtgaagggccgattcaccatctccagagac
aacgccaagaactcactgtatctgcaaataaacagcctgagagccgaggacacggccgtgtattactgtgcga
gacttggggggggataaattactacgaataacttcgatgtctggggcaaagggaccacggtcaccgtctcctccga
taaaaccataactaaaccgcaaaaaccggcgccggaactgctgggtggtcctggtaccggtactggtggtccg
actattaacactccgaaacactccgaaacactgctccgaacactgctgggtggtccggacattgtgctgaccag
ctccagcctccttgccgtgtctccaggacagagggccaccatcacctgcagagccagtaagagtgtcagtac
cagcggatactcctacatgcactggtatcagcagaaaccaggacaacctcctaaactcctgatttacctggca
tccaatctggagagcgggggtcccagccagggttcagcggcagtggtctgggaccgatttcaccctcacaatta
atcctgtggaagctaatactgatactgcaaattattactgtcagcacagtagggagctgcctttcacattcggcgg
agggaccaaggtggagatcaaacgaact (SEQ ID NO:242)

(amino acids)

EVQLVESGGGLVLPKPGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDSVKGRFTISR
NAKNSLYLQMNSLRAEDTAVYYCARLGGDNYYEYFDVWGKGTITVTVSSDKTHTKPPKPAPELLGGPGTGTGGP
TIKPPKPPKPAENLLGGPDIVLTQSPASLAVSPGQRATITCRASKSVSTSGYSYMHYQKPGQPPKLLIYLA
SNLESGVPARFSGSGSGTDFTLTINPVEANDTANYYCQHSRELPFTFGGGTKVEIKRT (SEQ ID
NO:243)

Humanized C3 single chain GS3

(DNA)

caggttcagctggtgcagctctggagctgaggtgaagaagcctggggcctcagtggaaggtctcctgcaaggctt
ctggttacacctttaccgactacgccatgaactgggtgacagaggccctggacaagggcttgagtggtggg
agtgatcagcaccttcagcggtaacacaaacttcaaccagaagttcaagggcagagtcaccatgaccacagac
acatccacgagcacagcctacatggagctgaggagcctgagatctgacgacacggccgtgtattactgtgcga
gaagcgactactacggccatacttcgactactggggccagggcaccaccctgaccgtgtccagcggcgggtgg
cggatccggcgggtggcggatccggcgggtggcggatccgatattgtgatgaccagactccactctctctgtcc

gtcacccttgacagcgcgcctccatctcctgcaggtctagtcagaccattgtccatagtaatggaaacacct
 atttggagtggtacctgcagaagccaggccaggtctccacagctcctgatctataaggtttccaaccggttctc
 tggagtgccagataggttcagtggcagcgggtcagggacagatttcacactgaaaatcagccgggtggaggt
 gaggatgttggggtttattactgcttccaaggtagccacgtgcctttcaccttcggcggagggaccaaggtgg
 agatcaaacgaact (SEQ ID NO:244)

(amino acids)

QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMNWVRQAPGQGLEWMGVISTFSGNTNFNQKFKGRVTMTTD
 TSTSTAYMELRSLRSDDTAVYYCARSDYYGPYFDYWGGQTTLTVSSGGGSGGGGSGGGGSDIVMTQTPLSL
 VTPGQPASISCRSSQTIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEA
 EDVGVYYCFQGSHVPFTFGGGTKVEIKRT (SEQ ID NO:245)

Humanized C3 single chain IgG1 (no Cysteine)

(DNA)

caggttcagctggtgcagctctggagctgaggtgaagaagcctggggcctcagtggaaggtctcctgcaaggctt
 ctggttacacctttaccgactacgccatgaactgggtgcgacaggccctggacaagggcttgagtggatggg
 agtgatcagcaccttcagcggtaacacaaacttcaaccagaagttcaagggcagagtcaccatgaccacagac
 acatccacgagcacagcctacatggagctgaggagcctgagatctgacgacacggcctgtattactgtgcga
 gaagcgactactacggccatacttcgactactggggccagggcaccaccctgaccgtgtccagcgataaaac
 ccatactaaaccgcaaaaccggcgccggaactgctgggtggctcctggtagcgggtgatattgtgatgaccag
 actccactctctctgtccgtcacccttgacagcgcgcctccatctcctgcaggtctagtcagaccattgtcc
 atagtaatggaaacacctatattggagtggtacctgcagaagccaggccaggtctccacagctcctgatctataa
 ggtttccaaccggttctctggagtgccagataggttcagtggcagcgggtcagggacagatttcacactgaaa
 atcagccgggtggaggctgaggatgttggggtttattactgcttccaaggtagccacgtgcctttcaccttcg
 gcggagggaccaaggtggagatcaaacgaact (SEQ ID NO:246)

(amino acids)

QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMNWVRQAPGQGLEWMGVISTFSGNTNFNQKFKGRVTMTTD
 TSTSTAYMELRSLRSDDTAVYYCARSDYYGPYFDYWGGQTTLTVSSDKTHTKPPKPAPELLGGPGTGDIVMTQ
 TPLSLSVTPGQPASISCRSSQTIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLK
 ISRVEAEDVGVYYCFQGSHVPFTFGGGTKVEIKRT (SEQ ID NO:247)

Humanized C3 single chain X4 (linker is IgG1 and IgG2 modified hinge region)

(DNA)

caggttcagctggtgcagctctggagctgaggtgaagaagcctggggcctcagtggaaggtctcctgcaaggctt
 ctggttacacctttaccgactacgccatgaactgggtgcgacaggccctggacaagggcttgagtggatggg
 agtgatcagcaccttcagcggtaacacaaacttcaaccagaagttcaagggcagagtcaccatgaccacagac
 acatccacgagcacagcctacatggagctgaggagcctgagatctgacgacacggcctgtattactgtgcga
 gaagcgactactacggccatacttcgactactggggccagggcaccaccctgaccgtgtccagcgataaaac
 ccatactaaaccgcaaaaccggcgccggaactgctgggtggctcctggtagcgggtactgggtggctccgactatt
 aaacctccgaaacctccgaaacctgctccgaacctgctgggtggctccggatattgtgatgaccagactccac
 tctctctgtccgtcacccttgacagcgcgcctccatctcctgcaggtctagtcagaccattgtccatagtaa
 tggaaacacctatattggagtggtacctgcagaagccaggccaggtctccacagctcctgatctataaggtttcc
 aaccggttctctggagtgccagataggttcagtggcagcgggtcagggacagatttcacactgaaaatcagcc
 ggggtggaggctgaggatgttggggtttattactgcttccaaggtagccacgtgcctttcaccttcggcggagg
 gaccaaggtggagatcaaacgaact (SEQ ID NO:248)

(amino acids)

QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMNWVRQAPGQGLEWMGVISTFSGNTNFNQKFKGRVTMTTD
 TSTSTAYMELRSLRSDDTAVYYCARSDYYGPYFDYWGGQTTLTVSSDKTHTKPPKPAPELLGGPGTGTGGPTI
 KPPKPPKPAPELLGGPDIVMTQTPLSLSVTPGQPASISCRSSQTIVHSNGNTYLEWYLQKPGQSPQLLIYKVS
 NRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPFTFGGGTKVEIKRT (SEQ ID NO:249)

Humanized C8 single chain GS3 (linker is [Gly₄Ser₁]₃)

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcct
 ctggattcaccttcagtggtatgccaatgagctgggtccgccaggctccagggaaggggctggagtggtctc
 aaccattagtagtggcggaacctacatataactaccctgactcagtgaggggccgattcaccatctccagagac
 aacgccaagaactcactgtatctgcaaataaacagcctgagagccgaggacacggccgtgtattactgtgcga
 gactgggcggcgataactattatgaatattggggcaaagggaccacggtcaccgtctcctccggcggtggcgg
 atccggcggtggcggtatccggcggtggcggtatccgacatcgtgatgaccagtcctccagactccctggctgtg
 tctctgggcgagagggccaccatcaactgcaggggccagcaagagtgttagcaccagcggctacagctacatgc
 actggtaccagcagaaaccaggacagcctcctaagctgctcatttacctgggtgttaacctggaatccgggggt
 ccctgaccgattcagtggtcagcgggtctgggacagatttcactctcaccatcagcagcctgcaggctgaagat
 gtggcagtttattactgtcaacacattcgggaactgaccaggagtgaattcggcgaggaggaccaaggtggaga
 tcaaacgaact (SEQ ID NO:250)

(amino acids)

EVQLVESGGGLVKPGLSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDSVKGRFTISR
 NAKNSLYLQMNSLRAEDTAVYYCARLGGDNYYEYWGKTTTVTVSSGGGSGGGGSGGGGSDIVMTQSPDSLAV
 SLGERATINCRASKSVSTSGYSYMHWYQQKPGQPPLLLIYLVSNLESGVPDRFSGSGSGTDFTLTISLQAE
 D VAVYYCQHIRELTRSEFGGGTKVEIKRT (SEQ ID NO:251)

Humanized C8 single chain IgG1 (no Cysteine)

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcct
 ctggattcaccttcagtggtatgccaatgagctgggtccgccaggctccagggaaggggctggagtggtctc
 aaccattagtagtggcggaacctacatataactaccctgactcagtgaggggccgattcaccatctccagagac
 aacgccaagaactcactgtatctgcaaataaacagcctgagagccgaggacacggccgtgtattactgtgcga
 gactgggcggcgataactattatgaatattggggcaaagggaccacggtcaccgtctcctccgataaaaccca
 tactaaaccgccccaaaccggcgccggaactgctgggtggtcctggtaccgggtgacatcgtgatgaccagtc
 ccagactccctggctgtgtctctgggcgagagggccaccatcaactgcaggggccagcaagagtgttagcacca
 gcggctacagctacatgcactggtaccagcagaaaccaggacagcctcctaagctgctcatttacctgggtgtc
 taacctggaatccgggggtccctgaccgattcagtggtcagcgggtctgggacagatttcactctcaccatcagc
 agcctgcaggctgaagatgtggcagtttattactgtcaacacattcgggaactgaccaggagtgaattcggcg
 gagggaccaaggtggagatcaaacgaact (SEQ ID NO:252)

(amino acids)

EVQLVESGGGLVKPGLSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDSVKGRFTISR
 NAKNSLYLQMNSLRAEDTAVYYCARLGGDNYYEYWGKTTTVTVSSDKTHTKPPKPAPELLGGPGTGDIVMTQS
 PDSLAVSLGERATINCRASKSVSTSGYSYMHWYQQKPGQPPLLLIYLVSNLESGVPDRFSGSGSGTDFTLTIS
 SLQAEADVAVYYCQHIRELTRSEFGGGTKVEIKRT (SEQ ID NO:253)

Humanized C8 single chain X4 (linker is IgG1 and IgG2 modified hinge region)

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcct
 ctggattcaccttcagtggtatgccaatgagctgggtccgccaggctccagggaaggggctggagtggtctc
 aaccattagtagtggcggaacctacatataactaccagactcagtgaggggccgattcaccatctccagagac
 aacgccaagaactcactgtatctgcaaataaacagcctgagagccgaggacacggccgtgtattactgtgcga
 gactgggcggcgacaattactatgagtattggggcaaagggaccacggtcaccgtctcctccgataaaaccca
 tactaaaccgccccaaaccggcgccggaactgctgggtggtcctggtaccgggtactgggtgggtccgactattaaa
 cctccgaaacctccgaaacctgctccgaacctgctgggtggtccggacatcgtgatgaccagtcctccagact
 ccctggctgtgtctctgggcgagagggccaccatcaactgcaggggccagcaagagtgttagcaccagcggcta
 cagctacatgcactggtaccagcagaaaccaggacagcctcctaagctgctcatttacctgggtgttaacctg
 gaatccgggggtccctgaccgattcagtggtcagcgggtctgggacagatttcactctcaccatcagcagcctgc

aggctgaagatgtggcagtttattactgtcaacacattcggaactgaccaggagtgaattcggcggaggagac
caaggtggagatcaaacgaact (SEQ ID NO:254)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDSVKGRFTISRDN
AKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYWGKTTVTVSSDKHTTKPPKPAPELLGGPGTGTGGPTIK
PPKPPKAPNLLGGPDIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSYMHWYQQKPGQPPLLIYLVSNL
ESGVPDRFSGSGSTDFTLTISSLQAEDVAVYYCQHIRELTRSEFGGGTKVEIKRT (SEQ ID NO:255)

pSECTag2 E6 scFV-FC

(DNA)

atggagacagacacactcctgctatgggtactgctgctctgggttccaggttccactggtgacgcgg
cccagccggccgaggtgcagctggtggagtctgggggaggcctggtcaagcctgggggggtccctgag
actctcctgtgcagcctctggattcaccttcagtaggtatggcatgagctgggtccgccaggctcca
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gagagccgaggacacggctgtgtattactgtaccagagataactatggccgcaactatgattatggc
atggattattggggccaggggcacccctggtgaccgtgagcagcggcggtggcggtatccggcggtggcg
gatccggcggtggcggtatccgaaattgtgttgacacagctctccagccaccctgtctttgtctccagg
ggaaagagccaccctcacctgcagcggccaccagcagtggttagctacatccactggtaccaacagagg
cctggccagagccccaggctcctcatctatagcacctccaacctggccagcggcatcccagccagggt
tcagtggcagtggtctgggagcgactacactctcaccatcagcagcctagagcctgaagattttgc
agtttattactgtcagcagcgtagcagctccccctttcacctttggcagcggcaccaaagtggaaatt
aaagagcccaaattctgtgacaaaactcacacatgccaccgctgccagcacctgaactcctggggg
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cacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggc
gtggaggtgcataatgccaaagacaaagccgcgggaggagcagtagaacagcacgtaccgtgtggtca
gcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaa
agccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacagggtg
tacaccctgcccccatccccgggaggagatgaccaagaaccagggtcagcctgacctgacctggtcaaag
gcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagac
cacgcctcccgtgctggactccgacgggtccttcttctctacagcaagctcaccgtggacaagagc
agggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatgataa (SEQ ID NO:256)

(amino acids)

METDTLLLWVLLLWVPGSTGDAAQPAEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAP
GKRLEWVSTISSGGTYIYYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYG
MDYWGQGLTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWYQQR
PGQSPRLLIYSTSNLASGIPARFSGSGSGSDYTLTISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEI
KEPKSCDKHTHTCPKPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSQSVMEALHNHYTQKSLSLSPGK** (SEQ ID NO:257)

E6 scFC-FC 1 gBLOCK sequence:

tgctctgggttccaggttccactggtgacgcggcccagccggccgaggtgcagctggtggagtctgg
gggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcctctggattcaccttcagt
aggatggcatgagctgggtccgccaggctccagggaagaggctggagtgggtctcaaccattagtg
gcggaggcacctacataactaccagactcagtgaaggggccgattcaccatctccagagacaacgc

caagaacaccctgtatctgcaaataaacagcctgagagccgaggacacggctgtgtattactgtacc
agagataactatggccgcaactatgattatggcatggattattggggccagggcaccctggtagccg
tgagcagcggcggtggcggtatccggcggtggcggtatccggcggtggcggtatccgaaattgtgttgac
acagtctccagccaccctgtctttgtc (SEQ ID NO:258)

E6 scFC-FC 2 gBLOCK sequence:

aattgtgttgacacagtcctccagccaccctgtctttgtctccaggggaaagagccaccctcacctgc
agcgccaccagcagtgtagctacatccactggtaccaacagaggcctggccagagccccaggtcc
tcatctatagcacctccaacctggccagcggcatcccagccaggttcagtggcagtggtctgggag
cgactacactctcaccatcagcagcctagagcctgaagattttgcagtttattactgtcagcagcgt
agcagctccccctttcacctttggcagcggcaccaaagtggaaattaaagagcccaaattctgtgaca
aaactcacacatgcccaccgtgcccagcacctgaactcctggggggaccgtcagtccttctcttccc
cccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgctgtggtggtggacgtg
agccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaga
caaagccgcgggaggagcagtcacaacagcacgtaccgtgtggtcagc (SEQ ID NO:259)

pSECTag2 C2 scFV-FC

(DNA)

atggagacagacacactcctgctatgggtaactgctgctctgggttccaggttccactggtgacgcgg
cccagccggccgaggtgcagctggtggagtctgggggaggcctggtcaagcctgggggggtccctgag
actctcctgtgcagcctctggattcaccttcagtggtctatgccatgagctgggtccgccaggctcca
gggaaggggctggagtgggtctcaaccattagtagtggcggaacctacataactaccccgactcag
tgaagggccgattcaccatctccagagacaacgccaagaactcactgtatctgcaaataaacagcct
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gatgtctggggcaaagggaccacgggtcacctctcctccggcggtggcggtatccggcggtggcggtat
ccggcggtggcggtatccgacattgtgctgaccacgtctccagcctccttggccgtgtctccaggaca
gagggccaccatcacctgcagagccagtaagagtgtcagtagcagcgatactcctacatgcactgg
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taatgatactgcaaattattactgtcagcacagtagggagctgcctttcacattcggcggaggggacc
aaggtggagatcaaacgaactgagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccag
cacctgaactcctggggggaccgtcagtccttcttccccccaaaacccaaggacaccctcatgat
ctcccgaccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttc
aactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtcacaaca
gcagtagcgtgtggtcagcgtcctcacctcctgcaccaggactggctgaatggcaaggagtacaa
gtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaagccaaagggcag
ccccgagaaccacaggtgtacacctgcccccatcccgaggagatgaccaagaaccaggtcagcc
tgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagcc
ggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaag
ctcacctggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctc
tgcacaaccactacacgcagaagagcctctcctgtctccgggtaaatgataa (SEQ ID
NO:260)

(amino acids)

METDTLLLWVLLLWVPGSTGDAAQPAEVQLVESGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAP
GKGLEWVSTISSGGTYIYYPDsvKGRFTISRDNakNSLYLQMNSLRAEDTAVYYCARLGGDNYYEYF
DVWGKGTITVTVSSGGGGSGGGSGGGSDIVLTQSPASLAVSPGQRATITCRASKSVSTSGYSYMHW
YQQKPGQPPKLLIYLASNLESgVpARfSGSGSgTDFTLTINPVEANDTANYYCQHSRELpFTFGGT
KVEIKRTEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVDVSHEDPEVKF
NWWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQ

PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK
LTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK** (SEQ ID NO:261)

C2 scFV-FC 1 gBLOCK sequence:

(DNA)

tgctctgggttccaggttccactggtgacgcggcccagccggccgaggtgcagctggtggagtctgg
gggaggcctggtcaagcctggggggtccctgagactctcctgtgcagcctctggattcaccttcagt
ggctatgccatgagctgggtccgccaggctccagggaaggggctggagtgggtctcaaccattagta
gtggcggaacctacatatactaccccgactcagtgaagggccgattcaccatctccagagacaacgc
caagaactcactgtatctgcaaatgaacagcctgagagccgaggacacggccgtgtattactgtgcg
agacttgggggggataattactacgaatacttcgatgtctggggcaaagggaccacggtcaccgtct
cctccggcggtggcggtatccggcggtggcggtatccggcggtggcggtatccgacattgtgctgacca
gtctccagcctccttggc (SEQ ID NO:262)

C2 scFV-FC 2 gBLOCK sequence:

(DNA)

cattgtgctgacccagttctccagcctccttggccgtgtctccaggacagagggccaccatcacctgc
agagccagtaagagtgtcagtaccagcgatactcctacatgcactggtatcagcagaaaccaggac
aacctcctaaactcctgatttacctggcatccaatctggagagcggggtccagccaggttcagcgg
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tactgtcagcacagtagggagctgcctttcacattcggcgaggaggaccaaggtggagatcaaacgaa
ctgagcccaaactcttgtgacaaaactcacacatgccaccgtgccagcacctgaactcctgggggg
accgtcagttctcctcttccccccaaaacccaaggacaccctcatgatctcccggaaccttgaggtc
acatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcg
tgaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcag
c (SEQ ID NO:263)

pSECTag2 C3 scFV-FC

(DNA)

atggagacagacacactcctgctatgggtactgctgctctgggttccaggttccactggtgacgcgg
cccagccggcccaggttcagctggtgcagctctggagctgaggtgaagaagcctggggcctcagtga
ggtctcctgcaaggcttctggttacacctttaccgactacgccatgaactgggtgacagggccct
ggacaagggcttgagtggatgggagtgatcagcaccttcagcggtaacacaaacttcaaccagaagt
tcaagggcagagtcaccatgaccacagacacatccacgagcacagcctacatggagctgaggagcct
gagatctgacgacacggccgtgtattactgtgagagaagcgactactacggcccatacttcgactac
tggggccagggcaccaccctgaccgtgtccagcggcggtggcggtatccggcggtggcggtatccggcg
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gtggagatcaaacgaactgagcccaaactcttgtgacaaaactcacacatgccaccgtgccagcac
ctgaactcctgggggggaccgtcagttctcctcttccccccaaaacccaaggacaccctcatgatctc
ccggaccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaac
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cctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccgga
gaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagctc

accgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgc
acaaccactacacgcagaagagcctctccctgtctccgggtaaataata (SEQ ID NO:264)

(amino acids)

METDTLLLLWVLLLVPGSTGDAAQPAQVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMNWRQAPGQGLEW
MGVISTFSGNTNFNQKFKGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARSDYYGPYFDYWGQGTTLTVSSG
GGGSGGGSGGGGSDIVMTQTPLSLSVTPGQPASISCRSSQTIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNR
FSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHPVFTFGGGTKVEIKRTEPKSCDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSLSPGK**
(SEQ ID NO:265)

C3 GS scFV FC 1 gBLOCK sequence:

(DNA)

tgtctctgggttccaggttccactggtgacgcggcccagccggcccaggttcagctggtgcagctctgg
agctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggccttctggttacacctttacc
gactacgcatgaactgggtgacgacaggcccctggacaagggttgagtggatgggagtgatcagca
ccttcagcggtaacacaaacttcaaccagaagttcaagggcagagtcaccatgaccacagacacatc
cacgagcacagcctacatggagctgaggagcctgagatctgacgacacggccgtgtattactgtgcg
agaagcgactactacggcccatacttgcactactggggccagggcaccaccctgaccgtgtccagcg
gcggtggcggatccggcggtggcggatccggcggtggcggatccgatattgtgatgaccagactcc
actctctctgt (SEQ ID NO:266)

C3 scFV FC2 gBLOCK sequence:

(DNA)

tattgtgatgaccagactccactctctctgtccgtcaccctggacagccggcctccatctcctgc
aggtctagtacagaccattgtccatagtaatggaaacacctatttggagtggtacctgcagaagccag
gccagtctccacagctcctgatctataaggtttccaaccgggtctcttgagtgccagataggttcag
tggcagcgggtcaggacagatttcacactgaaaatcagccgggtggaggctgaggatgttgggggtt
tattactgcttccaaggtagccacgtgcctttcaccttcggcgaggaggaccaaggtggagatcaaac
gaactgagcccaaactcttgtagacaaaactcacacatgccaccgtgccagcacctgaactcctggg
gggaccgtcagtccttcttccccccaaaacccaaggacaccctcatgatctcccggacccttgag
gtcacatgctgtggtgggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacg
gcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtagaacagcacgtaccgtgtggt
cagc (SEQ ID NO:267)

pSECTag2 C8 scFV-FC

(DNA)

atggagacagacacactcctgctatgggtactgctgctctgggttccaggttccactggtgacgcgg
cccagccggccgaggtgcagctggtggagtctgggggaggcctggtcaagcctgggggggtccctgag
actctcctgtgcagcctctggattcaccttcagtggctatgccatgagctgggtccgccaggctcca
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ggcaaagggaccacgggtcacctgtctcctccggcggtggcggatccggcggtggcggatccggcggtg
gcggatccgacatcgtgatgaccagctctccagactccctggctgtgtctctgggagagaggccac
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aaaccaggacagcctcctaagctgctcatttacctgggtgtctaacctggaatccgggggtccctgacc
gattcagtggcagcgggtctgggacagatttcactctcaccatcagcagcctgcaggctgaagatgt

ggcagttttattactgtcaacacattcgggaactgaccaggagtgaattcggcgaggaggaccaaggtg
 gagatcaaacgaactgagcccaaattcttgtgacaaaactcacacatgccaccgtgccagcacctg
 aactcctgggggggaccgtcagtccttcttccccccaaaacccaaggacaccctcatgatctcccg
 gacccttgaggtcacatgcggtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactgg
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 gcctgggtcaaaggcttctatcccagcgacatcgccgtggagtgaggagcaatgggagccggagaa
 caactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagctcacc
 gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcaca
 accactacacgcagaagagcctctccctgtctccgggtaaatgataa (SEQ ID NO:268)

(amino acids)

METDTLLLWVLLLWVPGSTGDAAQPAEVQLVESGGGLVKGPGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEW
 VSTISSGGTYIYYPDSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYWGKGTITVTVSSGG
 GSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSYMHWYQQKPGQPPKLLIYLVSNLES
 GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHIRELTRSEFGGGTKVEIKRTEPKSCDKTHTCPPCPAPEL
 LGGPVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
 VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
 WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK**

(SEQ ID NO:269)

C8 scFV FC 1 gBLOCK sequence:

(DNA)

tgctctgggttccaggttccactggtgacgcggcccagccggccgaggtgcagctggtggagtctgg
 gggaggcctggtcaagcctggggggtccctgagactctcctgtgcagcctctggattcaccttcagt
 ggctatgccatgagctgggtccgccaggtccaggggaaggggctggagtggttctcaaccattagta
 gtggcggaacctacatatactaccctgactcagtggaagggccgattcaccatctccagagacaacgc
 caagaactcactgtatctgcaaatgaacagcctgagagccgaggacacggccgtgtattactgtgcg
 agactgggcgggcgataactattatgaatattggggcaaagggaccacgggtcacctgtcctccggcg
 gtggcggtatccggcggtggcggtatccggcggtggcggtatccgacatcgtgatgaccaggtctccaga
 ctccctgg (SEQ ID NO:270)

C8 scFV FC2 gBLOCK sequence:

(DNA)

catcgtgatgaccaggtctccagactccctggctgtgtctctgggcgagagggccaccatcaactgc
 agggccagcaagagtgttagcaccagcggctacagctacatgcactggtaccagcagaaaccaggac
 agcctcctaagctgctcatttacctgggtgttaacctggaatccgggggtccctgaccgattcagtg
 cagcgggtctgggacagatttactctcaccatcagcagcctgcaggtgaagatgtggcagtttat
 tactgtcaacacattcgggaactgaccaggagtgaattcggcgaggaggaccaaggtggagatcaaac
 gaactgagcccaaattcttgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggg
 gggaccgtcagtccttcttccccccaaaacccaaggacaccctcatgatctcccggaccctgag
 gtcacatgcggtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacg
 gcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggt
 cagc (SEQ ID NO:271)

Human IgG1 Fc sequence:

(DNA)

gagcccaaattcttgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcag
 tcttctcttccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtgggtgt
 ggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaag
 acaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcacctcctgcaccaggact

ggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctc
 caaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaac
 caggtcagcctgacctgctgggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggc
 agccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagct
 caccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
 cactacacgcagaagagcctctccctgtctccgggtaaatgataa (SEQ ID NO:272)

(amino acids)

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFKNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
 QVSLTCLVKGIFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSFSVMHEALHN
 HYTQKSLSLSPGK** (SEQ ID NO:273)

Human IgG1 CH2-CH3 domain sequence:

(DNA)

ccgtgccagcacctgaactcctggggggaccgtcagttcttctcttccccccaaaacccaaggacacctca
 tgatctcccggacccctgaggtcacatgcgtgggtggtagcgtgagccacgaagacctgaggtcaagttcaa
 ctggtagcgtggacggcgtggaggtgcataatgccaaagacaaagccgaggagcagtagacaacagcacgtac
 cgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctcca
 acaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgta
 caccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgctgggtcaaaggcttctat
 ccagcgacatcgccgtggagtgggagagcaatgggcagccggagagaacaactacaagaccacgcctcccgtgc
 tggactccgacggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgt
 cttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggt
 aatgataa (SEQ ID NO:274)

(amino acids)

PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFKNWYVDGVEVHNAKTKPREEQYNSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGIFY
 PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSFSVMHEALHNHYTQKSLSLSPG
 K** (SEQ ID NO:275)

Human IgG1 CH3 domain sequence:

(DNA)

gggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcc
 tgacctgctgggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaa
 caactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagctcaccgtggac
 aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
 agaagagcctctccctgtctccgggtaaatgataa (SEQ ID NO:276)

(amino acids)

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGIFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
 KSRWQQGNVFSFSVMHEALHNHYTQKSLSLSPGK** (SEQ ID NO:277)

Human IgG1 Fc Y407R sequence:

(DNA)

gagcccaaattctgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcag
 tcttctcttccccccaaaacccaaggacacctcatgatctcccggacccctgaggtcacatgcgtgggtgg
 ggacgtgagccacgaagacctgaggtcaagttcaactggtagcgtggacggcgtggaggtgcataatgccaa
 acaaagccgaggaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggact
 ggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctc
 caaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaac
 caggtcagcctgacctgctgggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggc
 agccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctcaggagcaagct

caccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaatgataa (SEQ ID NO:278)

(amino acids)

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLRSKLTVDKSRWQQGNVFSQSVSMHEALHN
HYTQKSLSLSPGK** (SEQ ID NO:279)

Human IgG1 Fc F405Q sequence:

(DNA)

gagcccaaatcttgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcag
tcttcctcttccccccaaaacccaaggacaccctcatgatctcccggaaccctgaggtcacatgcgtggtggt
ggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggact
ggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctccagcccccatcgagaaaaccatctc
caaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatccggggaggagatgaccaagaac
caggtcagcctgacctgacctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggc
agccgggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttccagctctacagcaagct
caccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaatgataa (SEQ ID NO:280)

(amino acids)

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFQLYSLKLTVDKSR
WQQGNVFSQSVSMHEALHNHYTQKSLSLSPGK** (SEQ ID NO:281)

Human IgG1 Fc T394D sequence:

(DNA)

gagcccaaatcttgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcag
tcttcctcttccccccaaaacccaaggacaccctcatgatctcccggaaccctgaggtcacatgcgtggtggt
ggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggact
ggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctccagcccccatcgagaaaaccatctc
caaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatccggggaggagatgaccaagaac
caggtcagcctgacctgacctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggc
agccgggagaacaactacaagaccgaccctcccggtgctggactccgacggctccttcttctctacagcaagct
caccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaatgataa (SEQ ID NO:282)

(amino acids)

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTDPVLDSDGSFFLYSLKLTVDKSR
WQQGNVFSQSVSMHEALHNHYTQKSLSLSPGK** (SEQ ID NO:283)

Human IgG1 Fc T366W/L368W sequence:

(DNA)

gagcccaaatcttgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcag
tcttcctcttccccccaaaacccaaggacaccctcatgatctcccggaaccctgaggtcacatgcgtggtggt
ggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggact
ggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctccagcccccatcgagaaaaccatctc

caaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaac
caggtcagcctgtggtgctgggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggc
agccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagct
caccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaatgataa (SEQ ID NO:284)

(amino acids)

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLWCVKGFPYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK** (SEQ ID NO:285)

Human IgG1 Fc T364R/L368R sequence:

(DNA)

gagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctggggggaccgtcag
tcttctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgctggtggt
ggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaa
gacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggact
ggctgaatggcaaggagtacaagtgcagggtctccaacaaagccctcccagcccccatcgagaaaaccatctc
caaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaac
caggtcaggtgacctgcagggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggc
agccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagct
caccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaatgataa (SEQ ID NO:286)

(amino acids)

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVRLTTCRVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK** (SEQ ID NO:287)

Human IgG1 Fc hingeless sequence:

(DNA)

gcacctgaactcctggggggaccgtcagttcttcttccccccaaaacccaaggacaccctcatgatctccc
ggacccctgaggtcacatgctggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgt
ggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtc
agcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcagggtctccaacaaagccc
tcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcc
cccatcccgggaggagatgaccaagaaccagggtcagcctgacctgacctggtcaaaggcttctatcccagcgac
atcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccgtgctggactccg
acggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatg
ctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgataa
(SEQ ID NO:288)

(amino acids)

APPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK**
(SEQ ID NO:289)

Human IgG1 G237A FC sequence:

(DNA)

gagcccaaactcttgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggccc
 cgtcagtccttcttccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcac
 atgctggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtg
 gaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcg
 tcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagc
 cctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtac
 accctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgacctggtcaaaggct
 tctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccac
 gcctcccgtgctggactccgacggctccttcttctctacagcaagctcaccgtggacaagagcagg
 tggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcaga
 agagcctctccctgtctccgggtaaa (SEQ ID NO:290)

(amino acids)

EPKSCDKTHTCPPCPAPELLGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
 EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
 TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPFVLDSGDSFFLYSKLTVDKSR
 WQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:291)

Human IgG1 L234A/L235A FC sequence:

(DNA)

gagcccaaactcttgtgacaaaactcacacatgccaccgtgccagcacctgaagccgccggggggac
 cgtcagtccttcttccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcac
 atgctggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtg
 gaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcg
 tcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagc
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 accctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgacctggtcaaaggct
 tctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccac
 gcctcccgtgctggactccgacggctccttcttctctacagcaagctcaccgtggacaagagcagg
 tggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcaga
 agagcctctccctgtctccgggtaaa (SEQ ID NO:292)

(amino acids)

EPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
 EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
 TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPFVLDSGDSFFLYSKLTVDKSR
 WQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:293)

CAR-T E6 CD3z sequence:

(DNA)

atggccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgccgccaggccagagg
 tccagctggttgagagtggcgggtgggctggttaagcctggcggctccctgcggtgagctgcgccgc
 gagtggatttactttcagccgatatgggatgagttgggtgcggcaagctcccggaagaggctggaa
 tgggtctcaacaatctccggggggggcacttacatctattaccccgactcagtcaggggagattta
 ccatttcacgagacaacgctaagaataccctgtatttgcagatgaattctctgagagcagaggacac
 agctgtttactattgtacccgcgacaactatggcaggaactacgactacggtatggactattgggga
 caagggaacattgggttacagtgcagcagtgccggcgggggcagcggaggaggaggcagcgggtggggggg
 gcagcgagatagtgctcacgcagtcacccgcgactctcagtcctcacctggggaacgagctaccct
 gacgtgctctgctacctcctcagtgctcatatattcactgggtatcagcaacggcccgggcagtcacct
 agattgctcatttatagttacctctaattctggcctcaggtatccctgcacgattttctggatctggtt

cagggttctgattacaccctcactatctctagcctggagcctgaagactttgccgtttattactgccagcagaggtctagctccccattcacctttgggagtgaggaccaaggttgaaattaaaacgacaacccccgccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctgagaccagaagcctgtaggcctgccgcccgttgagctgtgcacacaagaggactggatttcgcctgtgatctctacatttgggccccgctcgcaggcacatgtggagtgtcctcctctccctgggtgattaccctgtactgccgcgttaagtctcccgatcagccgacgcgcctgcttacaagcagggccagaaccaactgtacaacgagctgaatctcggttagacgggaagagtacgacgtgttgacaaaacggagaggccgcgaccagaaatgggcgccaagcctcgcaggaaaaacccccaggagggactgtacaatgagttgcagaaagataagatggcagaa gcttatagcgagatcggaatgaagggggaaaggagacgagggaaaggacacgacggcctttatcagg gctgtccacagcaacaaaagatacgtatgacgcctccatattgcaggcacttccaccacggtgata a (SEQ ID NO:294)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLE WVSTISGGGTYYIYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWG QGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWYQQRPGQSP RLLIYSTSNLASGIPARFSGSGSGSDYTLTISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKTTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRV KFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRGKHDGLYQGLSTATKDTYDALHMQALPPR**

** (SEQ ID NO:295)

CAR-T E6 CD3z gBLOCK sequence:

(DNA)

tggagctgtgcacacaagaggactggatttcgcctgtgatctctacattttgggccccgctcgcaggcacatgt ggagtgtcctcctctccctgggtgattaccctgtactgccgcgttaagtctcccgatcagccgacgcgcctg cttacaagcagggccagaaccaactgtacaacgagctgaatctcggttagacgggaagagtacgacgtgttgga caaacggagaggccgcgaccagaaatgggcggcaagcctcgcaggaaaaacccccaggagggactgtacaat gagttgcagaaagataagatggcagaagcttatagcgagatcggaatgaagggggaaaggagacgagggaaag gacacgacggcctttatcagggcctgtccacagcaacaaaagatacgtatgacgcctccatattgcaggcact tccaccacggtgataagtttaaaccgcgtgatcagcctcgactgtgc (SEQ ID NO:296)

CAR-T E6 CD28/CD3z sequence:

(DNA)

atggccctgcccgtgaccgctttgctgctccccctggcgtgctgctgctgcacgccgccaggccagagg tccagctgggttgagagtggcgggtgggctgggttaagcctggcggtccctgcggtgagctgcgccgc gagtggtttactttcagccgatatgggatgagttgggtgcggaagctcccggaagaggctggaa tgggtctcaacaatctccggggggggcacttacatctattacccgactcagtcagggggagattta ccatttcagagacaacgctaagaataccctgtattttgcagatgaattctctgagagcagaggacac agctgtttactattgtaccgcgcacaactatggcaggaactacgactacggatggactattgggga caagggacattgggttacagtgagcagtggcggcgggggcagcggaggaggaggcagcgggtggggggg gcagcgagatagtgtcacgcagtcacccgcgactctcagtcctcacctggggaacgagctaccct gacgtgctctgctacctcctcagtgctcatatattcactgggtatcagcaacggccccgggcagtcacct agattgctcatttatagctacctctaattctggcctcaggtatccctgcacgattttctggatctgggt cagggttctgattacaccctcactatctctagcctggagcctgaagactttgccgtttattactgccagcagaggtctagctccccattcacctttgggagtgaggaccaaggttgaaattaaaacgacaacccccgccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctgagaccagaagcctgtaggcctgccgcccgttgagctgtgcacacaagaggactggatttcgcctgtgatctctacatttg gggccccgctcgcaggcacatgtggagtgtcctcctctccctgggtgattaccctgtactgcagaagc aagcgggtctcggtcctgcattctgattacatgaacatgacccaagaagaccaggccccaccagga aacattaccagccctacgctccgccacgcgacttcgctgcctaccggtcccgcggttaagtctcccg

atcagccgacgcgcctgcttacaagcagggccagaaccaactgtacaacgagctgaatctcggtaga
 cgggaagagtacgacgtgttgacaaacggagagggccgcgacccagaaatgggcggaagcctcgca
 ggaaaaacccccaggagggactgtacaatgagttgcagaaagataagatggcagaagcttatagcga
 gatcggaatgaagggggaaaggagacgagggaaaggacacgacggcctttatcagggcctgtccaca
 gcaacaaaagatacgtatgacgccctccatattgcaggcacttccaccacgggtgataa
 (SEQ ID NO:297)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLE
 WVSTISGGGTYYYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWG
 QGTLVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWYQQRPQGSP
 RLLIYSTSNLASGIPARFSGSGSGSDYTLTISSELEPEDFAVYYCQQRSSSPFTFGSGTKVEIKTTTP
 APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRS
 KRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGR
 REEYDVLDRRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKHDGLYQGLST
 ATKDITYDALHMQALPPR** (SEQ ID NO:298)

CAR-T E6 CD28/CD3z g BLOCK sequence:

(DNA)

tggagctgtgcacacaagaggactggatttcgcctgtgatattacatttgggccccgcctgcagggcacatgt
 ggagtgtcctcctctcctcctgggtgattaccctgtactgcagaagcaagcgggtctcggtcctgcattctgatt
 acatgaacatgaccccaagaagaccaggccccaccaggaaacattaccagccctacgctccgccacgcgactt
 cgctgcctaccgggtcccgcgttaagttctcccgatcagccgacgcgcctgcttacaagcagggccagaacca
 ctgtacaacgagctgaatctcggttagacgggaagagtacgacgtgttgacaaacggagagggccgcgacccag
 aaatgggcggaagcctcgcaggaaaaacccccaggagggactgtacaatgagttgcagaaagataagatggc
 agaagcttatagcgagatcggaatgaagggggaaaggagacgagggaaaggacacgacggcctttatcagggc
 ctgtccacagcaacaaaagatacgtatgacgccctccatattgcaggcacttccaccacgggtgataagtttaa
 cccgctgatcagcctcgactgtgc (SEQ ID NO:299)

CAR-T E6 4-1BB/CD3z sequence:

(DNA)

atggccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgccgccaggccagagg
 tccagctgggttgagagtggcggtgggctgggttaagcctggcggtcctcctgcggctgagctgcgccgc
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 ccatttcacgagacaacgctaagaataccctgtatttgcagatgaattctctgagagcagaggacac
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 caagggaacattgggttacagtgcagcagtgccggcgggggcagcggaggaggaggcagcgggtggggggg
 gcagcgagatagtgctcacgcagtcacccgcgactctcagtcctcacctggggaaacgagctacccct
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 ggccccgcctgcagggcacatgtggagtgtcctcctctcctcctgggtgattaccctgtactgaaaagg
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 ccgatcagccgacgcgcctgcttacaagcagggccagaaccaactgtacaacgagctgaatctcggt
 agacgggaagagtacgacgtgttgacaaacggagagggccgcgacccagaaatgggcggaagcctc
 gcaggaaaaacccccaggagggactgtacaatgagttgcagaaagataagatggcagaagcttatag

cgagatcggaatgaaggggaaaggagacgagggaaaggacacgacggcctttatcagggcctgtcc
acagcaacaaaagatacgtatgacgccctccatatgcaggcacttccaccacggtgataa
(SEQ ID NO:300)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLEWVSTIS
GGGTYYIYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWGQGLTVTVSSGGG
GSGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWYQQRPGQSPRLLIYSTSNLASGIPARF
SGSGSGSDYTLTISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACR
PAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF
PEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQ
KDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDALHMQALPPR**

(SEQ ID NO:301)

CAR-T E6 4-1BB/CD3z gBLOCK sequence:

(DNA)

tggagctgtgcacacaagaggactggatttcgcctgtgatctacatttgggccccgctcgcaggcacatgt
ggagtgtcctcctctccctgggtgattaccctgtactgcaaaaggggcccgcacaaaactcctttacattttta
agcagccttttatgaggccagtacagacgactcaagaggaagacgggtgtcatgccgctttcctgaggagga
ggaaggagggtgcgaactgcgcgttaagtctcccgatcagccgacgcgcctgcttacaagcagggccagaac
caactgtacaacgagctgaatctcggtagacgggaagagtacgacgtgttggacaaacggagagggccgcgacc
cagaaatggcgcgcaagcctcgcaggaaaaacccccaggagggactgtacaatgagttgcagaaagataagat
ggcagaagcttatagcgagatcggaatgaagggggaaaggagacgagggaaaggacacgacggcctttatcag
ggcctgtccacagcaacaaaagatacgtatgacgccctccatatgcaggcacttccaccacggtgataagttt
aaacccgctgatcagcctcgactgtgc (SEQ ID NO:302)

CAR-T E6 CD28/4-1BB/CD3z sequence:

(DNA)

atggccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgccgcccaggccagagg
tccagctgggttgagagtggcgggtgggctgggttaagcctggcggtccctgcggtgagctgcgcgcg
gagtggtttactttcagccgatatgggatgagttgggtgcggcaagctcccggaagaggctggaa
tgggtctcaacaatctccggggggggcacttacatctattaccccgactcagtcagggggagattta
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gtacgacgtgttggacaaacgggagaggccgcgaccagaaatggcgggcaagcctcgcaggaaaaac
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agatacgtatgacgccctccatatgcaggcacttccaccacggtgataa

(SEQ ID NO:303)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLEWVSTIS
GGGTYYIYPDSVKGRFTISRDNANKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWQGTLLTVTVSSGGG
SGSGSGSGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWYQQRPGQSPRLLIYSTSNLASGIPARF
SGSGSGSDYTLTISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACR
PAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRSKRSRLHSDYMNMTPRRPGPTRKHYPYAP
PRDFAAYRSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHGGLYQGLST
ATKDTYDALHMQALPPR** (SEQ ID NO:304)

CAR-T E6 CD28/4-1BB/CD3z gBLOCK sequence:

(DNA)

ataggagagaccaagctggctagttaagcttggtagcaggaggccaccatggccctgcccgtgaccgc
tttgctgctccccctggcgctgctgctgcacgccgagccagaggtccagctgggtgagagtggc
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atatgcaggcacttccaccacggtgataagtttaaaccgctgatcagcctcgaactgtgc (SEQ
ID NO:305)

CAR-T C2 CD28/4-1BB/CD3z sequence:

(DNA)

atggccttgccagtgaaggccctgctgctgccattggctcttctgttgacgctgccaggcctgaagtgcagc
tcgtagagagtggcggggggactgggtgaagcccggtggaagcctcagactcagttgcgcgcctcaggtttcac
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cctgcgcgcttttcagggagtgaggagcggcacagattttacgctgactatcaaccccgtagaagcaaacgata
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 taagatggcagaagcttatagcgagatcggaatgaagggggaaaggagacgagggaaggacacgacggcctt
 tatcagggcctgtccacagcaacaaaagatacgtatgacgccctccatatgcaggcacttccaccacgggtgat
 aa (SEQ ID NO:306)

(amino acids)

EVQLVESGGGLVQPKGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDVSKGRFTISR
 NAKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYFDVWGKGTITVTVSSGGGSGGGGSGGGGSDIVLTQSPAS
 LAVSPGQRATITCRASKSVSTSGYSYMHYQKPGQPKLLIYLASNLESGVPAFRFSGSGSGTDFTLTINPVE
 ANDTANYYCQHSRELPTFTGGGKTKVEIKRTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
 CDIYIWAPLAGTCGVLLLSLVITLYCRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSKRGRKK
 LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNLYNELNLGRREEYDVLDK
 RRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRRGKGDGLYQGLSTATKDTYDALHMQALP
 PR** (SEQ ID NO:307)

CAR-T C2-1 gBLOCK sequence:

(DNA)

atagggagacccaagctggctagtttaagcttgggtaccgagggccaccatggccttgccagtgacggccctgct
 gctgccattggctcttctgttgacgctgccaggcctgaagtgcagctcgtagagagtggcggggggactgggtg
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 caccaacgccagccccaccatcgccagccaacccctgtctctgagaccagaagcctgtaggcctgccgcgg
 tggagctgtgcacacaagaggactggatttcgcctgtgatatactacatttgggccccgctcgaggcacatgt
 ggagtgc (SEQ ID NO:308)

CAR-T C2-2 gBLOCK sequence:

(DNA)

aagtgatattgtgctcacacaatccccagcctccctggctgtgtctcccgccaacgcgctacaattacatgt
 cgggcctccaaaagcgtgagcaccagcggtacagctacatgcactgggtatcaacagaaaccaggacaacccc
 ccaaactgttgatttatctcgttcaaacttggagtcggcggtgctgcgcgcttttcagggagtgaggagcgg
 cacagattttacgctgactatcaaccccgtagaagcaaacgatacagcgaattattattgtcaacattcccgg
 gaactcccctttacgttcggcggggggcacaaagggtcgaaattaagagaaccacgacaacccccggccccagac
 caccaacgccagccccaccatcgccagccaacccctgtctctgagaccagaagcctgtaggcctgccgcgg
 tggagctgtgcacacaagaggactggatttcgcctgtgatatactacatttgggccccgctcgaggcacatgt
 ggagtgc (SEQ ID NO:309)

CAR E6 Fc/8/4-1BB/CD3z sequence:

(DNA)

atggccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgccgcccaggccagagg
 tcagctgggttgagagtggcggtgggctgggttaagcctggcggtccttgccggtgagctgcgcgcg
 gagtggattttactttcagccgatatgggatgagttgggtgcggaagctcccggaagaggctggaa
 tgggtctcaacaatctccggggggggcacttacatctattaccccgactcagtcagggggagattta
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 caaggacattgggttacagtgagcagtgccggcgggggcagcggaggaggaggcagcgggtggcgag
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 cgacggcctttatcagggcctgtccacagcaacaaaagatacgtatgacgcctccatattgcaggca
 cttccaccacgggtgataa (SEQ ID NO:310)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLE
 WVSTISGGGTYYYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWG
 QGTLTVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWWYQQRPGQSP
 RLLIYSTSNLASGIPARFSGSGSGSDYTLTISSELPEDFAVYYCQQRSSSPFTFGSGTKVEIKEPKS
 CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
 SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHREALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM
 RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGR
 DPENGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKHDGLYQGLSTATKDTYDALHMQA
 LPPR** (SEQ ID NO:311)

E6 CAR pCDH gBLOCK sequence:

(DNA)

acgctgttttgacctccatagaagattctagagctagctgtagagcttggtaccgagggccaccatg
 gccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgcgccaggccagaggtcc
 agctgggttgagagtggcggtgggctgggttaagcctggcggtccctgcggctgagctgcgccgcgag
 tggatttactttcagccgatatgggatgagttgggtgcggcaagctcccggaagaggctggaatgg
 gtctcaacaatctccggggggggcacttacatctattaccccgactcagtcagggggagatttacca
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E6 CAR Fc pCDH gBLOCK sequence:

(DNA)

agtaccttaaatctggcctcaggtatccctgcacgattttctggatctgggttcaggttctgattaca
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gggagagcaatgggcagccggagaaacaactacaagaccacgcctcccgtgctg (SEQ ID
NO:313)

E6 CAR 8BB3 pCDH gBLOCK sequence:

(DNA)

agaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagct
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CAR E6 FcH/8/4-1BB/CD3z sequence:

(DNA)

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 tgacgcctccatattgcaggcacttccaccacggtgataa (SEQ ID NO:315)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGLSLRLSCAASGFTFSRYGMSWVRQAPGKRLE
 WVSTISGGGTYYYPDSVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCTRDNYGRNYDYGMDYWG
 QGTLVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHQYQQRPGQSP
 RLLIYSTSNLASGIPARFSGSGSGSDYTLTISLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKAPEL
 LGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
 VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
 VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNH
 YTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR
 FPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGRDPGEMGGKPRRKNPQE
 GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR** (SEQ ID
 NO:316)

E6 CAR FcH pCDH gBLOCK sequence:

(DNA)

agtacctaatctggcctcaggtatccctgcacgattttctggatctgggttcaggttctgattaca
 ccctcactatctctagcctggagcctgaagactttgccgtttattactgccagcagaggtctagctc
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CAR E6 Fc/4/4-1BB/CD3z sequence:

(DNA)

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 ccacggtgataa (SEQ ID NO:318)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVLPKPGSLRLSCAASGFTFSRYGMSWVRQAPGKRLE
 WVSTISGGGTYYYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGM DYWG
 QGTLVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYYHWYQQRPGQSP
 RLLIYSTSNLASGIPARFSGSGSGSDYTLTISLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKEPKS
 CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
 SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMH EALHNHYTQKSLSLSPGKMALIVLGGVAGLLLF IGLGIFFKRGRKKLLYIFKQPFMRP
 VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVL DKRRGRDP
 EMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP
 PR** (SEQ ID NO:319)

E6 CAR 44BB3 pCDH gBLOCK sequence:

(DNA)

agaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagct
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CAR E6 FcH/4/4-1BB/CD3z sequence:

(DNA)

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(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLE
WVSTISGGGTYYYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWG
QGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWHYQQRPGQSP
RLLIYSTSNLASGIPARFSGSGSGSDYTLTISLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNH
YTQKSLSLSPGKMALIVLGGVAGLLLFIFGLGIFFKRGRKLLYIFKQPFMRPVQTTQEEDGCSCRFP
EEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGRDPENGGKPRRKNPQEG
YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR** (SEQ ID
NO:322)

CAR E6 IgD/8/4-1BB/CD3z sequence:

(DNA)

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 tgggttgagagtggcggtgggctgggttaagcctggcggtccctgcggctgagctgcgcgcgagtggtttac
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 tacgtatgacgcctccatattgcaggcacttccaccacgggtgataa (SEQ ID NO:323)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLE
 WVSTISGGTYIYYPDsvKGRFTISRDNakNTLYLQMNslRAEDTAVYYCTRDNYGRNYDYGMdYWG
 QGTLVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHwyQQRPGQSP
 RLLIYSTSNLASGIPARFSGSGSGSDYTLTISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKESPK
 AQASSVPTAQPPQAEGSLAKATTAPATTRNTGRGGEKKKEKEKEEQEERETKPIYIWAPLAGTCGV
 LLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYK
 QGQNQLYNELNLGRREEYDVLdkRRGRDPemGgKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
 RGKGHDGLYQGLSTATKDTYDALHMQALPPR** (SEQ ID NO:324)

E6 CAR IgD8 pCDH gBLOCK sequence:

(DNA)

agtacctctaactctggcctcaggtatccctgcacgattttctggatctggttcaggttctgattaca
 cctcactatctctagcctggagcctgaagactttgccgtttattactgccagcagaggtctagctc
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 ggccag (SEQ ID NO:325)

E6 CAR BB 3 pCDH gBLOCK sequence:

(DNA)

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gaaaggagacgagggaaaggacacgacggcctttatcagggcctgtccacagcaacaaaagatacgt
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cgaaggatctgcgatcgctccgggtgcccgtcag (SEQ ID NO:326)

CAR E6 IgD/4/4-1BB/CD3z sequence:

(DNA)

atggccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgcccaggccagagg
tccagctggttgagagtgccgggtgggctggttaagcctggcggtccctgcggtgagctgcgcgcg
gagtggtttactttcagccgatatgggatgagttgggtgcggaagctcccggaagaggctggaa
tgggtctcaacaatctccggggggggcacttacatctattaccccgactcagtcaaggggagattta
ccatttcacgagacaaacgctaagaataccctgtattttgcagatgaattctctgagagcagaggacac
agctgtttactattgtacccgcgacaactatggcaggaactacgactacggatggactattgggga
caagggacattgggttacagtgcagcagtgccggcgggggcagcggaggaggaggcagcgggtggcgga
gcagcgagatagtgctcacgcagtcacccgcgactctcagtcctcacctggggaacgagctaccct
gacgtgctctgctacctcctcagtggtcatatattcactgggtatcagcaacggcccgggcagtcacct
agattgctcatttatagtacctctaattctggcctcaggtatccctgcacgattttctggatctgggt
caggttctgattacacctcactatctctagcctggagcctgaagactttgccgtttattactgcc
gcagaggtctagctccccattcacctttgggagtgaggaccaaggttgaaattaaagagctctcaaag
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ccccagccaccaccgtaaacacaggaagaggcggcgaagagaagaaaaaggagaaggagaaagagga
acaagaagagagagagacaaagacaccaatggccctgattgtgctggggggcgctcgccggcctcctg
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cttttatgaggccagtacagacgactcaagaggaagacgggtgctcatgccgctttcctgaggagga
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cagaaccaactgtacaacgagctgaatctcggtagacgggaagagtagcagctggttgacaaacgga
gagggcgcgaccacagaaatgggcggaagcctcgcaggaaaaacccccaggagggactgtacaatga
gttgacagaaagataagatggcagaagcttatagcgagatcggaatgaagggggaaaggagacgagg
aaaggacacgacggcctttatcagggcctgtccacagcaacaaaagatacgtatgacgccctccata
tgcaggcacttccaccacgggtgataa (SEQ ID NO:327)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLE
WVSTISGGGTYYYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGM DYWG
QGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWHYQQRPGQSP
RLLIYSTSNLASGIPARFSGSGSGSDYTLTISLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKESPK
AQASSVPTAQQAEGSLAKATTAPATTRNTGRGGEKKKEKEKEEQEERETKTPMALIVLGGVAGLL
LFIGLGIFFKRGRKLLYIFKQPFMRPVQTTQEEDGCSRFP EEEEEGGCELRVKFSRSADAPAYKQG
QNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG
KGHDGLYQGLSTATKDTYDALHMQALPPR** (SEQ ID NO:328)

E6 CAR IgD4 pcDH gBLOCK sequence:

(DNA)

agtacctctaattctggcctcaggtatccctgcacgattttctggatctgggttcaggttctgattaca
ccctcactatctctagcctggagcctgaagactttgccgtttattactgccagcagaggtctagctc
ccattcacctttgggagtgaggaccaaggttgaaattaaagagctctcaaaggcagaggcctcctca
gtgccactgcacaaccccaagcagagggcagcctcgccaaggcaaccacagccccagccaccaccc
gtaaacacaggaagaggcggcgaagagaagaaaaaggagaaggagaaagaggaacaagaagagagaga
gacaaagacaccaatggccctgattgtgctggggggcgctcgccggcctcctgcttttcattgggcta

ggcatcttcttcaaaaggggcccgcacaaaaactcctttacatttttaagcagccttttatgaggccag
(SEQ ID NO:329)

CAR E6 X4/8/4-1BB/CD3z sequence:

(DNA)

atggccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgcccagggccagagg
tccagctggttgagagtggcgggtgggctggttaagcctggcggctccctgcggtgagctgcgcgcg
gagtggttactttcagccgatatgggatgagttgggtgcggaagctcccggaagaggctggaa
tgggtctcaacaatctccggggggggcacttacatctattaccccgactcagtcaaggggagattta
ccatttcacgagacaaacgctaagaataccctgtattttgcagatgaattctctgagagcagaggacac
agctgtttactattgtaccgcgcacaactatggcaggaactacgactacggtatggactattgggga
caagggacattgggttacagtgcagtggtggcgggggcagcggaggaggaggcagcgggtggcgga
gcagcgagatagtgctcacgcagtcacccgcgactctcagtcctcacctggggaacgagctaccct
gacgtgctctgctacctcctcagtggtcatatattcactgggtatcagcaacggcccgggcagtcctct
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caggttctgattacacccctcactatctctagcctggagcctgaagactttgccgtttattactgcc
gcagaggtctagctccccattcacctttgggagtgaggaccaaggttgaaattaaagacaagacgcac
accaagccacctaaccagctccagaactgctcggaggtcctggcaccggaaccggaggacctaaca
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gctcgcaggcacatgtggagtgtcctcctctccctggtgattaccctgtactgcaaaagggggccgc
aaaaaactcctttacatttttaagcagccttttatgaggccagtacagacgactcaagaggaagacg
ggtgctcatgccgctttcctgaggaggaggaaggaggtgcgaactgcgcgttaagttctcccgatc
agccgacgcgcctgcttacaagcagggccagaaccaactgtacaacgagctgaatctcggtagacgg
gaagagtacgacgtgttgacaaaacggagaggccgcgaccagaaatgggcggaagcctcgcagga
aaaacccccaggagggactgtacaatgagttgcagaaagataagatggcagaagcttatagcgagat
cggaatgaagggggaaaggagacgagggaaaggacacgacggcctttatcagggcctgtccacagca
acaaaagatacgtatgacgcctccatatgcaggcacttccaccacgggtgataa (SEQ ID
NO:330)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLE
WVSTISGGGTYYYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGM DYWG
QGTLVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWHYQQRPGQSP
RLLIYSTSNLASGIPARFSGSGSGSDYTLTISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKDKTH
TKPPKPAPELLGGPGTGTGGPTIKPPKPPKPAPNLLGGPIYIWAPLAGTCGVLLLSLVITLYCKRGR
KKLLYIFKQPFMRPVQTTQEEDGCSRFP EEEEEGGCEL RVKFSRSADAPAYKQGQNQLYNELNLGRR
EEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTA
TKDITYDALHMQALPPR** (SEQ ID NO:331)

E6 CAR X48 pCDH gBLOCK sequence:

(DNA)

agtacctctaattctggcctcaggtatccctgcacgattttctggatctggttcaggttctgattaca
ccctcactatctctagcctggagcctgaagactttgccgtttattactgccagcagaggtctagctc
ccatttcacctttgggagtgaggaccaaggttgaaattaaagacaagacgcacaccaagccaccta
ccagctccagaactgctcggaggtcctggcaccggaaccggaggacctaaccatcaaaccacctaagc
cacctaagcctgctcctaacctgctcggaggacctaatacattttgggccccgctcgcaggcacatg
tggagtgtcctcctctccctggtgattaccctgtactgcaaaagggggcccgcacaaaaaactcctttac
atttttaagcagccttttatgaggccag (SEQ ID NO:332)

CAR E6 X4/4/4-1BB/CD3z sequence:

(DNA)

atggccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgccgccaggccagagg
 tccagctggttgagagtggcggtgggctggttaagcctggcggtccctgcggtgagctgcgccgc
 gagtggattttactttcagccgatatgggatgagttgggtgcggaagctcccggaagaggctggaa
 tgggtctcaacaatctccggggggggcacttacatctattaccccgactcagtcaaggggagattta
 ccatttcacgagacaacgctaagaataccctgtatttgcagatgaattctctgagagcagaggacac
 agctgtttactattgtaccgcgacaactatggcaggaactacgactacggtatggactattgggga
 caagggacattgggttacagtgagcagtgccggcgggggcagcggaggaggaggcagcgggtggcggag
 gcagcgagatagtgctcagcagtcacccgcgactctcagctctcacctggggaacgagctaccct
 gacgtgctctgctacctcctcagtgctcatatattcactgggtatcagcaacggcccgggcagctccct
 agattgctcatttatagctacctctaactctggcctcaggtatccctgcagattttctggatctgggt
 caggttctgattacacctcactatctctagcctggagcctgaagactttgccgtttattactgcc
 gcagaggtctagctccccattcacctttgggagtgaggaccaaggttgaaattaaagacaagacgcac
 accaagccacctaaccagctccagaactgctcggaggtcctggcaccggaaccggaggacctaaca
 tcaaacacctaagccacctaagcctgctcctaacctgctcggaggacctaaggcctgattgtgct
 gggggcgctgcggcctcctgcttttattgggctaggcatcttcttcaaaaggggcccgaaaaaa
 ctccctttacatttttaagcagccttttatgaggccagtacagacgactcaagaggaagacgggtgct
 catgccgctttcctgaggaggaggaaggaggggtgcgaactgcgcgttaagttctccgatcagccga
 cgcgctgcttacaagcagggccagaaccaactgtacaacgagctgaatctcggtagacgggaagag
 tacgacgtgttgacaaacggagaggccgcgaccagaaatggcggaagcctcgaggaacaaacc
 ccaggaggaggtgtacaatgagttgcagaaagataagatggcagaagcttatagcgagatcggaat
 gaagggggaaaggagacgagggaaaggacacgacggcctttatcagggcctgtccacagcaacaaaa
 gatacgtatgacgccctccatatgcaggcacttccaccacgggtgataa (SEQ ID NO:333)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVLPKPGSLRLSCAASGFTFSRYGMSWVRQAPGKRLE
 WVSTISGGGTYYIYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWG
 QGTLVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWHYQQRPGQSP
 RLLIYSTSNLASGIPARFSGSGSGSDYTLTISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKDKTH
 TKPPKPAPELLGGPGTGTGGPTIKPPKPPKPAPNLLGGPMALIVLGGVAGLLLF IGLGIFFKRGRKK
 LLYIFKQPFMRPVQTTQEEDGCSRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREE
 YDVLDRRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKHDGLYQGLSTATK
 DTYDALHMQALPPR** (SEQ ID NO:334)

E6 CAR X44 pCDH gBLOCK sequence:

(DNA)

agtacctctaactctggcctcaggtatccctgcagattttctggatctgggttcaggttctgattaca
 ccctcactatctctagcctggagcctgaagactttgccgtttattactgccagcagaggtctagctc
 ccatttcacctttgggagtgaggaccaaggttgaaattaaagacaagacgcacaccaagccaccta
 ccagctccagaactgctcggaggtcctggcaccggaaccggaggacctaaccatcaaaccacctaagc
 cacctaagcctgctcctaacctgctcggaggacctaaggcctgattgtgctggggggcgctgcgcgg
 cctcctgcttttattgggctaggcatcttcttcaaaaggggcccgaaaaaactcctttacattttt
 aagcagccttttatgaggccag (SEQ ID NO:335)

CAR E6 8+4/4/4-1BB/CD3z sequence:

(DNA)

atggccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgccgccaggccagagg
 tccagctggttgagagtggcggtgggctggttaagcctggcggtccctgcggtgagctgcgccgc
 gagtggattttactttcagccgatatgggatgagttgggtgcggaagctcccggaagaggctggaa
 tgggtctcaacaatctccggggggggcacttacatctattaccccgactcagtcaaggggagattta

ccatttcacgagacaacgctaagaataccctgtattttgcagatgaattctctgagagcagaggacac
 agctgtttactattgtacccgcgacaactatggcaggaactacgactacggatggactattgggga
 caaggacattgggttacagtgagcagtgccggcgggggcagcggaggaggaggcagcgggtggcgga
 gcagcgagatagtgtcacgcagtcacccgcgactctcagtcctcacctggggaacgagctacct
 gacgtgctctgctacctcctcagtggtcatatattcactgggtatcagcaacggcccgggcagtcacct
 agattgctcatttatagtacctctaatactggcctcaggtatccctgcacgattttctggatctggtt
 caggttctgattacacctcactatctctagcctggagcctgaagactttgccgtttattactgcc
 gcagaggtctagctccccattcacctttgggagtgaggaccaaggttgaaattaaaacgacaaccccc
 gccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctgagaccagaagcct
 gtaggcctgccgcgggtggagctgtgcacacaagaggactggatttcgcctgtgatatggccctgat
 tgtgctggggggcgctgcgggcctcctgcttttcatctgggctagggcatcttcttcaaaagggggcgc
 aaaaaactcctttacatttttaagcagccttttatgaggccagtacagacgactcaagaggaagacg
 ggtgctcatgccgctttcctgaggaggaggaaaggaggggtgcgaactgcgcgttaagttctcccgatc
 agccgacgcgcctgcttacaagcagggccagaaccaactgtacaacgagctgaatctcggtagacgg
 gaagagtacgacgtgttgacaaaacggagaggccgcgaccagaaatgggcggaagcctcgcagga
 aaaacccccagggaggactgtacaatgagttgcagaaagataagatggcagaagcttatagcgagat
 cggaatgaagggggaaaggagacgagggaaggacacgagcgcctttatcagggcctgtccacagca
 acaaaagatacgtatgacgccctccatatgcaggcacttccaccacggtgataa (SEQ ID
 NO:336)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLE
 WVSTISGGGTYYYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGM DYWG
 QGTLVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWHYQQRPGQSP
 RLLIYSTSNLASGIPARFSGSGSGSDYTLTISSELEPEDFAVYYCQQRSSSPFTFGSGTKVEIKTTTP
 APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDMALIVLGGVAGLLLFGLGIFFKRGR
 KLLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRR
 EEYDVLDDKRRGRDPENGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKHDGLYQGLSTA
 TKDITYDALHMQALPPR** (SEQ ID NO:337)

E6 CAR CD844 pCDH gBLOCK sequence:

(DNA)

agtacctaatctggcctcaggtatccctgcacgattttctggatctgggttcaggttctgattaca
 cctcactatctctagcctggagcctgaagactttgccgtttattactgccagcagaggtctagctc
 ccattcacctttgggagtgaggaccaaggttgaaattaaaacgacaacccccggccccagaccacca
 acgccagccccaccatcgccagccaacccctgtctctgagaccagaagcctgtaggcctgccgcg
 gtggagctgtgcacacaagaggactggatttcgcctgtgatatggccctgattgtgctggggggcg
 cgccggcctcctgcttttcatctgggctagggcatcttcttcaaaagggggcgcaaaaaactcctttac
 atttttaagcagccttttatgaggccag (SEQ ID NO:338)

Humanized C2 scFV sequence in CAR:

(DNA)

gagggccaccatggccttgccagtgacggccctgctgctgccattggctcttctgttgcaagctgccaggcct
 gaagtgcagctcgtagagagtgccgggggactggtagagcccggtggaagcctcagactcagttgcgcgcct
 caggtttcacttttccaggttacgccatgtcctgggtaagacaggcaccggggaaaggactcgagtggtgtc
 tactatcagctcaggaggcacttatatatattatcctgactctgtaaaaggccgatttacgatttctcgcgac
 aatgcaaagaactccctctacctccaaatgaacagtccttagggcagaagacactgctgtatactattgtgcac
 gcctcggcggcgacaactactacgagtactttgacgtgtgggggaaagggactaccgtgacagtttcaagcg
 aggaggtggctcaggtggaggcggtcaggggggggaggaagtgtatattgtgctcacacaatccccagcctcc
 ctggctgtgtctcccgcccaacgcgctacaattacatgtcgggcctccaaaagcgtgagcaccagcggtaca
 gctacatgcactgggtatcaacagaaaccaggacaacccccaaactgttgatttatctcgcttcaaacttga

gtccggcgtgcctgcgcgcttttcagggagtgaggcggcacagattttacgctgactatcaaccccgtagaa
gcaaacgatacagcgaattattattgtcaacattcccgggaactcccctttacgttcggcgggggcacaaagg
tcgaaattaagagaacc (SEQ ID NO:339)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDSVKGR
FTISRDNAKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYFDVWGKGTITVTVSSGGGGSGGGGSGGG
GSDIVLTQSPASLAVSPGQRATITCRASKSVSTSGYSYMHWYQQKPGQPPKLLIYLASNLESGVPAR
FSGSGSGTDFTLTINPVEANDTANYYCQHSRELPFTFGGGTKVEIKRT (SEQ ID NO:340)

Humanized E6 scFV sequence in CAR:

(DNA)

gaggtccagctggttgagagtgggcgggtgggctggttaagcctggcggtccctgcggctgagctgcg
ccgcgagtggtatttactttcagccgatatgggatgagttgggtgcggcaagctcccgggaagaggct
ggaatgggtctcaacaatctccgggggggggcacttacatctattaccccgactcagtcagggggaga
tttaccatttcacgagacaacgctaagaataccctgtatttgcagatgaattctctgagagcagagg
acacagctgtttactattgtacccgcgacaactatggcaggaactacgactacggatatggactattg
gggacaagggacattggttacagtgagcagtgggcggcgggggcagcggaggaggaggcagcgggtggg
gggggcagcgagatagtgtcacgcagtcacccgcgactctcagtcctcaccctggggaacgagcta
ccctgacgtgctctgctacctcctcagtggtcatatattcactgggtatcagcaacggcccgggcagtc
ccctagattgctcatttatagtagtacctctaactctggcctcaggtatccctgcacgattttctggatct
ggttcaggttctgattacacctcactatctctagcctggagcctgaagactttgccgtttattact
gccagcagaggtctagctccccattcacctttgggagtgggaccaaggttgaaattaaa (SEQ ID
NO:341)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLEWVSTISGGGTYYIYPDSVKGR
FTISRDNAKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWQGTLLTVTVSSGGGGSGGGGSGG
GGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWWYQQRPQGSPRLLIYSTSNLASGIPARFSGS
GSGSDYTLTISLLEPEDFAVYYCQQRSSSPFTFGSGTKVEIK (SEQ ID NO:342)

CD8 leader sequence:

(DNA)

atggccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgccgccaggcca
(SEQ ID NO:343)

(amino acids)

MALPVTALLLPLALLLHAARP (SEQ ID NO:344)

CD8 hinge domain sequence:

(DNA)

acgacaacccccggccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctga
gaccagaagcctgtaggcctgccgcgggtggagctgtgcacacaagaggactggatttcgcctgtga
t (SEQ ID NO:345)

(amino acids)

TTTPAPRPPTPAPTIIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO:346)

CD4 hinge domain sequence:

(DNA)

tcgggacaggtcctgctggaatccaacatcaaggttctgcccacatggtccaccccggtgcagcca
(SEQ ID NO:347)

(amino acids)

SGQVLLESNIKVLPTWSTPVQP (SEQ ID NO:348)

CD28 hinge domain sequence:

(DNA)

aaacacctttgtccaagtcccctatttcccggaccttctaagccc (SEQ ID NO:349)

(amino acids)

KHLCPSPLFPGPSKP (SEQ ID NO:350)

CD8+CD4 hinge domain sequence:

(DNA)

acgacaaccccgggccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctga
gaccagaagcctgtaggcctgccgccggtggagctgtgcacacaagaggactggatttcgcctgtga
ttcgggacaggtcctgctggaatccaacatcaaggttctgcccacatggtccaccccggtgcagcca
(SEQ ID NO:351)

(amino acids)

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDSGQVLLESNIKVLPTWSTPVQP
(SEQ ID NO:352)

CD8+CD28 hinge domain sequence:

(DNA)

acgacaaccccgggccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctga
gaccagaagcctgtaggcctgccgccggtggagctgtgcacacaagaggactggatttcgcctgtga
taaacacctttgtccaagtcccctatttcccggaccttctaagccc (SEQ ID NO:353)

(amino acids)

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDKHLCPSPLFPGPSKP (SEQ
ID NO:354)

CD28+CD4 hinge domain sequence:

(DNA)

aaacacctttgtccaagtcccctatttcccggaccttctaagccctcgggacaggtcctgctggaat
ccaacatcaaggttctgcccacatggtccaccccggtgcagcca (SEQ ID NO:355)

(amino acids)

KHLCPSPLFPGPSKPSGQVLLESNIKVLPTWSTPVQP (SEQ ID NO:356)

Human IgD hinge domain sequence:

(DNA)

gagtctccaaaggcacaggcctcctcagtgccactgcacaaccccaagcagagggcagcctcgcca
aggcaaccacagccccagccaccacccgtaacacaggaagaggcggcgaagagaagaaaaaggagaa
ggagaaagaggaacaagaagagagagagacaaagacacca (SEQ ID NO:357)

(amino acids)

ESPKAQASSVPTAQPPQAEGLAKATTAPATTRNTGRGGEEKKKEKEKEEQEERETKTP (SEQ ID NO:358)

X4 linker (IgG1 and IgG2 modified hinge region) sequence:

(DNA)

gacaagacgcacaccaagccacctaaccagctccagaactgctcggagggtcctggcaccggaaccg
gaggacctaccatcaaaccacctaagccacctaagcctgctcctaacctgctcggaggacct (SEQ ID NO:359)

(amino acids)

DKTHTKPPKPAPELLGGPGTGTGGPTIKPPKPPKPAPNLLGGP (SEQ ID NO:360)

CD3 zeta transmembrane domain sequence:

(DNA)

ctctgctacctgctggatggaatcctcttcatctatggtgtcattctcactgccttgcttcctg
(SEQ ID NO:361)

(amino acids)

LCYLLDGILFIYGVILTALFL (SEQ ID NO:362)

CD8 transmembrane domain sequence:

(DNA)

atctacatttgggccccgctcgcaggcacatgtggagtgtcctcctctccctgggtgattaccctgt
actgc (SEQ ID NO:363)

(amino acids)

IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO:364)

CD4 transmembrane domain sequence:

(DNA)

atggccctgattgtgctggggggcgctcgccggcctcctgcttttcattgggctaggcatcttcttc
(SEQ ID NO:365)

(amino acids)

MALIVLGGVAGLLLLFIGLGIFF (SEQ ID NO:366)

CD28 transmembrane domain sequence:

(DNA)

ttttgggtgctggtggtggttggtggagtcctggcttgctatagcttgctagtaacagtggccttta
ttatctctggtg (SEQ ID NO:367)

(amino acids)

FWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:368)

4-1BB transmembrane domain sequence:

(DNA)

atcatctccttctttcttgctgctgacgtcgactgcgttgctcttcctgctgttcttcctcacgctcc
gtttctctgttggtt (SEQ ID NO:369)

(amino acids)

IISFFLALTSTALLFLLFFLTLRFSVV (SEQ ID NO:370)

OX40 transmembrane domain sequence:

(DNA)

gttgccgccatcctgggcctgggcctggtgctggggctgctgggccccctggccatcctgctggccc
tgtacctgctc (SEQ ID NO:371)

(amino acids)

VAILGLGLVLGLLGPLAILLALYLL (SEQ ID NO:372)

CD3 zeta domain sequence:

(DNA)

cgcgttaagttctcccgatcagccgacgcgcctgcttacaagcagggccagaaccaactgtacaacg
agctgaatctcggtagacgggaagagtacgacgtgttgacaaacggagagggccgcgaccagaaat
ggcgggcaagcctcgcaggaaaaacccccaggaggggactgtacaatgagttgcagaaagataagatg
gcagaagcttatagcgagatcggaatgaagggggaaaggagacgagggaaaggacacgacggccttt
atcagggcctgtccacagcaacaaaagatacgtatgacgccctccatatgcaggcacttccaccacg
g (SEQ ID NO:373)

(amino acids)

RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:374)

CD3 zeta domain variant sequence:

(DNA)

agagtgaagttcagcaggagcgcagacgcccccgcggtaccagcagggccagaaccagctctataacg
agctcaatctaggacgaagagaggagtacgatgttttgacaagagacgtggccgggaccctgagat
ggggggaaagccgagaaggaagaaccctcaggaaggcctgtacaatgaactgcagaaagataagatg
gcggaggcctacagtgagattgggatgaaaggcgagcgccggagggggcaaggggcacgatggccttt
accaggggtctcagtacagccaccaaggacacctacgacgcccttcacatgcaggccctgccccctcg
c (SEQ ID NO:375)

(amino acids)

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:376)

CD28 domain sequence:

(DNA)

agaagcaagcgggtctcggctcctgcattctgattacatgaacatgaccccaagaagaccaggcccca
ccaggaaacattaccagccctacgctccgccacgcgacttcgctgcctaccggtcc (SEQ ID
NO:377)

(amino acids)

RSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO:378)

4-1BB domain sequence:

(DNA)

aaaagggggccgcaaaaaactcctttacatttttaagcagccttttatgaggccagtacagacgactc
aagaggaagacgggtgctcatgccgctttcctgaggaggaggaaggaggggtgcgaactg (SEQ ID
NO:379)

(amino acids)

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO:380)

OX40 domain sequence:

(DNA)

cggagggaccagaggctgccccccgatgccacaagccccctgggggaggcagtttccggacccccca
tccaagaggagcaggccgacgcccactccaccctggccaagatc (SEQ ID NO:381)

(amino acids)

RRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI (SEQ ID NO:382)

Humanized anti CD3 scFV clone 12F6 (VH-VL) sequence:

(DNA)

caggtgcagctggtgcagagcggaggtggagtgggtccaacctggaagatctctgagactgagctgta
aggctagcgggtacacgttcacatcttacacgatgcactgggtgaggcaagcccccggttaagggcct
ggaatggatcggatatataaaaccccagctcaggggtataccaaatataatcagaagttcaaagatcgg
ttcacgattttctgctgataaaagtaagtcaccgcttttctgagatggactcactcaggccagaag
atactggtgtttatctgtgcaaggtggcaggactacgacgtgtactttgactattgggggcaggg
gacgcctgtaacagtatcaagcggcgggtggcggtccggcggtggcggtccggcggtggcggtcc
gatattcagatgaccagagccccgagcagcctgagcgcgagcgtgggcgatcgcgtgaccatgacct
gccgcgcgagcagcagcgtgagctatatgcattggtatcagcagacccccgggcaaagcgcgcgaaacc
gtggatttatgcgaccagcaacctggcgagcggcgtgccgagccgcttttagcggcagcggcagcggc
accgattataacctgaccattagcagcctgcagccggaagatattgcgacctattattgccagcagt
ggagcagcaacccgcccagcctttggccaggggcaccaaactgcagattaccgcgc (SEQ ID
NO:383)

(amino acids)

QVQLVQSGGGVVPGRSLRLSCKASGYTFTSYTMHWVRQAPGKGLEWIGYINPSSGYTKYNQKFKDR
FTISADKSKSTAFLLQMDSLRPEDTGVYFCARWQDYDYFDYWGQGPVTVSSGGGSGGGGSGGGGS
DIQMTQSPSSLSASVGDRVTMTCRASSSVSYMHWYQQTPGKAPKPIYATSNLASGVPSRFSGSGSG
TDYTLTISSLQPEDIATYYCQQWSSNPPTFGQGTKLQITR (SEQ ID NO:384)

Humanized anti CD3 scFV clone 12F6 (VL-VH) sequence:

(DNA)

gatattcagatgaccagagccccgagcagcctgagcgcgagcgtgggcgatcgcgtgaccatgacct
gccgcgcgagcagcagcgtgagctatatgcattggtatcagcagacccccgggcaaagcgcgcgaaacc
gtggatttatgcgaccagcaacctggcgagcggcgtgccgagccgcttttagcggcagcggcagcggc
accgattataacctgaccattagcagcctgcagccggaagatattgcgacctattattgccagcagt
ggagcagcaacccgcccagcctttggccaggggcaccaaactgcagattaccgcgcggcggtggcggtatc
cggcggtggcggtatccggcggtggcggtatccaggtgcagctggtgcagagcggaggtggagtggtc
caacctggaagatctctgagactgagctgtaaggctagcgggtacacgttcacatcttacacgatgc
actgggtgaggcaagcccccggttaagggcctggaatggatcggatatataaaaccccagctcagggta
taccaaatataatcagaagttcaaagatcggttcacgattttctgctgataaaagtaagtcaccgcgt
ttcctgagatggactcactcaggccagaagatactggtgtttatctgtgcaaggtggcaggact
acgacgtgtactttgactattgggggcaggggacgcctgtaacagtatcaagc (SEQ ID
NO:385)

(amino acids)

DIQMTQSPSSLSASVGDRVTMTCRASSSVSYMHWYQQTPGKAPKPIYATSNLASGVPSRFSGSGSG
 TDYTLTISSLQPEDIAITYYCQQWSSNPFTFGQGTKLQITRGGGSGGGGSGGGGSGVQLVQSGGGV
 QPGRSLRLSCKASGYTFTSYTMHWVRQAPGKGLEWIGYINPSSGYTKYNQKFKDRFTISADKSKSTA
 FLQMDSLRLPEDTGVYFCARWQDYDYFDYWGQGPVTVSS (SEQ ID NO:386)

Humanized anti CD3 scFV clone OKT3 (VH-VL) sequence:

(DNA)

caggtgcagctggtgcagagcggagggcggagtggtgcagcctggaagaagcctgcgcctgagctgca
 aagcgagcggctatacctttaccgcctataccatgcattgggtgcgccaggcgccgggcaaaggcct
 ggaatggattggctatattaacccgagccgcggctataccaactataaccagaaagtgaagatcgc
 tttaccattagcaccgataaaaagcaaaagcaccgcgtttctgcagatggatagcctgcgcccggaag
 ataccgcggtgtattattgcgcgcgctattatgatgatcattattgcctggattattggggccaggg
 caccaccctgaccgtgagcagcggcgggtggcggtatccggcggtggcggtatccggcggtggcggtatcc
 gatattcagatgaccagagcccagcagcctgagcgcgagcgtgggcgatcgcgtgaccattacct
 gcagcgcgagcagcagcgtgagctatatgaactggtatcagcagaccccgggcaaagcgccgaaacg
 ctggatttatgataaccagcaaactggcgagcggcgtgccgagccgctttagcggcagcggcagcggc
 accgattatacctttaccattagcagcctgcagccggaagatattgcgacctattattgccagcagt
 ggagcagcaaccgcgtttacctttggccagggcaccaaactgcagattaccgc (SEQ ID
 NO:387)

(amino acids)

QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKVKDR
 FTISTDKSKSTAFQLQMDSLRLPEDTAVYYCARYYDDHYCLDYWGQGTTLTVSSGGGSGGGGSGGGGSG
 DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKSLASGVPSRFSGSGSG
 TDYTFTISSLQPEDIAITYYCQQWSSNPFTFGQGTKLQITR (SEQ ID NO:388)

Humanized anti CD3 scFV clone OKT3 (VH-VL) sequence:

(DNA)

gatattcagatgaccagagcccagcagcctgagcgcgagcgtgggcgatcgcgtgaccattacct
 gcagcgcgagcagcagcgtgagctatatgaactggtatcagcagaccccgggcaaagcgccgaaacg
 ctggatttatgataaccagcaaactggcgagcggcgtgccgagccgctttagcggcagcggcagcggc
 accgattatacctttaccattagcagcctgcagccggaagatattgcgacctattattgccagcagt
 ggagcagcaaccgcgtttacctttggccagggcaccaaactgcagattaccgcggcggtggcggtatc
 cggcggtggcggtatccggcggtggcggtatccaggtgcagctggtgcagagcggaggcggtggtg
 cagcctggaagaagcctgcgcctgagctgcaaagcgagcggctatacctttaccgcctataccatgc
 attgggtgcgccaggcgccgggcaaaggcctggaatggattggctatattaacccgagccgcggctat
 taccactataaccagaaagtgaagatcgctttaccattagcaccgataaaaagcaaaagcaccgcg
 tttctgcagatggatagcctgcgcccggaagataccgcggtgtattattgcgcgcgctattatgatg
 atcattattgcctggattattggggccagggcaccaccctgaccgtgagcagc (SEQ ID
 NO:389)

(amino acids)

DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKSLASGVPSRFSGSGSG
 TDYTFTISSLQPEDIAITYYCQQWSSNPFTFGQGTKLQITRGGGSGGGGSGGGGSGVQLVQSGGGV
 QPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTA
 FLQMDSLRLPEDTAVYYCARYYDDHYCLDYWGQGTTLTVSS (SEQ ID NO:390)

HumanizeE6 scFV (VH-VL) sequence:

(DNA)

gaggtgcagctggtggagtctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtg
 cagcctctggattcaccttcagtaggtatggcatgagctgggtccgccaggctccagggaagaggct
 ggagtgggtctcaaccattagtggcggaggcacctacataactaccagactcagtgaagggccga
 ttcaccatctccagagacaacgccaagaacaccctgtatctgcaaataaacagcctgagagccgagg
 acacggctgtgtattactgtaccagagataactatggccgcaactatgattatggcatggattattg
 gggccaggggcaccctggtgaccgtgagcagcggcggtggcggatccggcggtggcggatccggcggt
 ggcggtatccgaaattgtgttgacacagtctccagccaccctgtctttgtctccaggggaaagagcca
 ccctcacctgcagcggccaccagcagtgttagctacatccactggtaccaacagaggcctggccagag
 cccagggctcctcatctatagcacctccaacctggccagcggcatcccagccagggttcagtggcagt
 gggctctgggagcgactacactctcaccatcagcagcctagagcctgaagattttgcagtttattact
 gtcagcagcgtagcagctcccctttcacctttggcagcggcaccaaagtggaaattaaa (SEQ ID
 NO:391)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLEWVSTISGGGTYYYPDSVKGR
 FTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWGQGLTVTVSSGGGGSGGGSGG
 GGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHWHYQQRPGQSPRLLIYSTSNLASGIPARFSGS
 GSGSDYTLTISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIK (SEQ ID NO:392)

HumanizeE6 scFV (VL-VH) sequence:

(DNA)

gaaattgtgttgacacagtctccagccaccctgtctttgtctccaggggaaagagccaccctcacct
 gcagcggccaccagcagtgttagctacatccactggtaccaacagaggcctggccagagccccaggct
 cctcatctatagcacctccaacctggccagcggcatcccagccagggttcagtggcagtgggtctggg
 agcgactacactctcaccatcagcagcctagagcctgaagattttgcagtttattactgtcagcagc
 gtagcagctcccctttcacctttggcagcggcaccaaagtggaaattaaaggcggtggcggatccgg
 cggtggcggtatccggcggtggcggatccgagggtgcagctggtggagtctgggggaggcctggtcaag
 cctgggggggtccctgagactctcctgtgcagcctctggattcaccttcagtaggtatggcatgagct
 ggggtccgccagggtccagggaagaggctggagtgggtctcaaccattagtggcggaggcacctacat
 atactaccagactcagtgaagggccgattcaccatctccagagacaacgccaagaacaccctgtat
 ctgcaaataaacagcctgagagccgaggacacggctgtgtattactgtaccagagataactatggcc
 gcaactatgattatggcatggattattggggccaggggcaccctggtgaccgtgagcagc (SEQ ID
 NO:393)

(amino acids)

EIVLTQSPATLSLSPGERATLTCSATSSVSYIHWHYQQRPGQSPRLLIYSTSNLASGIPARFSGSGS
 SDYTLTISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKGGGGSGGGSGGGGSEVQLVESGGGLVK
 PGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLEWVSTISGGGTYYYPDSVKGRFTISRDNKNTLY
 LQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWGQGLTVTVSS (SEQ ID NO:394)

HumanizeC2 scFV (VH-VL) sequence:

(DNA)

gaggtgcagctggtggagtctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtg
 cagcctctggattcaccttcagtggtatgcatgagctgggtccgccaggctccagggaaggggct
 ggagtgggtctcaaccattagtagtggcggaacctacataactacccgactcagtgaagggccga
 ttcaccatctccagagacaacgccaagaactcactgtatctgcaaataaacagcctgagagccgagg
 acacggccgtgtattactgtgcgagacttgggggggataattactacgaatacttcgatgtctgggg
 caaagggaaccacgggtcacctgtctcctccggcggtggcggatccggcggtggcggatccggcggtggc
 ggatccgacattgtgtgtgacccagtctccagcctccttggccgtgtctccaggacagaggggccacca
 tcacctgcagagccagtaagagtggtcagtaccagcggatactcctacatgcactggtatcagcagaa

accaggacaacctcctaaactcctgatttacctggcatccaatctggagagcggggtcccagccagg
 ttcagcggcagtggtctctgggaccgatttcaccctcacaattaatcctgtggaagctaataactg
 caaattattactgtcagcacagtagggagctgcctttcacattcggcggagggaccaaggtggagat
 caaacgaact (SEQ ID NO:395)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDSVKGR
 FTISRDNAKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYFDVWGKGTTVTVSSGGGSGGGGSGGG
 GSDIVLTQSPASLAVSPGQRATITCRASKSVSTSGYSYMHWYQQKPGQPPKLLIYLASNLESGV
 PARFSGSGSGTDFTLTINPVEANDTANYYCQHSRELPFTFGGGTKVEIKRT (SEQ ID NO:396)

HumanizeE6 scFV (VL-VH) sequence:

(DNA)

gacattgtgctgacccagtcctccagcctccttggccgtgtctccaggacagagggccaccatcacct
 gcagagccagtaagagtgtcagtagcagcgatactcctacatgcactggtatcagcagaaaccagg
 acaacctcctaaactcctgatttacctggcatccaatctggagagcggggtcccagccagggttcagc
 ggcagtggtctctgggaccgatttcaccctcacaattaatcctgtggaagctaataactgatactgcaaatt
 attactgtcagcacagtagggagctgcctttcacattcggcggagggaccaaggtggagatcaaacg
 aactggcgggtggcggatccggcgggtggcggatccggcgggtggcggatccgaggtgcagctggtggag
 tctgggggagggcctggtcaagcctgggggggtccctgagactctcctgtgcagcctctggattcacct
 tcagtggctatgccatgagctgggtccgccaggctccagggaaggggctggagtgggtctcaaccat
 tagtagtggcggaacctacatatactaccccgactcagtgaagggccgattcaccatctccagagac
 aacgccaagaactcactgtatctgcaaatgaacagcctgagagccgaggacacggccgtgtattact
 gtgagagacttgggggggataattactacgaatacttcgatgtctgggggcaaagggaccacggtcac
 cgtctcctcc (SEQ ID NO:397)

(amino acids)

DIVLTQSPASLAVSPGQRATITCRASKSVSTSGYSYMHWYQQKPGQPPKLLIYLASNLESGV
 PARFSGSGSGTDFTLTINPVEANDTANYYCQHSRELPFTFGGGTKVEIKRTGGGSGGGGSGGGGSEVQLVE
 SGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDSVKGRFTISR
 DAKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYFDVWGKGTTVTVSS (SEQ ID NO:398)

G₄S₁ linker sequence:

(DNA)

ggcgggtggcggatcc (SEQ ID NO:399)

(amino acids)

GGGS (SEQ ID NO:400)

[G₄S₁]₃ linker sequence:

(DNA)

ggcgggtggcggatccggcgggtggcggatccggcgggtggcggatcc (SEQ ID NO:401)

(amino acids)

GGGSGGGSGGGGS (SEQ ID NO:402)

8 aa GS linker sequence:

(DNA)

ggcgggtccggcgggtggatccgga (SEQ ID NO:403)

(amino acids)
GGSGGGSG (SEQ ID NO:404)

12 aa GS linker sequence:

(DNA)
ggcggttcggcggtggatccggcggtggcgatccgga (SEQ ID NO:405)

(amino acids)
GGSGGGSGGGSG (SEQ ID NO:406)

13 aa GS linker sequence:

(DNA)
ggcggtggatccggcggtggcgatccggcggtggatcc (SEQ ID NO:407)

(amino acids)
GGSGGGSGGGSG (SEQ ID NO:408)

22 aa GS linker sequence:

(DNA)
ggcggtggaagcggcggtggcgatccggcagcggcggaagcggcggtggcgatccggcggtgga
(SEQ ID NO:409)

(amino acids)
GGSGGGSGSGGGSGGGSGGG (SEQ ID NO:4110)

24 aa GS linker sequence:

(DNA)
ggcggttcggcggtggatccggcggtggcgatccggaggcggttcggcggtggatccggcggtg
cgatccgga (SEQ ID NO:411)

(amino acids)
GGSGGGSGGGSGGGSGGGSGGGSG (SEQ ID NO:412)

Mouse C3 Heavy chain variable region sequence:

(DNA)
caggtccagctgcagcagctctgggcctgagctggtgaggcctggggtctcagtgaagatttcctgcaaggggt
ccggctacagattcactgattatgctatgaactgggtgaagcagagtcattgcaaagagctctagagtggttg
agttattagtagtcttctctggttaatacaaaacttcaaccagaagtttaagggaaggccacaatgactgtagac
aaatcctccagcacagcctatatggaacttgccagattgacatctgaggattctgccatgtattactgtgcaa
gatcggattactacggccatactttgactactggggccaaggcaccactctcacagtctcctca (SEQ ID
NO:413)

(amino acids)
QVQLQQSGPELVRPGVSVKISCKGSGYRFTDYAMNWVKQSHAKSLEWIGVISTFSGNTNFNQKFKGKATMTVD
KSSSTAYMELARLTSEDSAMYICARSDYYGPYFDYWGGQTTLTVSS (SEQ ID NO:414)

Mouse C3 heavy chain variable framework region 1 (FWR1) sequence:

(DNA)
caggtccagctgcagcagctctgggcctgagctggtgaggcctggggtctcagtgaagatttcctgcaaggggt
ccggctacagattcact (SEQ ID NO:415)

(amino acids)

QVQLQQSGPELVPRPGVSVKISCKGSGYRFT (SEQ ID NO:416)

Mouse C3 heavy chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

gattatgctatgaac (SEQ ID NO:417)

(amino acids)

DYAMN (SEQ ID NO:418)

Mouse C3 heavy chain variable framework region 2 (FWR2) sequence:

(DNA)

tgggtgaagcagagtcatgcaaagagtctagagtggttga (SEQ ID NO:419)

(amino acids)

WVKQSHAKSLEWIG (SEQ ID NO:420)

Mouse C3 heavy chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

gttattagtagtcttctctggttaatacaaaacttcaaccagaagtttaagggc (SEQ ID NO:421)

(amino acids)

VISTFSGNTNFNQKFKG (SEQ ID NO:422)

Mouse C3 heavy chain variable framework region 3 (FWR3) acid sequence:

(DNA)

aaggccacaatgactgtagacaaatcctccagcacagcctatatggaacttgccagattgacatctgaggatt
ctgccatgtattactgtgcaaga (SEQ ID NO:423)

(amino acids)

KATMTVDKSSSTAYMELARLTSEDSAMYYCAR (SEQ ID NO:424)

Mouse C3 heavy chain variable complementarity determining regions 3 (CDR3) sequence:

(DNA)

tcggattactacggccatactttgactac (SEQ ID NO:425)

(amino acids)

SDYYGPYFDY (SEQ ID NO:426)

IGHV1-18*04 heavy chain variable region sequence:

(DNA)

cagggttcagctggtgacgtctggagctgaggtgaagaagcctggggcctcagtgaaggtctcctgca
aggcttctggttacacctttaccagctacggtatcagctgggtgacagggccctggacaagggct
tgagtggatgggatggatcagcgcttacaatggtaacacaaaactatgcacagaagctccagggcaga
gtcaccatgaccacagacacatccacgagcacagcctacatggagctgaggagcctgagatctgacg
acacggcccggtgtattactgtgacgagaga (SEQ ID NO:427)

(amino acids)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTD
TSTSTAYMELRSLRSDDTAVYYCAR (SEQ ID NO:428)

IGHV1-18*04 heavy chain variable framework region 1 (FWR1) sequence:

(DNA)

caggttcagctggtgcagtcctggagctgaggtgaagaagcctggggcctcagtgaaaggtctcctgcaaggctt
ctggttacacctttacc (SEQ ID NO:429)

(amino acids)

QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO:430)

IGHV1-18*04 heavy chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

agctacggtatcagc (SEQ ID NO:431)

(amino acids)

SYGIS (SEQ ID NO:432)

IGHV1-18*04 heavy chain variable framework region 2 (FWR2) sequence:

(DNA)

tgggtgcgacaggccccctggacaagggcttgagtggatggga (SEQ ID NO:433)

(amino acids)

WVRQAPGQGLEWMG (SEQ ID NO:434)

IGHV1-18*04 heavy chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

tggatcagcgcttacaatggtaacacaaactatgcacagaagctccagggc (SEQ ID NO:435)

(amino acids)

WISAYNGNTNYAQLQG (SEQ ID NO:436)

IGHV1-18*04 heavy chain variable framework region 3 (FWR3) sequence:

(DNA)

agagtcaccatgaccacagacacatccacgagcacagcctacatggagctgaggagcctgagatctgacgaca
cgcccggtgtattactgtgcgaga (SEQ ID NO:437)

(amino acids)

RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR (SEQ ID NO:438)

Humanized C3 heavy chain variable region sequence:

(DNA)

caggttcagctggtgcagtcctggagctgaggtgaagaagcctggggcctcagtgaaaggtctcctgcaaggctt
ctggttacacctttaccgactacgcatgaactgggtgcgacaggccccctggacaagggcttgagtggatggg
agtgatcagcaccttcagcggttaacacaaacttcaaccagaagttcaagggcagagtcaccatgaccacagac
acatccacgagcacagcctacatggagctgaggagcctgagatctgacgacacggccgtgtattactgtgcga
gaagcgactactacggccatacttcgactactggggccagggcaccaccctgaccgtgtccagc (SEQ ID
NO:439)

(amino acids)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMNWVRQAPGQGLEWMGVISTFSGNTNFNQKFGRVTMTTD
TSTSTAYMELRSLRSDDTAVYYCARSDYYGPFYDWGQGTTLTVSS (SEQ ID NO:440)

Humanized C3 heavy chain variable framework region 1 (FWR1) acid sequence:

(DNA)

cagggttcagctggtgcagtcctggagctgaggtgaagaagcctggggcctcagtggaaggtctcctgcaaggctt
ctgggttacacctttacc (SEQ ID NO:441)

(amino acids)

QVQLVQSGAEVKKPGASVKVSKASGYTFT (SEQ ID NO:442)

Humanized C3 heavy chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

gactacgccatgaac (SEQ ID NO:443)

(amino acids)

DYAMN (SEQ ID NO:444)

Humanized C3 heavy chain variable framework region 2 (FWR2) acid sequence:

(DNA)

tgggtgcgacaggccccctggacaagggcttgagtggatggga (SEQ ID NO:445)

(amino acids)

WVRQAPGQGLEWMG (SEQ ID NO:446)

Humanized C3 heavy chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

gtgatcagcaccttcagcggtaacacaaacttcaaccagaagttcaagggc (SEQ ID NO:447)

(amino acids)

VISTFSGNTNFNQKFKG (SEQ ID NO:448)

Humanized C3 heavy chain variable framework region 3 (FWR3) acid sequence:

(DNA)

agagtcaccatgaccacagacacatccacgagcacagcctacatggagctgaggagcctgagatctgacgaca
cggcogtgtattactgtgcgaga (SEQ ID NO:449)

(amino acids)

RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR (SEQ ID NO:450)

Humanized C3 heavy chain variable complementarity determining regions 3 (CDR3) sequence:

(DNA)

agcgactactacggcccatacttcgactac (SEQ ID NO:451)

(amino acids)

SDYYGPYFDY (SEQ ID NO:452)

Humanized C3 IgG1 heavy chain sequence

(DNA)

cagggttcagctggtgcagtcctggagctgaggtgaagaagcctggggcctcagtggaaggtctcctgcaaggctt
ctgggttacacctttaccgactacgccatgaactgggtgcgacaggccccctggacaagggcttgagtggatggg
agtgatcagcaccttcagcggtaacacaaacttcaaccagaagttcaagggcagagtcaccatgaccacagac
acatccacgagcacagcctacatggagctgaggagcctgagatctgacgacacggccgtgtattactgtgcga
gaagcgactactacggcccatacttcgactactggggccagggcaccacccctgaccgtgtccagcgctagcac

caagggcccatcggtcttccccctggcaccctcctccaagagcacctctgggggcacagcgccctgggctgc
ctgggtcaaggactacttccccgaaccgggtgacgggtgctggtggaactcaggcgccctgaccagcggtgcaca
ccttcccgggtgtcctacagtcctcaggactctactccctcagcagcgtgggtgacagtgcctccagcagctt
gggcacccagacctaactctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagccc
aaatcttgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagtcctcc
tcttccccccaaaacccaaggacaccctcatgatctccgggacccctgaggtcacatgctggtgggtggacgt
gagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaag
ccgcgaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctga
atggcaaggagtacaagtgaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagc
caaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtc
agcctgacctgctggtcaaaggcttctatcccagcgacatcgccgtggagtgaggagcaatgggcagccgg
agaacaactacaagaccagcctcccgtgctggactccgaaggctccttcttctctacagcaagctcaccgt
ggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaaccactac
acgcagaagagcctctcctgtctccgggtaaatgataa (SEQ ID NO: 453)

(amino acids)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMNWVRQAPGQGLEWMGVISTFSGNTNFNQKFGRVTMTTD
TSTSTAYMELRSLRSDDTAVYYCARSDYYGPFYDWGQGTTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGC
LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSCDKHTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHY
TQKSLSLSPGK** (SEQ ID NO: 454)

Humanized C3 IgG2 heavy chain sequence

(DNA)

caggttcagctgggtgcagtcctggagctgaggtgaagaagcctggggcctcagtgaaaggtctcctgcaaggctt
ctgggttacacctttaccgactacgccaatgaactgggtgacacaggccctggacaagggttgagtggtggg
agtgatcagcaccttcagcggtaacacaaacttcaaccagaagttcaagggcagagtcaccatgaccacagac
acatccacgagcacagcctacatggagctgaggagcctgagatctgacgacacggccgtgtattactgtgcga
gaagcgactactacggccatacttgcactactggggccaggggcaccaccctgaccgtgtccagcgcctccac
caagggcccatcggtcttccccctggcgccctgctccaggagcacctccgagagcacagccgccctgggctgc
ctgggtcaaggactacttccccgaaccgggtgacgggtgctggtggaactcaggcgctctgaccagcggtgcaca
ccttcccagctgtcctacagtcctcaggactctactccctcagcagcgtgggtgaccgtgcctccagcaactt
cggcaccacagacctaactgcaacgtagatcacaagcccagcaacaccaaggtggacaagacagttgagcgc
aaatgttgtgtcgagtgccaccgtgccagcaccacctgtggcaggaccgtcagtcctcctcttccccccaa
aaccacaggacaccctcatgatctccggacccctgaggtcacgtgctggtgggtggacgtgagccacgaaga
ccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccacgggaggag
cagttcaacagcacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagt
acaagtgaaggtctccaacaaaggcctcccagcccccatcgagaaaaccatctccaaaaccaaagggcagcc
ccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgc
ctgggtcaaaggcttctaccccagcgacatcgccgtggagtgaggagcaatgggcagccgggagacaaactaca
agaccacacctcccatgctggactccgacggctccttcttctctacagcaagctcaccgtggacaagagcag
gtggcagcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaaccactacacgcagaagagc
ctctccctgtctccgggtaaatagtaa (SEQ ID NO: 455)

(amino acids)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMNWVRQAPGQGLEWMGVISTFSGNTNFNQKFGRVTMTTD
TSTSTAYMELRSLRSDDTAVYYCARSDYYGPFYDWGQGTTTLTVSSASTKGPSVFPLAPCSRSTSESTAALGC
LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTV
KCCVECPPEPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCL
LVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKS
LSLSPGK** (SEQ ID NO: 456)

Humanized C3 heavy chain IgG1 gBLOCK sequence:

(DNA)

tgctctgggttccaggttccactggtgacgcggcccagccggcccaggttcagctggtgcagtctggagctga
ggtgaagaagcctggggcctcagtgaaggtctcctgcaaggcttctggttacacctttaccgactacgcoatg
aactgggtgcgacaggcccctggacaagggcttgagtggatgggagtgatcagcaccttcagcggtaacacaa
acttcaaccagaagttcaagggcagagtcaccatgaccacagacacatccacgagcacagcctacatggagct
gaggagcctgagatctgacgacacggccgtgtattactgtgcgagaagcgactactacggcccatacttcgac
tactggggccagggcaccaccctgaccgtgtccagcgtatgcaccaagggcccatcggtcttccccctggcac
cctcctccaagagcacctctgggggcacagcggccctgggctgcctggtcaaggactacttccccgaaccggt
gacggtgtcgtggaactcaggcgccctgaccagc (SEQ ID NO:457)

Mouse C3 Light Chain variable region sequence:

(DNA)

gatgttttgatgacccaaactccactctccctgcctgtcagtccttgagatcaagcctccatctcttgcat
ctagtcagaccattgtacatagtaatggaaacacctatctagaatggtagctgcagaaaccaggccagtcctc
aaagctcctgatctacaaagtttccaaccgattttctgggggtcccagacaggttcagtggcagtggtcaggg
acagatttcacactcaagatcaacagagtggaggtgaggatctgggagtttattactgctttcaaggttcac
atgttccattcacgttcggctcggggacaaagttggaataaaa (SEQ ID NO:458)

(amino acids)

DVLMTQTPLSLPVSILGDQASISCRSSQTIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGS
TDFTLKINRVEAEDLGVIYCFQGSHPFTFGSGTKLEIK (SEQ ID NO:459)

Mouse C3 light chain variable framework region 1 (FWR1) sequence :

(DNA)

gatgttttgatgacccaaactccactctccctgcctgtcagtccttgagatcaagcctccatctcttg
(SEQ ID NO:460)

(amino acids)

DVLMTQTPLSLPVSILGDQASISC (SEQ ID NO:461)

Mouse C3 light chain variable complementarity determining regions 1 (CDR1) sequence :

(DNA)

agatctagtcagaccattgtacatagtaatggaaacacctatcttagaa (SEQ ID NO:462)

(amino acids)

RSSQTIVHSNGNTYLE (SEQ ID NO:463)

Mouse C3 light chain variable framework region 2 (FWR2) sequence:

(DNA)

tggtacctgcagaaaccaggccagtcctccaaagctcctgatctac (SEQ ID NO:464)

(amino acids)

WYLQKPGQSPKLLIY (SEQ ID NO:465)

Mouse C3 light chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

aaagtttccaaccgattttct (SEQ ID NO:466)

(amino acids)

KVSNRFS (SEQ ID NO:467)

Mouse C3 light chain variable framework region 3 (FWR3) sequence:

(DNA)

gggggtcccagacaggttcagtggtcagtggtcagggacagatttcacactcaagatcaacagagtggaggctg
aggatctgggagtttattactgc (SEQ ID NO:468)

(amino acids)

GVPDRFSGSGSGTDFTLKINRVEAEDLGVIY (SEQ ID NO:469)

Mouse C3 light chain variable complementarity determining regions 3 (CDR3) sequence:

(DNA)

tttcaaggttcacatgttccattcacg (SEQ ID NO:470)

(amino acids)

FQGSHPFT (SEQ ID NO:471)

IGKV2-29*03 light chain variable region sequence:

(DNA)

gatattgtgatgaccagactccactctctctgtccgtcaccctggacagccggcctccatctcctgcaagt
ctagtcagagcctcctgcatagtgatggaaagacctatttgtattggtacctgcagaagccaggccagtctcc
acagctcctgatctatgaagtttccagccggttctctggagtgccagatagggtcagtggcagcgggtcaggg
acagatttcacactgaaaatcagccgggtggaggctgaggatggtggggtttattactgcatgcaaggtatac
accttcct (SEQ ID NO:472)

(amino acids)

DIVMTQTPLSLSVTPGQPASISCKSSQSLHSDGKTYLYWYLQKPGQSPQLLIYEVSSRFSGVDPDRFSGSGSG
TDFTLKISRVEAEDVGVYYCMQGIHLP (SEQ ID NO:473)

IGKV2-29*03 light chain variable framework region 1 (FWR1) acid sequence:

(DNA)

gatattgtgatgaccagactccactctctctgtccgtcaccctggacagccggcctccatctcctgc
(SEQ ID NO:474)

(amino acids)

DIVMTQTPLSLSVTPGQPASISC (SEQ ID NO:475)

IGKV2-29*03 light chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

aagtctagtcagagcctcctgcatagtgatggaaagacctatttgtat (SEQ ID NO:476)

(amino acids)

KSSQSLHSDGKTYLY (SEQ ID NO:477)

IGKV2-29*03 light chain variable framework region 2 (FWR2) sequence:

(DNA)

tggtacctgcagaagccaggccagtctccacagctcctgatctat (SEQ ID NO:478)

(amino acids)

WYLQKPGQSPQLLIY (SEQ ID NO:479)

IGKV2-29*03 light chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

gaagtttccagccggttc (SEQ ID NO:480)

(amino acids)

EVSSRFS (SEQ ID NO:481)

IGKV2-29*03 light chain variable framework region 3 (FWR3) sequence:

(DNA)

ggagtgccagatagggtcagtggcagcgggtcagggacagatttcacactgaaaatcagccgggtggaggctg
aggatggttggggtttattactgc (SEQ ID NO:482)

(amino acids)

GVPDRFSGSGSGTDFTLKISRVEAEDVGYYC (SEQ ID NO:483)

IGKV2-29*03 light chain variable complementarity determining regions3 (CDR3) sequence:

(DNA)

atgcaaggatacaccttcct (SEQ ID NO:484)

(amino acids)

MQGIHLP (SEQ ID NO:485)

Humanized C3 light chain variable region sequence:

(DNA)

gatattgtgatgaccagactccactctctctgtccgtcaccctggacagccggcctccatctcct
gcaggtctagtcagaccattgtccatagtaatggaaacacctatttggagtggtacctgcagaagcc
aggccagtctccacagctcctgatctataagggtttccaaccggttctctggagtgccagatagggtc
agtggcagcgggtcagggacagatttcacactgaaaatcagccgggtggaggctgaggatggtggg
tttattactgcttccaaggtagccacgtgcctttcaccttcggcggagggaaccaaggtggagatcaa
acgaact (SEQ ID NO:486)

(amino acids)

DIVMTQTPLSLSVTPGQPASISCRSSQTIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCFQGSHPVFTFGGGTKVEIKRT (SEQ ID NO:487)

Humanized C3 light chain variable framework region 1 (FWR1) acid sequence:

(DNA)

gatattgtgatgaccagactccactctctctgtccgtcaccctggacagccggcctccatctcct
gc (SEQ ID NO:488)

(amino acids)

DIVMTQTPLSLSVTPGQPASISC (SEQ ID NO:489)

Humanized C3 light chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

ggtctagtcagaccattgtccatagtaatggaaacacctatttggag (SEQ ID NO:490)

(amino acids)

RSSQTIVHSNGNTYLE (SEQ ID NO:491)

Humanized C3 light chain variable framework region 2 (FWR2) acid sequence:

(DNA)

tggtacctgcagaagccaggccagtcctccacagctcctgatctat (SEQ ID NO:492)

(amino acids)

WYLQKPGQSPQLLIY (SEQ ID NO:493)

Humanized C3 light chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

aaggtttccaaccgggttctct (SEQ ID NO:494)

(amino acids)

KVSNRFS (SEQ ID NO:495)

Humanized C3 light chain variable framework region 3 (FWR3) acid sequence:

(DNA)

ggagtgccagataggttcagtgccagcgggtcagggacagatttcacactgaaaatcagccgggtggaggctg
aggatgttgggttttattactgc (SEQ ID NO:496)

(amino acids)

GVPDRFSGSGTDFTLKISRVEAEDVGVIYC (SEQ ID NO:497)

Humanized C3 light chain variable complementarity determining regions 3 (CDR3) sequence:

(DNA)

ttccaaggtagccacgtgcctttcacc (SEQ ID NO:498)

(amino acids)

FQGSHPFT (SEQ ID NO:499)

Humanized C3 lambda light chain sequence

(DNA)

gatattgtgatgaccagactccactctctctgtccgtcaccctggacagccggcctccatctcctgcaggt
ctagtcagaccattgtccatagtaatggaaacacctatgttgagtggtacctgcagaagccaggccagtctcc
acagctcctgatctataaggtttccaaccggttctctggagtgccagataggttcagtgccagcgggtcaggg
acagatttcacactgaaaatcagccgggtggaggctgaggatgttgggttttattactgcttccaaggtagcc
acgtgcctttcaccttcggcggagggaccaaggtggagatcaaacgaactggtcagcccaaggctgccccctc
ggtcactctgttcccgccctcctctgaggagcttcaagccaacaaggccacactggtgtgtctcataagtgac
ttctaccggggagccgtgacagtggtcctggaaggcagatagcagccccgtcaaggcgggagtgagaccacca
caccctccaaacaaagcaacaacaagtagcgcggccagcagctatctgagcctgacgcctgagcagtggaagtc
ccacagaagctacagctgccaggtcacgcatgaaggagcaccgtggagaagacagtggtccctacagaatgt
tcatagtaa (SEQ ID NO:500)

(amino acids)

DIVMTQTPLSLSVTPGQPASISCRSSQTIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVDPDRFSGSGS
TDFTLKISRVEAEDVGVIYCFQGSHPFTFGGGTKVEIKRTGQPKAAPSVTLFPPSSEELQANKATLVCLISD
FYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTEC
S** (SEQ ID NO:501)

Humanized C3 Kappa light chain

(DNA)

gatattgtgatgaccagactccactctctctgtccgtcaccctggacagccggcctccatctcctgcaggt
ctagtcagaccattgtccatagtaatggaaacacctatgttgagtggtacctgcagaagccaggccagtctcc
acagctcctgatctataaggtttccaaccggttctctggagtgccagataggttcagtgccagcgggtcaggg
acagatttcacactgaaaatcagccgggtggaggctgaggatgttgggttttattactgcttccaaggtagcc
acgtgcctttcaccttcggcggagggaccaaggtggagatcaaacgaactacggtggctgcaccatctgtctt
catcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgctgctgaataacttctat
cccagagaggccaaagtacagtggaaggtggataacgcctccaatcgggttaactcccaggagagtggtcacag
agcaggacagcaaggacagcacctacagcctcagcagcacctgacgctgagcaaagcagactacgagaaaca
caaagtctacgcctgcgaagtcacccatcagggcctgagctcgccgtcacaagagcttcaacaggggagag
tgtagtaa (SEQ ID NO:502)

(amino acids)

DIVMTQTPLSLSVTPGQPASISCRSSQTIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSG
 TDFTLKISRVEAEDVGVYYCFQGSHPFTFGGGTKVEIKRTTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY
 PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
 C** (SEQ ID NO: 503)

Humanized C3 Kappa light gBLOCK sequence:

(DNA)

agctgggctaggttaagcttggtaccgagctcggatccacgccaccatggagacagacacactcctgct
 atgggtactgctgctctgggttccaggttccactggtgacgatatgtgatgaccagactccactc
 tctctgtccgtcaccctggacagccggcctccatctcctgcagggtctagtcagaccattgtccata
 gtaatggaaacacctatttggagtggtacctgcagaagccaggccagtctccacagctcctgatcta
 taaggtttccaaccggttctctggagtgccagataggttcagtggcagcgggtcagggacagatttc
 acactgaaaatcagccgggtggaggctgaggatgttggggtttattactgcttccaaggtagccacg
 tgcctttcaccttcggcgaggggaccaaggtggagatcaaacgaactacggtggctgcaccatctgt
 cttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttggtgtgcctgctgaat
 aacttctatcccagagaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactccc
 aggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgag
 caaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgccc
 gtcacaaagagcttcaacaggggagagtgttagtaagtttaaacccgctgatcagcctcgactgtgc
 cttctagttgc (SEQ ID NO:504)

Mouse C8 heavy chain variable region sequence

(DNA)

gaagtgatggctcgtggaaagcggcggttggtctggtaaagccggggggatcccttaagctttcttgcg
 ccgcatccgggttcacgttctccggctatgccatgtcctgggtccgacagactcccgaaaagcgctt
 ggaatgggtggccactatctcctccggggggacgtacatctactaccccgacagtggtgaaaggaaga
 tttaacaatatctcgcgacaacgcaaaaaataccttgtatcttcaaatagagctccctgcgggtcagagg
 acactgccatgtactattgcgcccgcctgggcggcgacaattactatgagtat (SEQ ID
 NO:505)

(amino acids)

EVMVVESSGGLVKPGGSLKLSCAASGFTFSGYAMSWVRQTPEKRLEWVATISSGGTYIYYPDSVKGRF
 TISRDNAKNTLYLQMSSLRSEDAMYYCARLGGDNYYEY (SEQ ID NO:506)

Mouse C8 heavy chain variable complementarity determining region 1 (CDR1) sequence:

(DNA)

ggctatgccatgtcc (SEQ ID NO:507)

(amino acids)

GYAMS (SEQ ID NO:508)

Mouse C8 heavy chain variable complementarity determining region 2 (CDR2) sequence:

(DNA)

actatctcctccgggggacgtacatctactaccccgacagtggtgaaagga (SEQ ID NO:509)

(amino acids)

TISSGGTYIYYPDSVKG (SEQ ID NO:510)

Mouse C8 heavy chain variable complementarity determining region 3 (CDR3) sequence:

(DNA)

ctggggcggcgacaattactatgagtat (SEQ ID NO:511)

(amino acids)

LGGDNYEY (SEQ ID NO:512)

IGHV3-21*04 heavy chain variable region sequence:

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtg
cagcctctggattcaccttcagtagctatagcatgaactgggtccgccaggctccaggaaggggct
ggagtgggtctcatccattagtagtagtagttacatatactacgcagactcagtgaagggccga
ttcaccatctccagagacaacgccaaagaactcactgtatctgcaaataaacagcctgagagccgagg
acacggccgtgtattactgtgcga (SEQ ID NO:513)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKLEWVSSISSSSSYIYYADSVKGRFTISR
NAKNSLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:514)

IGHV3-21*04 heavy chain variable framework region 1 (FWR1) sequence:

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcct
ctggattcaccttcagt (SEQ ID NO:515)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFS (SEQ ID NO:516)

IGHV3-21*04 heavy chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

agctatagcatgaac (SEQ ID NO:517)

(amino acids)

YSYMN (SEQ ID NO: 518)

IGHV3-21*04 heavy chain variable framework region 2 (FWR2) sequence:

(DNA)

tgggtccgccaggctccaggaaggggctggagtgggtc (SEQ ID NO:519)

(amino acids)

WVRQAPGKLEWV (SEQ ID NO:520)

IGHV3-21*04 heavy chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

tcattccattagtagtagtagtagttacatatactacgcagactcagtgaagggc (SEQ ID NO: 521)

(amino acids)

SSISSSSSYIYYADSVKG (SEQ ID NO: 522)

IGHV3-21*04 heavy chain variable framework region 3 (FWR3) sequence:

(DNA)

cgattccaccatctccagagacaacgccaaagaactcactgtatctgcaaataaacagcctgagagccgaggaca
cggccgtgtattactgtgcga (SEQ ID NO:523)

(amino acids)

RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:524)

Humanized C8 heavy chain variable region sequence:

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggccctgagactctcctgtgcagcct
ctggattcaccttcagtggctatgccatgagctgggtccgccaggctccagggaaggggctggagtgggtctc
aaccattagtagtggcggaacctacatatactaccctgactcagtgaagggccgattcaccatctccagagac
aacgccaagaactcactgtatctgcaaataaacagcctgagagccgaggacacggccgtgtattactgtgcga
gactgggcggcgataactattatgaatattggggcaaagggaccacggtcaccgtctcctcc (SEQ ID
NO: 525)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDSVKGRFTISR
DNAKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYWGKTTTVTVSS (SEQ ID NO: 526)

Humanized C8 heavy chain variable framework region 1 (FWR1) sequence:

(DNA)

gaggtgcagctggtggagctctgggggaggcctggtcaagcctgggggggccctgagactctcctgtgcagcct
ctggattcaccttcagt (SEQ ID NO:527)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFS (SEQ ID NO:528)

Humanized C8 heavy chain variable complementarity determining region 1 (CDR1) sequence:

(DNA)

ggctatgccatgagc (SEQ ID NO:529)

(amino acids)

GYAMS (SEQ ID NO:530)

Humanized C8 heavy chain variable framework region 2 (FWR2) sequence:

(DNA)

tgggtccgccaggctccagggaaggggctggagtgggtctca (SEQ ID NO:531)

(amino acids)

WVRQAPGKGLEWVS (SEQ ID NO:532)

Humanized C8 heavy chain variable complementarity determining region 2 (CDR2) sequence:

(DNA)

accattagtagtggcggaacctacatatactaccctgactcagtgaagggc (SEQ ID NO:533)

(amino acids)

TISSGGTYIYYPDSVKG (SEQ ID NO:534)

Humanized C8 heavy chain variable framework region 3 (FWR3) sequence:

(DNA)

cgattcaccatctccagagacaacgccaagaactcactgtatctgcaaataaacagcctgagagccgaggaca
cggccgtgtattactgtgcgaga (SEQ ID NO:535)

(amino acids)

RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:536)

Humanized C8 heavy chain variable complementarity determining region 3 (CDR3) sequence:

(DNA)

ctgggcgcgataactattatgaatat (SEQ ID NO:537)

(amino acids)

LGGDNYEY (SEQ ID NO:538)

Humanized C8 IgG1 heavy chain sequence

(DNA)

gaggtgcagctggtggagtctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcct
 ctggattcaccttcagtggctatgccatgagctgggtccgccaggctccagggaaggggctggagtgggtctc
 aaccattagtagtggcggaacctacatataactacctgactcagtgaagggccgattcaccatctccagagac
 aacgccaagaactcactgtatctgcaaatgaacagcctgagagccgaggacacggccgtgtattactgtgcga
 gactgggcgcgataactattatgaatatgggggcaaagggaccacggtcacctctcctccgctagcaccaa
 gggcccatcggtcttccccctggcaccctcctccaagagcacctctgggggcacagcgccctgggctgcctg
 gtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcgcggtgcacacct
 tcccggtgtcctacagtcctcaggactctactccctcagcagcgtggtgacagtgcctccagcagcttggtg
 caccagacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaa
 tcttgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgctcagctcttctct
 tccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtgag
 ccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccg
 cgggaggagcagtacaacagcacgtacctgtggtcagcgtcctcacctcctgcaccaggactggctgaatg
 gcaaggagtacaagtgaaggtctccaacaaagccctcccagcccccatcgagaaaacctctccaaagccaa
 agggcagccccgagaaccacaggtgtacacctgcccccatcccgaggagatgaccaagaaccaggtcagc
 ctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggaga
 acaactacaagaccacgctcccggtgtggaactccgacggctccttcttctctacagcaagctcacctgga
 caagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctctgcacaaccactacacg
 cagaagagcctctccctgtctccgggtaaatgataa (SEQ ID NO:539)

(amino acids)

EVQLVESGGGLVQPKGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPD SVKGRFTISR
 D NAKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYWGKGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
 VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSLGTQTYICNVNHKPSNTKVDKKVEPK
 SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYT
 QKSLSLSPGK** (SEQ ID NO:540)

Humanized C8 IgG2 heavy chain sequence

(DNA)

gaggtgcagctggtggagtctgggggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcct
 ctggattcaccttcagtggctatgccatgagctgggtccgccaggctccagggaaggggctggagtgggtctc
 aaccattagtagtggcggaacctacatataactacctgactcagtgaagggccgattcaccatctccagagac
 aacgccaagaactcactgtatctgcaaatgaacagcctgagagccgaggacacggccgtgtattactgtgcga
 gactgggcgcgataactattatgaatatgggggcaaagggaccacggtcacctctcctccgctccaccaaa
 gggcccatcggtcttccccctggcgcctgtcctcaggagcacctccgagagcacagccgcctgggctgcctg
 gtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgctctgaccagcgcggtgcacacct
 tcccagctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgcctccagcaacttcgg
 caccagacctacacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagacagttgagcgcaaa
 tgttgtgtcgagtgccaccgtgccagcaccacctgtggcaggaccgtcagctcttctcttccccccaaaac
 ccaaggacacctcatgatctcccgaccctgaggtcacgtgcgtggtggtggacgtgagccacgaagaccc
 cgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccacgggaggagcag

ttcaacagcacggttccgtgtggtcagcgtcctcacggttgtgcaccaggactggctgaacggcaaggagtaca
 agtgcaaggctctccaacaaaggcctcccagcccccatcgagaaaaccatctccaaaaccaaagggcagccccg
 agaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccagggtcagcctgacctgcctg
 gtcaaaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaaga
 ccacacctcccatgctggactccgacggctccttcttcctctacagcaagctcacggtggacaagagcaggtg
 gcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctc
 tcctgtctccgggtaaatagtaa (SEQ ID NO:541)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPD SVKGRFTISR
 D NAKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYWGKGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCL
 VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSNFGTQTYTCNV DHKPSNTKVDKTV
 ERK CCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQ
 FNSTFRVVS VLTIVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCL
 VKGFYPSPDI AVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSL
 SLSPGK** (SEQ ID NO:542)

Mouse C8 light chain variable region sequence

(DNA)

gacatcgtcattacgcagaccctgccagtcttgccgtttctctgggccagagggccactatcagttacaggg
 cgagtaagtcctgtgagtaccagcggctatagttacatgcattggaaccagcagaaaccgggacagccaccacg
 cctgcttatttatctggtgtctaattcttgagtcgggggtgcccgccagggttcagcggcagcggctctgggacc
 gacttcacactcaacattcatccagtggagaagaggacgctgctacatactactgtcaacacattcgggaac
 tgaccaggagtga (SEQ ID NO:543)

(amino acids)

DIVITQTPASLAVSLGQRATISYRASKSVSTSGYSYMHWNQQKPGQPPRLLIYLVSNLESGVPARF'S
 GSGSGTDFTLNIHPVEEEDAATYYCQHIRELTRSE (SEQ ID NO:544)

Mouse C8 light chain variable complementarity determining region 1 (CDR1) sequence:

(DNA)

agggcgagtaagtcctgtgagtaccagcggctatagttacatgcat (SEQ ID NO:545)

(amino acids)

RASKSVSTSGYSYMH (SEQ ID NO: 546)

Mouse C8 light chain variable complementarity determining region 2 (CDR2) sequence:

(DNA)

ctggtgtctaattcttgagtcc (SEQ ID NO:547)

(amino acids)

LVSNNLES (SEQ ID NO:548)

Mouse C8 light chain variable complementarity determining region 3 (CDR3) sequence:

(DNA)

caacacattcgggaactgaccaggagtga (SEQ ID NO:549)

(amino acids)

QHIRELTRSE (SEQ ID NO:550)

NCBI germline z00023 light chain variable region sequence:

(DNA)

gacatcgtgatgacccagtcctccagactccctggctgtgtctctgggcgagagggccaccatcaactgcaagt
ccagccagagtgtttttatacagctccaacaataagaactacttagcttggtaccagcagaaaccaggacagcc
tctaagctgctcatttactgggcacatctaccgggaatccgggggtccctgaccgattcagtggcagcgggtct
gggacagatttcactctcaccatcagcagcctgcaggctgaagatgtggcagtttattactgtcagcaatatt
atagtactcct (SEQ ID NO:551)

(amino acids)

DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
GTDFLTITISLQAEDVAVYYCQYYSTP (SEQ ID NO:552)

NCBI germline z00023 light chain variable framework region 1 (FWR1) acid sequence:

(DNA)

gacatcgtgatgacccagtcctccagactccctggctgtgtctctgggcgagagggccaccatcaactgc
(SEQ ID NO:553)

(amino acids)

DIVMTQSPDSLAVSLGERATINC (SEQ ID NO:554)

NCBI germline z00023 light chain variable complementarity determining regions 1 (CDR1) sequence:

(DNA)

aagtccagccagagtgtttttatacagctccaacaataagaactacttagct (SEQ ID NO:555)

(amino acids)

KSSQSVLYSSNNKNYLA (SEQ ID NO:556)

NCBI germline z00023 light chain variable framework region 2 (FWR2) sequence:

(DNA)

tggtaccagcagaaaccaggacagcctcctaagctgctcatttac (SEQ ID NO:557)

(amino acids)

WYQQKPGQPPKLLIY (SEQ ID NO:558)

NCBI germline z00023 light chain variable complementarity determining regions 2 (CDR2) sequence:

(DNA)

tgggcacatctaccgggaatcc (SEQ ID NO:559)

(amino acids)

WASTRES (SEQ ID NO:560)

NCBI germline z00023 light chain variable framework region 3 (FWR3) sequence:

(DNA)

gggggtccctgaccgattcagtggcagcgggtctgggacagatttcactctcaccatcagcagcctgcaggctg
aagatgtggcagtttattactgt (SEQ ID NO:561)

(amino acids)

GVPDRFSGSGSGTDFLTITISLQAEDVAVYYC (SEQ ID NO:562)

NCBI germline z00023 light chain variable complementarity determining regions3 (CDR3) sequence:

(DNA)

cagcaatattatagtactcct (SEQ ID NO:563)

(amino acids)

QQYYSTP (SEQ ID NO:564)

Humanized C8 light chain variable region sequence

(DNA)

gacatcgtgatgaccagtcctccagactccctggctgtgtctctctgggcgagagggccaccatcaact
gcagggccagcaagagtgttagcaccagcggctacagctacatgcactggtaccagcagaaaccagg
acagcctcctaagctgctcatttacctgggtgtctaacctggaatccgggggtccctgaccgattcagt
ggcagcgggtctgggacagatttcactctcaccatcagcagcctgcaggctgaagatgtggcagttt
attactgtcaacacattcgggaactgaccaggagtgaattcggcggagggaccaaggtggagatcaa
acgaact (SEQ ID NO:565)

(amino acids)

DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSYMHWYQQKPGQPPKLLIYLVSNLESGVPDRFS
GSGSGTDFTLTISSLQAEDVAVYYCQHIRELTRSEFGGGTKVEIKRT (SEQ ID NO:566)

Humanized C8 light chain variable framework region 1 (FWR1) sequence:

(DNA)

gacatcgtgatgaccagtcctccagactccctggctgtgtctctctgggcgagagggccaccatcaact
gc (SEQ ID NO:567)

(amino acids)

DIVMTQSPDSLAVSLGERATINC (SEQ ID NO:568)

Humanized C8 light chain variable complementarity determining region 1 (CDR1) sequence:

(DNA)

agggccagcaagagtgttagcaccagcggctacagctacatg (SEQ ID NO:569)

(amino acids)

RASKSVSTSGYSYM (SEQ ID NO:570)

Humanized C8 light chain variable framework region 2 (FWR2) sequence:

(DNA)

cactggtaccagcagaaaccaggacagcctcctaagctgctcatttac (SEQ ID NO:571)

(amino acids)

HWYQQKPGQPPKLLIY (SEQ ID NO:572)

Humanized C8 light chain variable complementarity determining region 2 (CDR2) sequence:

(DNA)

ctggtgtctaacctggaatcc (SEQ ID NO:573)

(amino acids)

LVSNLES (SEQ ID NO:574)

Humanized C8 light chain variable framework region 3 (FWR3) sequence:

(DNA)

gggggtccctgaccgattcagtgggcagcgggtctgggacagatttcactctcaccatcagcagcctgcaggctg
aagatgtggcagtttattactgt (SEQ ID NO:575)

(amino acids)

GVPDRFSGSGSGTDFLTISLQAEDVAVYYC (SEQ ID NO:576)

Humanized C8 light chain variable complementarity determining region 3 (CDR3) sequence:

(DNA)

caacacattcgggaactgaccaggagtga (SEQ ID NO:577)

(amino acids)

QHIRELTRSE (SEQ ID NO:578)

Humanized C8 Lambda light chain sequence

(DNA)

gacatcgtgatgaccagtcctccagactccctggctgtgtctctgggcgagagggccaccatcaactgcaggg
ccagcaagagtgttagcaccagcggctacagctacatgcactggtaccagcagaaaccaggacagcctcctaa
gctgctcatttacctgggtgtctaacctggaatccgggggtccctgaccgattcagtgggcagcgggtctgggaca
gatttcactctcaccatcagcagcctgcaggctgaagatgtggcagtttattactgtcaacacattcgggaac
tgaccaggagtgaattcggcggagggaccaaggtggagatcaaacgaactggtcagcccaaggctgccccctc
ggtcactctgttcccgccctcctctgaggagcttcaagccaacaaggccacactgggtgtgtctcataagtgc
ttctacccgggagccgtgacagtggcctggaaggcagatagcagccccgtcaaggcgggagtgagaccacca
cacctccaacaaaagcaacaacaagtagcggccagcagctatctgagcctgacgctgagcagtggaagtc
ccacagaagctacagctgccaggtcacgcatgaaggagcaccgtggagaagacagtgggccctacagaatgt
tcatagtaa (SEQ ID NO:579)

(amino acids)

DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSYMHWYQQKPGQPPKLLIYLVSNLESGVPDRFSGSGSGT
DFLTISLQAEDVAVYYCQHIRELTRSEFGGGTKVEIKRTGQPKAAPSVTLFPPSSEELQANKATLVCLISD
FYPGAVTVAWKADSSPVKAGVETTTTPSKQSNKNYAASSYLSLTPEQWKSQRSYSCQVTHEGSTVEKTVAPTEC
S** (SEQ ID NO:580)

Humanized C8 Kappa light chain sequence

(DNA)

gacatcgtgatgaccagtcctccagactccctggctgtgtctctgggcgagagggccaccatcaactgcaggg
ccagcaagagtgttagcaccagcggctacagctacatgcactggtaccagcagaaaccaggacagcctcctaa
gctgctcatttacctgggtgtctaacctggaatccgggggtccctgaccgattcagtgggcagcgggtctgggaca
gatttcactctcaccatcagcagcctgcaggctgaagatgtggcagtttattactgtcaacacattcgggaac
tgaccaggagtgaattcggcggagggaccaaggtggagatcaaacgaactacggtggtgcaccatctgtctt
catcttcccgccatctgatgagcagttgaaatctggaactgcctctgttggtgtgctgctgaataacttctat
cccagagagggccaaagtacagtgggaaggtggataacgcctccaatcgggtaactcccaggagagtgtcacag
agcaggacagcaaggacagcacctacagcctcagcagcacctgacgctgagcaaagcagactacgagaaaca
caaagtctacgctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggagag
tgtagtaa (SEQ ID NO:581)

(amino acids)

DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSYMHWYQQKPGQPPKLLIYLVSNLESGVPDRFSGSGSGT
DFLTISLQAEDVAVYYCQHIRELTRSEFGGGTKVEIKRTTVAAPSVFIFPPSDEQLKSGTASVCLLNIFY
PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C** (SEQ ID NO:582)

Humanized C8 Kappa light chain gBLOCK sequence:

(DNA)

agctggctaggttaagcttgggtaccgagctcggatccacgccaccatggagacagacacactcctgctatgggt
 actgctgctctgggttccaggttccactggtgacgacatcgtgatgaccagctctccagactccctggctgtg
 tctctgggcgagagggccaccatcaactgcagggccagcaagagtgttagcaccagcggctacagctacatgc
 actggtaccagcagaaaccaggacagcctcctaagctgctcatttacctgggtgttaacctggaatccgggt
 ccctgaccgattcagtggtcagcgggtctgggacagatttactctcaccatcagcagcctgcaggctgaagat
 gtggcagtttattactgtcaacacattcgggaactgaccaggagtgaattcggcggagggaccaaggtggaga
 tcaaacgaactacgggtggtgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaac
 tgctctgtgtgtgctgctgaataacttctatccagagaggccaaagtacagtggaaggtggataacgcc
 ctccaatcgggtaactccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagca
 ccctgacgctgagcaaagcagactacgagaaacacaaagtctacgctgcaagtcacccatcagggcctgag
 ctgccccgtcacaaagagcttcaacaggggagagtgttagtaagtttaaaccgctgatcagcctcgaactgtg
 ccttctagttgc (SEQ ID NO:583)

CAR-T E6 CD8 sequence:

(DNA)

gaggtccagctgggtgagagtggtgggtgggtgggttaagcctggcggtccctgcggtgagctgcgcgcga
 gtggatttactttcagccgatatgggatgagttgggtgcggaagctcccggaagaggctggaatgggtctc
 aacaatctccgggggggcacttacatctattacccgactcagtcagggggagatttaccatttcacgagac
 aacgctaagaataccctgtatttgcagatgaattctctgagagcagaggacacagctgtttactattgtacc
 gcgacaactatggcaggaactacgactacgggtatggactattggggacaagggacattgggttacagtgagcag
 tggcggcgggggcagcggaggaggaggcagcgggtggggggggcagcgagatagtgtcacgcagtcacccgcg
 actctcagctctctcacctggggaacgagctaccctgacgtgctctgtacctcctcagtgctcatatattcact
 ggtatcagcaacggcccgggcagtcacctagattgtcatttatagtagcttaactctggcctcaggtatccc
 tgcaagattttctggatctggttcagggtctgtattacacctcactatctctagcctggagcctgaagacttt
 gccgtttattactgccagcagaggtctagctccccattcacctttgggagtgggaccaagggttgaaattaaaa
 cgacaacccccggccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctgagaccaga
 agcctgtaggcctgccgcgggtggagctgtgcacacaagaggactggatttcgctgtgatattctacatttgg
 gccccgctcgaggcacatgtggagtgtcctcctctcctcctggtgattaccctgtactgctgataa (SEQ
 ID NO:584)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLEWVSTISGGGTYYIYPDSVKGR
 FTISRDNAKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWGQGLTVTVSSGGGGSGGGGSGG
 GGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHQYQRPQGSPRLLIYSTSNLASGIPARFSGS
 GSGSDYTLTISLLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPE
 ACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC** (SEQ ID NO:585)

CAR-T C2 CD8 sequence:

(DNA)

gaagtgcagctcgtagagagtggcgggggactggtgaagcccggtggaagcctcagactcagttgcg
 ccgcctcaggttttactttttcaggttacgccatgtcctgggtgaagacaggcaccggggaaaggact
 cgagtgggtgtctactatcagctcaggaggcacttatatatattatcctgactctgtaaaaggccga
 ttctacgatttctcgcgacaatgcaaagaactccctctacctccaaatgaacagtccttagggcagaag
 aactgctgtatactattgtgcagcctcggcggcgacaactactacgagtactttgacgtgtgggg
 gaaagggactaccgtgacagtttcaagcggaggaggtggctcaggtggaggcgggtcagggggggga
 ggaagtgatattgtgctcacacaatccccagcctccctggctgtgtctcccgccaacgcgctacaa
 ttacatgtcgggcctccaaaagcgtgagcaccagcgggtacagctacatgcactgggtatcaacagaa
 accaggacaaccccccaaactgttgatttatctcgcttcaaacttgaggtccggcggtgcctgcgcgc
 ttttcaggagtggtggagcggcacagattttacgctgactatcaaccccgtagaagcaaacgatacag
 cgaattattattgtcaacattcccggaactccctttacgttcggcgggggcacaaagggtcgaaat
 taagagaaccacgacaacccccggccccagaccaccaacgccagccccaccatcgccagccaaccc

ctgtctctgagaccagaagcctgtaggcctgccgccggtggagctgtgcacacaagaggactggatt
tcgcctgtgatatctacatttgggccccgctcgcaggcacatgtggagtgtcctcctctccctggt
gattaccctgtactgctgataa (SEQ ID NO:586)

(amino acids)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSTISSGGTYIYYPDSVKGR
FTISRDNKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYFDVWGKGTITVTVSSGGGGSGGGSGGG
GSDIVLTQSPASLAVSPGQRATITCRASKSVSTSGYSYMHWYQQKPGQPPKLLIYLASNLESGVPAR
FSGSGSGTDFTLTINPVEANDTANYYCQHSRELPTFTGGGKTKVEIKRTTTTPAPRPPTPAPTIASQP
LSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC** (SEQ ID NO:587)

CD8/4-1BB sequence

(DNA)

acgacaacccccggccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctga
gaccagaagcctgtaggcctgccgccggtggagctgtgcacacaagaggactggatttcgcctgtga
tatctacatttgggccccgctcgcaggcacatgtggagtgtcctcctctccctggtgattaccctg
tactgcaaaaggggcccgaaaaaactcctttacatttttaagcagccttttatgaggccagtacaga
cgactcaagaggaagacgggtgctcatgccgctttcctgaggaggaggaaggagggtgcgaactgtg
ataa (SEQ ID NO:588)

(amino acids)

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITL
YCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL** (SEQ ID NO:589)

CD8/CD28 sequence

(DNA)

acgacaacccccggccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctga
gaccagaagcctgtaggcctgccgccggtggagctgtgcacacaagaggactggatttcgcctgtga
tatctacatttgggccccgctcgcaggcacatgtggagtgtcctcctctccctggtgattaccctg
tactgcagaagcaagcggctctcggtcctgcattctgattacatgaacatgaccccaagaagaccag
gccccaccaggaaacattaccagccctacgctccgccacgcgacttcgctgcttaccgggtcctgata
a (SEQ ID NO: 590)

(amino acids)

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITL
YCRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS** (SEQ ID NO:591)

CD8/CD3z sequence:

(DNA)

acgacaacccccggccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctga
gaccagaagcctgtaggcctgccgccggtggagctgtgcacacaagaggactggatttcgcctgtga
tatctacatttgggccccgctcgcaggcacatgtggagtgtcctcctctccctggtgattaccctg
tactgccgcgttaagttctcccgatcagccgacgcgcctgcttacaagcagggccagaaccaactgt
acaacgagctgaatctcggtagacgggaagagtagcagctggttgacaaacggagaggccgcgaccc
agaaatgggcggaagcctcgcaggaaaaacccccaggaggactgtacaatgagttgcagaaagat
aagatggcagaagcttatagcgagatcggaatgaagggggaaaggagacgagggaaaggacacgacg
gcctttatcagggcctgtccacagcaacaaaagatacgtatgacgcctccatgatgcaggcacttcc
accacggtgataa (SEQ ID NO:592)

(amino acids)

TTTPAPRPPTPAPTIIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLLSLVITL
YCRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD
KMAEAYSEIGMKGERRRRGKGHDLGLYQGLSTATKDTYDALHMQALPPR** (SEQ ID NO:593)

CD8/CD28/CD3z sequence:

(DNA)

acgacaacccccggccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctga
gaccagaagcctgtaggcctgccgccggtggagctgtgcacacaagaggactggatttcgcctgtga
tatctacatattgggccccgctcgccagcacatgtggagtgtcctcctctccctgggtgattaccctg
tactgcagaagcaagcgggtctcggtcctgcattctgattacatgaacatgaccccaagaagaccag
gccccaccaggaaacattaccagccctacgctccgccacgcgacttcgctgcctaccggtcccgcgt
taagttctcccgatcagccgacgcgcctgcttacaagcagggccagaaccaactgtacaacgagctg
aatctcggtagacgggaagagtacgacgtgttgacaaacggagaggccgcgaccagaaatgggcg
gcaagcctcgccaggaaaaacccccaggagggactgtacaatgagttgcagaaagataagatggcaga
agcttatagcgagatcggaatgaagggggaaaggagacgagggaaaggacacgacggcctttatcag
ggcctgtccacagcaacaaaagatacgtatgacgccctccatattgcaggcacttccaccacgggtgat
aa (SEQ ID NO:594)

(amino acids)

TTTPAPRPPTPAPTIIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLLSLVITL
YCRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNE
NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDLGLY
GLSTATKDTYDALHMQALPPR** (SEQ ID NO:595)

CD8/4-1BB/CD3z sequence:

(DNA)

acgacaacccccggccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctga
gaccagaagcctgtaggcctgccgccggtggagctgtgcacacaagaggactggatttcgcctgtga
tatctacatattgggccccgctcgccagcacatgtggagtgtcctcctctccctgggtgattaccctg
tactgcaaaaggggcccgaaaaaactcctttacatttttaagcagccttttatgaggccagtacaga
cgactcaagaggaagacgggtgtcatgccgctttcctgaggaggaggaaggagggtgcgaactgcg
cgtaagttctcccgatcagccgacgcgcctgcttacaagcagggccagaaccaactgtacaacgag
ctgaatctcggtagacgggaagagtacgacgtgttgacaaacggagaggccgcgaccagaaatgg
gcggcaagcctcgccaggaaaaacccccaggagggactgtacaatgagttgcagaaagataagatggc
agaagcttatagcgagatcggaatgaagggggaaaggagacgagggaaaggacacgacggcctttat
cagggcctgtccacagcaacaaaagatacgtatgacgccctccatattgcaggcacttccaccacgggt
gataa (SEQ ID NO:596)

(amino acids)

TTTPAPRPPTPAPTIIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLLSLVITL
YCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNE
LNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDLGLY
QGLSTATKDTYDALHMQALPPR** (SEQ ID NO:597)

CD8/CD28/4-1BB/CD3z sequence:

(DNA)

acgacaacccccggccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctga
gaccagaagcctgtaggcctgccgccggtggagctgtgcacacaagaggactggatttcgcctgtga
tatctacatattgggccccgctcgccagcacatgtggagtgtcctcctctccctgggtgattaccctg
tactgcagaagcaagcgggtctcggtcctgcattctgattacatgaacatgaccccaagaagaccag

gccccaccaggaaacattaccagccctacgctccgccacgcgacttcgctgcctaccggtccaaaag
 gggccgcaaaaaactcctttacatttttaagcagccttttatgaggccagtacagacgactcaagag
 gaagacgggtgctcatgccgctttcctgaggaggaggaaggaggggtgcgaactgcgcgttaagttct
 cccgatcagccgacgcgcctgcttacaagcagggccagaaccaactgtacaacgagctgaatctcgg
 tagacgggaagagtacgacgtggtggacaaacggagaggccgcgacccagaaatggcgggcaagcct
 cgcaggaaaaacccccaggagggactgtacaatgagttgcagaaagataagatggcagaagcttata
 gcgagatcggaatgaaggggggaaaggagacgaggggaaaggacacgacggcctttatcagggcctgtc
 cacagcaacaaaagatacgtatgacgccctccatattgcaggcacttccaccacgggtgataa (SEQ
 ID NO:598)

(amino acids)

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLLSLVITL
 YCRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRPVQTTQE
 EDGCSCRFPEEEEEGGCELVRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP
 PEMGGKP
 RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR**
 (SEQ ID NO: 599)

CAR-T C3 4-1BB/CD3z sequence:

(DNA)

atggccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgccgccaggccacagg
 ttcagctgggtgcagtctggagctgaggtgaagaagcctggggcctcagtgaaaggtctcctgcaaggc
 ttctggttacacctttaccgactacgccatgaactgggtgcgacaggccccctggacaagggcttgag
 tggatgggagtgatcagcaccttcagcggtaacacaaacttcaaccagaagttcaagggcagagtgca
 ccatgaccacagacacatccacgagcacagcctacatggagctgaggagcctgagatctgacgacac
 ggccgtgtattactgtgcgagaagcgactactacggcccatacttcgactactggggccagggcacc
 accctgaccgtgtccagcggcggtggcggtatccggcggtggcggtatccggcggtggcggtatccgata
 ttgtgatgaccagactccactctctctgtccgtcacccctggacagccggcctccatctcctgcag
 gtctagtgcagaccattgtccatagtaatggaaacacctatctggagtggtacctgcagaagccaggc
 cagctctccacagctcctgatctataaggtttccaaccggttctctggagtgccagatagggttcagtg
 gcagcgggtcagggaacagatttcacactgaaaatcagccgggtggaggctgaggatgttggggttta
 ttactgcttccaaggtagccacgtgcctttcaccttcggcgaggaggaccaaggtggagatcaaacga
 actacgacaacccccggccccagaccaccaacgccagccccaccatcgccagccaacccctgtctc
 tgagaccagaagcctgtaggcctgccgcgggtggagctgtgcacacaagaggactggatttcgcctg
 tgatatctacatttgggccccgctcgcaggcacatgtggagtgtcctcctctccctgggtgattacc
 ctgtactgcaaaaggggcccgaaaaaactcctttacatttttaagcagccttttatgaggccagtac
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 gcgcgttaagttctccgatcagccgacgcgcctgcttacaagcagggccagaaccaactgtacaac
 gagctgaatctcggtagacgggaagagtacgacgtgttgacaaacggagaggccgcgacccagaaa
 tgggcgggaagcctcgcaggaaaaacccccaggagggactgtacaatgagttgcagaaagataagat
 ggcagaagcttatagcgagatcggaatgaaggggggaaaggagacgaggggaaaggacacgacggcctt
 tatcagggcctgtccacagcaacaaaagatacgtatgacgccctccatattgcaggcacttccaccac
 ggtgataa (SEQ ID NO:600)

(amino acids)

QVQLVQSGAEVKKPGASVKVSCKASGYTFDYMNNWVRQAPGQGLEWMGVISTFSGNTNFNQKFKGR
 VTMTTDTSTSTAYMELRSLRSDDTAVYYCARSDYYGPYFDYWGGQTTLTVSSGGGSGGGGSGGGGS
 DIVMTQTPLSLSVTPGQPASISCRSSQTIIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNNRFS
 GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHPVFTFGGGTKVEIKRTTTTPAPRPPTPAPTIASQPL
 SLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLLSLVITLYCKRGRKKLLYIFKQPFMRP
 VQTTQEEDGCSCRFPEEEEEGGCELVRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP

EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDLGLYQGLSTATKDTYDALHMQUALP
PR** (SEQ ID NO:601)

C3 CAR gBLOCK 1 sequence:

(DNA)

atccacgctgttttgacctccatagaagattctagagctagctgtagagcttggtaccgagggccac
catggccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgcccgccaggccacag
gttcagctggtgacgtctggagctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaagg
cttctgggttacacctttaccgactacgccatgaactgggtgcgacaggccccctggacaagggttga
gtggatgggagtgatcagcaccttcagcggttaacacaaacttcaaccagaagttcaagggcagagtc
accatgaccacagacacatccacgagcacagcctacatggagctgaggagcctgagatctgacgaca
cggccgtgtattactgtgcgagaagcgactactacggcccatacttcgactactggggccaggggcac
caccctgaccgtgtccagcggcggtggcggtatccggcggtggcggtatccggcggtggcggtatccgat
attgtgatgaccagactccactctctctgt (SEQ ID NO:602)

C3 CAR gBLOCK 2 sequence:

(DNA)

tattgtgatgaccagactccactctctctgtccgtcacccttgacagccggcctccatctcctgc
aggtctagtcagaccattgtccatagtaatggaaacacctatttggagtggtacctgcagaagccag
gccagtctccacagctcctgatctataagggtttccaaccgggtctcttgagtgccagataggttcag
tggcagcgggtcaggacagatttcacactgaaaatcagccgggtggaggctgaggatggtgggggtt
tattactgcttccaaggtagccacgtgcctttcaccttcggcgaggggaccaaggtggagatcaaac
gaactacgacaaccccgggccccccagaccaccaacgccagccccaccatcgccagccaacccctgtc
tctgagaccagaagcctgtaggcctgccgcgggtggagctgtgcacacaagaggactggatttcgcc
tgtgatattctacatttgggccccgctcgcaggcacatgtg (SEQ ID NO:603)

E6 scFV gBLOCK 1 sequence:

(DNA)

tgctctgggttccagggttccactgggtgacgcggcccagccggccgaggtgcagctggtggagtctgg
gggaggcctggtcaagcctgggggggtccctgagactctcctgtgcagcctctggattcaccttcagt
aggtatggcatgagctgggtccgccaggctccagggaagaggctggagtggtctcaaccattagtg
gcggaggcacctacatatactaccagactcagtgaaggggccgattcacctctccagagacaacgc
caagaacaccctgtatctgcaaatgaacagcctgagagccgaggacacggctgtgtattactgtacc
agagataactatggccgcaactatgattatggcatggattattggggccaggggcacccctggtgaccg
tgagcagcggcggtggcggtatccggcggtggcggtatccggcggtggcggtatcc (SEQ ID
NO:604)

E6 scFV gBLOCK 2 sequence:

(DNA)

ggcggtggcggtatccggcggtggcggtatccggcggtggcggtatccgaaattgtgttgacacagtctc
cagccaccctgtctttgtctccaggggaagagccaccctcacctgcagcgccaccagcagtggttag
ctacatccactggtaccaacagaggcctggccagagccccaggctcctcatctatagcacctccaac
ctggccagcggcatcccagccagggttcagtggcagtggtctgggagcgactacactctcaccatca
gcagcctagagcctgaagattttgcagtttattactgtcagcagcgtagcagctcccctttcacctt
tggcagcggcaccaaagtggaaattaaaaccgggtcatcatcaccatcaccactgataagtttaaacc
cgctgatcagcctcgactgtgccttctagt (SEQ ID NO:605)

CAR-T C2 CD3z sequence:

(DNA)

atggccttgccagtgacggccctgctgctgccattggctcttctgttgcaagctgccaggcctgaagtgcagc
tcgtagagagtgggcggggactggtgaagcccggtggaagcctcagactcagttgcgcgcgcctcaggtttcac
tttttcaggttacgccatgtcctgggtaagacaggcaccggggaaaggactcgagtgggtgtctactatcagc
tcaggaggcacttatatatattatcctgactctgtaaaaggccgatttacgatttctcgcgacaatgcaaaga
actccctctacctccaaatgaacagtccttagggcagaagacactgctgtatactattgtgcacgcctcggcgg
cgacaactactacgagtactttgacgtgtgggggaaagggactaccgtgacagtttcaagcggaggaggtggc
tcaggtggaggcggtcagggggggagggaagtgatattgtgctcacacaatcccagcctcctggctgtgt
ctcccgccaacgcgctacaattacatgtcgggcctccaaaagcgtgagcaccagcggctacagctacatgca
ctggtatcaacagaaaccaggacaaccccccaactgttgatttatctcgccttcaaacttgagtcgggcgtg
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gagaaccacgacaacccccggccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctg
agaccagaagcctgtaggcctgccgcgggtggagctgtgcacacaaggaggactggatttcgcctgtgatattc
acatttgggccccgctcgcaggcacatgtggagtgctcctcctcctcctgggtgattacccctgtactgcgcgt
taagttctccgatcagccgacgcgcctgcttacaagcaggggccagaaccaactgtacaacgagctgaatctc
ggtagacgggaagagtagcagctgttgacaaaacggagaggccgcgaccagaaatggcgggcaagcctcgca
ggaaaaacccccaggagggactgtacaatgagttgcagaaagataagatggcagaagcttatagcgagatcgg
aatgaaggggaaaggagacgagggaaaggacacgacggcctttatcagggcctgtccacagcaacaaaagat
acgtatgacgcctccatattgcaggcacttccaccacggtgataa (SEQ ID NO:606)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLE
WVSTISSGGTYIYYPDSVKGRFTISRDNKNSLYLQMSLR AEDTAVYYCARLGGDNYEYFDVWGK
GTTVTVSSGGGSGGGGSGGGSDIVLTQSPASLAVSPGQRATITCRASKSVSTSGYSYMHWYQQKP
GQPPKLLIYLASNLESGVPARFSGSGSGTDFTLTINPVEANDTANYYCQHSREL PFTFGGGTKVEIK
RTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI
TLYCRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVL DKRRGRDP EMGGKPRRKNPQEGLYNELQ
KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR** (SEQ ID NO:607)

CAR-T C2 CD28/CD3z sequence:

(DNA)

atggccttgccagtgacggccctgctgctgccattggctcttctgttgcaagctgccaggcctgaagtgcagc
tcgtagagagtgggcggggactggtgaagcccggtggaagcctcagactcagttgcgcgcgcctcaggtttcac
tttttcaggttacgccatgtcctgggtaagacaggcaccggggaaaggactcgagtgggtgtctactatcagc
tcaggaggcacttatatatattatcctgactctgtaaaaggccgatttacgatttctcgcgacaatgcaaaga
actccctctacctccaaatgaacagtccttagggcagaagacactgctgtatactattgtgcacgcctcggcgg
cgacaactactacgagtactttgacgtgtgggggaaagggactaccgtgacagtttcaagcggaggaggtggc
tcaggtggaggcggtcagggggggagggaagtgatattgtgctcacacaatcccagcctcctggctgtgt
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ctggtatcaacagaaaccaggacaaccccccaactgttgatttatctcgccttcaaacttgagtcgggcgtg
cctgcgcgcttttcaggagtgaggagcggcacagattttacgtgactatcaaccccgtagaagcaaacgata
cagcgaattattattgtcaacattcccgggaactcccctttacgttcggcgggggcacaaaggtcgaaattaa
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agaccagaagcctgtaggcctgccgcgggtggagctgtgcacacaaggaggactggatttcgcctgtgatattc
acatttgggccccgctcgcaggcacatgtggagtgctcctcctcctcctgggtgattacccctgtactgcagaag
caagcggctcgcgctcctgcattctgattacatgaacatgaccccaagaagaccaggccccaccaggaaacat
taccagccctacgctccgccacgcgacttcgctgcctaccgggtcccgcgttaagttctccgatcagccgacg
cgctgcttacaagcaggggccagaaccaactgtacaacgagctgaatctcggtagacgggaagagtagcagct
gttgacaaaacggagaggccgcgaccagaaatggcgggcaagcctcgaggaaaaacccccaggagggactg
tacaatgagttgcagaaagataagatggcagaagcttatagcgagatcggaatgaaggggaaaggagacgag
ggaaaggacacgacggcctttatcagggcctgtccacagcaacaaaagatacgtatgacgcctccatattgca
ggcacttccaccacggtgataa (SEQ ID NO:608)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLE
 WVSTISSGGTYIYYPDSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYFDVWGK
 GTTIVTVSSGGGGSGGGSGGGSDIVLTQSPASLAVSPGQRATITCRASKSVSTSGYSYMHWYQQKP
 GQPPKLLIYLASNLESGVPARFSGSGSDTFTLTINPVEANDTANYYCQHSRELPTFTGGGKVEIK
 RTTTTAPRPPTPAPTIIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI
 TLYCRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYN
 ELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDL
 YQGLSTATKDTYDALHMQALPPR** (SEQ ID NO:609)

CAR-T C2 4-1BB/CD3z sequence:

(DNA)

atggccttgccagtgacggccctgctgctgccattggctcttctgttgacgctgccaggcctgaagtgcagc
 tcgtagagagtggtgggggactggtgaagcccggtggaagcctcagactcagttgcgcgcgcctcaggtttcac
 tttttcaggttacgccatgtcctgggtaagacaggcaccggggaaaggactcgagtgggtgtctactatcagc
 tcaggaggcacttatatatattatcctgactctgtaaaaggccgatttacgatttctcgcgacaatgcaaaga
 actccctctacctccaaatgaacagtccttagggcagaagacactgctgtatactattgtgacgcctcggcgg
 cgacaactactacgagtactttgacgtgtgggggaaagggactaccgtgacagtttcaagcggaggaggtggc
 tcaggtggaggcgggtcagggggggagggaagtgatattgtgctcacacaatcccagcctccttggtgtgt
 ctcccgcccaacgcgctacaattacatgtcgggcctccaaaagcgtgagcaccagcggctacagctacatgca
 ctggtatcaacagaaaccaggacaaccccccaaaactgttgatttatctcgcttcaaacttggagtcgggcgtg
 cctgcgcgcttttcaggagtggtggagcggcacagattttacgtgactatcaaccccgtagaagcaaacgata
 cagcgaattattattgtcaacattcccggaactccctttacgttcggcgggggcacaaaggtcgaaattaa
 gagaaccacgacaacccccggccccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctg
 agaccagaagcctgtaggcctgccgccggtggagctgtgcacacaagaggactggatttcgcctgtgatattct
 acatttgggccccgctcgaggcacatgtggagtgctcctcctcctcctgggtgattaccctgtactgcaaaag
 gggcgcgcaaaaaactcctttacatttttaagcagccttttatgaggccagtagacagcactcaagaggaagac
 ggggtgctcatgccgctttcctgaggaggaggaaggagggtgcgaactgcgcgttaagttctcccgatcagccg
 acgcgcctgcttacaagcagggccagaaccaactgtacaacgagctgaatctcggtagacgggaagagtagca
 cgtgttggacaaacggagaggccgcgaccagaaatggcgggcaagcctcgcaggaaaaacccccaggaggga
 ctgtacaatgagttgcagaaagataagatggcagaagcttatagcgagatcggaatgaagggggaaaggagac
 gagggaaaggacacgacggcctttatcagggcctgtccacagcaacaaaagatacgtatgacgcctccatat
 gcaggcacttccaccacgggtgataa (SEQ ID NO:610)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLE
 WVSTISSGGTYIYYPDSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYFDVWGK
 GTTIVTVSSGGGGSGGGSGGGSDIVLTQSPASLAVSPGQRATITCRASKSVSTSGYSYMHWYQQKP
 GQPPKLLIYLASNLESGVPARFSGSGSDTFTLTINPVEANDTANYYCQHSRELPTFTGGGKVEIK
 RTTTTAPRPPTPAPTIIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI
 TLYCKRGRKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLY
 NELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDL
 LYQGLSTATKDTYDALHMQALPPR** (SEQ ID NO:611)

CAR-T C2 OX40/CD3z sequence:

(DNA)

atggccttgccagtgacggccctgctgctgccattggctcttctgttgacgctgccaggcctgaagtgcagc
 tcgtagagagtggtgggggactggtgaagcccggtggaagcctcagactcagttgcgcgcgcctcaggtttcac
 tttttcaggttacgccatgtcctgggtaagacaggcaccggggaaaggactcgagtgggtgtctactatcagc
 tcaggaggcacttatatatattatcctgactctgtaaaaggccgatttacgatttctcgcgacaatgcaaaga
 actccctctacctccaaatgaacagtccttagggcagaagacactgctgtatactattgtgacgcctcggcgg
 cgacaactactacgagtactttgacgtgtgggggaaagggactaccgtgacagtttcaagcggaggaggtggc
 tcaggtggaggcgggtcagggggggagggaagtgatattgtgctcacacaatcccagcctccttggtgtgt
 ctcccgcccaacgcgctacaattacatgtcgggcctccaaaagcgtgagcaccagcggctacagctacatgca
 ctggtatcaacagaaaccaggacaaccccccaaaactgttgatttatctcgcttcaaacttggagtcgggcgtg

cctgcgcgcttttcagggagtgaggagcggcacagattttacgctgactatcaaccccgtagaagcaaacgata
 cagcgaattattattgtcaacattcccgggaactcccctttacgttcggcggggggcacaaagggtcgaaattaa
 gagaaccacgacaacccccggccccccagaccaccaacgccagccccaccatcgccagccaacccctgtctctg
 agaccagaagcctgtaggcctgccgcccgtggagctgtgcacacaagaggactggatttcgctgtgatatact
 acatttgggccccgctcgcaggcacatgtggagtgctcctcctcctcctgggtgattaccctgtactgccggag
 ggaccagaggctgccccccgatgccacaagccccctgggggaggcagtttcgggacccccatccaagaggag
 caggccgacgcccactccaccctggccaagatccgcgttaagttctcccgatcagccgacgcgcctgttaca
 agcagggccagaaccaactgtacaacgagctgaatctcggtagacgggaagagtagcagctgttggacaaacg
 gagaggccgcgacccagaaatgggcggcaagcctcgcaggaaaaacccccaggagggactgtacaatgagttg
 cagaaagataagatggcagaagcttatagcgagatcggaatgaagggggaaaggagacgagggaaaggacacg
 acggcctttatcagggcctgtccacagcaacaaaagatacgtatgacgcctccatatagcaggcacttccacc
 acggtgataa (SEQ ID NO:612)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVLPKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLE
 WVSTISSGGTYIYYPDSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYFDVWGK
 GTTVTVSSGGGSGGGGSGGGGSDIVLTQSPASLAVSPGQRATITCRASKSVSTSGYSYMHWYQQK
 GPFPKLLIYLASNLESGVPAARFSGSGGTDFTLTINPVEANDTANYYCQHSRELPTFGGGTKVEIK
 RTTTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI
 TLYCRRDQRLPPDAHKKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYKQGQNQLYNELNL
 GRREEYDVLDKRRGRDPGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGL
 STATKDTYDALHMQALPPR** (SEQ ID NO:613)

CAR-T C2 CD28/OX40/CD3z sequence:

(DNA)

atggccttgccagtgacggccctgctgctgccattggctcttctgttgcaagctgccaggcctgaagtgcagc
 tcgtagagagtgggcggggactgggtgaagcccggtggaagcctcagactcagttgcgcgcctcaggtttcac
 tttttcaggttacgccatgtcctgggtaagacaggcaccggggaaaggactcgagtgggtgtctactatcagc
 tcaggaggcacttatatatattatcctgactctgtaaaaggccgatttacgatttctcgcgacaatgcaaaga
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 cgacaactactacgagtaactttgacgtgtgggggaaagggactaccgtgacagtttcaagcggaggaggtggc
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 taccagccctacgctccgccacgcgacttcgctgcctaccgggtcccgaggaggaccagaggctgccccccgatg
 ccacaagccccctgggggaggcagtttcgggacccccatccaagaggagcaggccgacgcccactccaccct
 ggccaagatccgcgttaagttctcccgatcagccgacgcgcctgttacaagcagggccagaaccaactgtac
 aacgagctgaatctcggtagacgggaagagtagcagctgttggacaaacgggagaggccgcgacccagaaatgg
 gcggcaagcctcgcaggaaaaacccccaggagggactgtacaatgagttgcagaaagataagatggcagaagc
 ttatagcgagatcggaatgaagggggaaaggagacgagggaaaggacacgacggcctttatcagggcctgtcc
 acagcaacaaaagatacgtatgacgcctccatatagcaggcacttccaccacggtgataa (SEQ ID
 NO:614)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVLPKPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLE
 WVSTISSGGTYIYYPDSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARLGGDNYEYFDVWGK
 GTTVTVSSGGGSGGGGSGGGGSDIVLTQSPASLAVSPGQRATITCRASKSVSTSGYSYMHWYQQK

GQPPKLLIYLASNLESGVPA RFSGSGSGTDFLTINPVEANDTANYYCQHSREL PFTFGGGTKVEIK
 RTTTT PAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI
 TLYCRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRRDQRLPPDAH KPPGGGSFRTP
 IQEEQADAHSTLAKIRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRK
 NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR** (SEQ
 ID NO:615)

CAR-T E6 OX40/CD3z sequence:

(DNA)

atggccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgccgccaggccagagg
 tccagctgggttgagagtggcggtgggctgggttaagcctggcggtccctgcggtgagctgcgccgc
 gagggtgatttactttcagccgatatgggatgagttgggtgcggaagctcccggaagaggctggaa
 tgggtctcaacaatctccggggggggcacttacatctattaccccgactcagtcagggggagattta
 ccatttcacgagacaacgctaagaataccctgtatttgcagatgaattctctgagagcagaggacac
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 caagggacattgggttacagtgagcagtgccggcgggggcagcggaggaggaggcagcgggtgggggg
 gcagcgagatagtgctcagcagtcacccgcgactctcagtcctcacctggggaacgagctaccct
 gacgtgctctgctacctcctcagtgctcatatattcactgggtatcagcaacggccccgggcagtcacct
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 ggccccgctcgcaggcacatgtggagtgtcctcctctcctgggtgattaccctgtactgccggagg
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 gcctgcttacaagcaggggccagaaccaactgtacaacgagctgaatctcggtagacgggaagagtac
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 gggggaaaggagacgagggaaaggacacgacggcctttatcagggcctgtccacagcaacaaaagat
 acgtatgacgcctccatattgcaggcacttccaccacgggtgataa (SEQ ID NO:616)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLE
 WVSTISGGGTYYYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGM DYWG
 QGTLVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSIHWHYQQRPGQSP
 RLLIYSTSNLASGIPARFSGSGSGSDYTLTISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKTTTP
 APRPPTPAPT IASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRR
 DQRLPPDAH KPPGGGSFRTP IQEEQADAHSTLAKIRVKFSRSADAPAYKQGQNQLYNELNLGRREEY
 DVL DKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD
 TYDALHMQALPPR** (SEQ ID NO:617)

CAR-T E6 CD28/OX40/CD3z sequence:

(DNA)

atggccctgcccgtgaccgctttgctgctccccctggcgctgctgctgcacgccgccaggccagagg
 tccagctgggttgagagtggcggtgggctgggttaagcctggcggtccctgcggtgagctgcgccgc
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gcagcgagatagtgctcacgcagtcacccgcgactctcagttctcacctggggaacgagctaccct
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 aacattaccagccctacgctccgccacgcgacttcgctgcctaccgggtcccggaggaccagaggct
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 gacgcccactccacccctggccaagatccgcgttaagttctcccgatcagccgacgcgcctgcttaca
 agcagggccagaaccaactgtacaacgagctgaatctcggtagacgggaagagtacgacgtgttgga
 caaacggagaggccgcgaccagaaatgggcccgaagcctcgaggaacccccaggaggaggactg
 tacaatgagttgcagaaagataagatggcagaagcttatagcgagatcggaatgaagggggaaagga
 gacgagggaaaggacacgacggcctttatcagggcctgtccacagcaacaaaagatacgtatgacgc
 cctccatatgcaggcacttccaccacggtgataa (SEQ ID NO:618)

(amino acids)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYGMSWVRQAPGKRLE
 WVSTISGGGTYYYPDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRDNYGRNYDYGMDYWG
 QGTLVTVSSGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLTCSATSSVSYIHQYQQRPGQSP
 RLLIYSTSNLASGIPARFSGSGSGSDYTLTISSLEPEDFAVYYCQQRSSSPFTFGSGTKVEIKTTTP
 APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRS
 KRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRRDQRLPPDAHKKPPGGGSFRTPIQEEQA
 DAHSTLAKIRVKFSRSADAPAYKQGQNLQYLNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG
 YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR** (SEQ ID
 NO:619)

MUC1 truncated cytoplasmic sequence

(amino acids)

SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620)

MUC1 truncated cytoplasmic sequence

(amino acids)

SVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621)

MUC1 truncated cytoplasmic sequence

(amino acids)

VQLTLAFREGTINVHDTVETQFNQY (SEQ ID NO:622)

MUC1 truncated cytoplasmic sequence

(amino acids)

SNIKFRPGSVVVQLTLAFREGTIN (SEQ ID NO:623)

Primers

attctaagcttgggccaccatggaactg (SEQ ID NO:624)

tctagagtttaaaacttactattttacccggagacagggagag (SEQ ID NO:625)

agtatggcccagccggccgaggtgcagctgggtggagctctgg (SEQ ID NO:626)

tagaaggcacagtcgaggctgatcag (SEQ ID NO:627)

attctaagcttgggccaccatggaagc (SEQ ID NO:628)
 tctagagtttaaaacttactaactctccccctgttgaagc (SEQ ID NO:629)
 agtatggcccagccggccgaaattgtgttgacacagtctccag (SEQ ID NO:630)
 tagaaggcacagtcgaggctgatcag (SEQ ID NO:631)
 actgtcatatggaggtgcagctggtggagtctg (SEQ ID NO:632)
 actgtctcgagtttaatttccactttggtgccgctgc (SEQ ID NO:633)
 actgtcatatggaggtgcagctggtggagtctg (SEQ ID NO:634)
 actgtaccggttttaatttccactttggtgccgctgc (SEQ ID NO:635)
 cttcttcctcaggagcaagctcaccgtgg (SEQ ID NO:636)
 gagccgtcggagtccagc (SEQ ID NO:637)
 gcacctgaactcctgggg (SEQ ID NO:638)
 ttttaatttccactttggtgccg (SEQ ID NO:639)
 cgcggttagcttaagcttgggtaccgagggcca (SEQ ID NO:640)
 cgcgggcgccgcctgatcagcggttttaacttatc (SEQ ID NO:641)

[00257] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims. The following examples are offered by way of illustration of the present invention, and not by way of limitation.

EXAMPLES

[00258] EXAMPLE 1 – ELISA competition with NME1 and NME7

[00259] PSMGFR peptide was covalently coupled to BSA using Inject Maleimide activated BSA kit (Thermo Fisher). PSMGFR peptide coupled BSA was diluted to 7.5ug/mL in 0.1M carbonate/bicarbonate buffer pH 9.6 and 50uL was added to each well of a 96 well plate. After overnight incubation at 4°C, the plate was wash twice with PBS-T and a 3% BSA solution was added to block remaining binding site on the well. After 1h at RT the plate was washed twice with PBS-T and NME1 or NME7, diluted in PBS-T + 1% BSA, was added at saturating concentration. After 1h at RT the plate was washed 3x with PBS-T and anti-MUC1* antibody (or antibody fragments), diluted in PBS-T + 1% BSA, was added (5x molar excess compared to NME1/NME7). After 1h at RT the plate was washed 3x with PBS-T and goat anti HisTag-HRP, diluted in PBS-T + 1% BSA, was added at 1/10000 dilution. After 1h at RT the plate was washed 3x with PBS-T and remaining NME1 or NME7 bound to the PSMGFR peptide was measured at 415nm using a ABTS solution (Thermo Fisher).

[00260] EXAMPLE 2 - Humanization of anti-MUC1* extracellular domain monoclonal antibodies

[00261] We generated humanized antibodies that bind to the extracellular domain of MUC1* by a process called complementarity determining region, 'CDR', grafting. First, homology searches were performed to independently align the heavy chain variable region and light chain variable region nucleotide sequences of mouse monoclonal anti-MUC1* antibody (E6 HC SEQ ID NOS:12-13; LC SEQ ID NOS:65-66 and MN-C2 HC SEQ ID NO:118-119; LC SEQ ID NO: 168-169) against a repertoire of human antibody sequences (IMGT, the international ImMunoGeneTics information system). The sequences with the highest homology were selected. IGHV3-21*01 is a human IgG heavy chain variable region sequence with 82.9% (DNA) and 74.5% (amino acids) identity to MouseMN-E6 heavy chain variable region. IGKV3-11*02 is a human IgG light chain variable region sequence with 68.8% (DNA) and 61.1% (amino acids) identity to MouseMN-E6 light chain variable region. IGHV3-21*04 is a human IgG heavy chain variable region sequence with 85% (DNA) and 81.6% (amino acids) identity to Mouse MN-C2 heavy chain variable region. IGKV7-3*01 is a human IgG light chain variable region sequence with 76.9% (DNA) and 71.3% (amino acids) identity to Mouse MN-C2 light chain variable region. Second, a model of the mouse scFv was generated to select and keep the mouse residues important for the stability of the CDR and framework. Finally, CDRs from the human germlines were replaced by the corresponding mouse CDRs.

[00262] Humanized MN-E6 IgG2 heavy chain cloning

[00263] The Kozak consensus sequence followed by the IGHV3-21*03 leader sequence, the humanizedMN-E6 heavy chain variable region and the constant region of human IgG2 was synthesized by our request by GenScript, NJ (SEQ ID NOS:52-53). The cDNA was amplified by polymerase chain reaction (PCR) using the following primer: 5'-ATTCTAAGCTTGGGCCACCATGGAAGT-3' (SEQ ID NO:624) and 5'-TCTAGAGTTTAACTTACTATTTACCCGGAGACAGGGAGAG-3' (SEQ ID NO:625). After digestion with HindIII and PmeI restriction enzymes (New England Biolabs), the purified fragment was cloned into the pCDNA 3.1 V5 vector (Life Technologies) digested with the same restriction enzymes.

[00264] Humanized MN-E6 heavy chain cDNA was amplified by polymerase chain reaction (PCR) using the following primer: 5'-AGTATGGCCCAGCCGGCCGAGGTGCAGCTGGTGGAGTCTGG-3' (SEQ ID NO:626) and 5'-TAGAAGGCACAGTCGAGGCTGATCAG-3' (SEQ ID NO:627). After digestion with SfiI and PmeI restriction enzymes (New England Biolabs), the purified fragment was cloned into the pSECTag2 vector (Life Technologies) digested with the same restriction enzymes.

[00265] Humanized MN-E6 kappa light chain cloning

[00266] The Kozak consensus sequence followed by the IGHV3-11*02 leader sequence, the humanizedMN-E6 light chain variable region and the constant region of human Kappa light chain was synthesized by our request by GenScript, NJ (SEQ ID NOS: 107-108). The cDNA was amplified by polymerase chain reaction (PCR) using the following primer: 5'-ATTCTAAGCTTGGGCCACCATGGAAGC-3' (SEQ ID NO:628) and 5'-TCTAGAGTTTAACTTACTAACACTCTCCCCTGTTGAAGC-3' (SEQ ID NO:629). After digestion with HindIII and PmeI restriction enzymes (New England Biolabs), the purified fragment was cloned into the pCDNA 3.1 V5 vector (Life Technologies) digested with the same restriction enzymes.

[00267] HumanizedMN-E6 light chain cDNA was amplified by polymerase chain reaction (PCR) using the following primer: 5'-AGTATGGCCCAGCCGGCCGAAATTGTGTTGACACAGTCTCCAG-3' (SEQ ID NO:630) and 5'-TAGAAGGCACAGTCGAGGCTGATCAG-3' (SEQ ID NO:631). After digestion with SfiI and PmeI restriction enzymes (New England Biolabs), the purified fragment was cloned into the pSECTag2 vector (Life Technologies) digested with the same restriction enzymes.

[00268] Humanized MN-E6 IgG1 heavy chain cloning

[00269] HumanizedMN-E6 IgG2 constructs (pCDNA 3.1 V5 and pSECTag2) were digested with BstEII and PmeI (New England Biolabs) to remove the IgG2 heavy chain constant region. The vector with humanizedMN-E6 heavy chain variable region was purified. Human IgG1 heavy chain constant region was synthesized by our request by IDT, IA (SEQ ID NOS: 60-61). Both gBLOCKS and the purified vector with humanizedMN-E6 variable region were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00270] HumanizedMN-E6 lambda light chain cloning

[00271] HumanizedMN-E6 kappa light chain constructs (pCDNA 3.1 V5 vector and pSECTag2 vector) were digested with KpnI and PmeI (New England Biolabs) to remove the kappa light chain constant region. The vector with humanizedMN-E6 light chain variable region was purified. Human lambda light chain constant region was synthesized by our request by IDT, IA (SEQ ID NO: 115). Both, gBLOCK and the purified vector with humanizedMN-E6 light chain variable region were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00272] Humanized MN-C2 IgG1 and IgG2 heavy chain cloning

[00273] HumanizedMN-E6 IgG1 and IgG2 heavy chain in pSECTag2 were digested with SfiI and AgeI to remove theMN-E6 variable region. HumanizedMN-E6 IgG1 and IgG2 heavy chain in

pCDNA 3.1 V5 were digested with HindIII and AgeI to remove the MN-E6 variable region. The vectors with human IgG1 or IgG2 constant region were purified. Humanized MN-C2 heavy chains were synthesized by our request by IDT, IA (SEQ ID NOS:160 and 165). Sequence to be cloned into pCDNA 3.1 V5 contains in 5' the murine Ig kappa chain leader sequence (SEQ ID NO 160). Both, gBLOCK and purified vector with human IgG1 or IgG2 constant region were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00274] Humanized MN-C2 kappa/lambda light chain cloning

[00275] Two humanized MN-C2 variable region fused to the kappa light chain constant region and two humanized MN-C2 variable region fused to the lambda light chain constant region were synthesized by our request by IDT, IA (SEQ ID NOS: 210 and 213 and SEQ ID NOS: 216 and 219, respectively). pCDNA 3.1 V5 was digested with HindIII and PmeI restriction enzymes (New England Biolabs) and pSEC Tag2 was digested with SfiI and PmeI restriction enzymes (New England Biolabs). Both plasmids were then purified. SEQ ID NOS: 210 and 216 were ligated into digested pCDNA 3.1 V5 and SEQ ID NOS: 213 and 219 were ligated into digested pSEC Tag2 using the Gibson assembly cloning kit (New England Biolabs).

[00276] Humanized C3 IgG1 heavy chain cloning

[00277] Humanized E6 IgG1 construct (pSECTag2) was digested with SfiI and AgeI (New England Biolabs) to remove the E6 heavy chain variable region. The vector without humanized E6 heavy chain variable region was purified. Humanized C3 heavy chain variable region was synthesized by our request by IDT, IA (SEQ ID NO:457). gBLOCK and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00278] Humanized C3 kappa light chain cloning

[00279] pEF V5-His was digested with BamHI and PmeI (New England Biolabs) and purified. Humanized C3 kappa light chain was synthesized by our request by IDT, IA (SEQ ID NO:504). Both, gBLOCK and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00280] Humanized C8 kappa light chain cloning

[00281] pEF V5-His was digested with BamHI and PmeI (New England Biolabs) and purified. Humanized C8 kappa light chain was synthesized by our request by IDT, IA (SEQ ID NO:583). Both, gBLOCK and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00282] EXAMPLE 3 – Cloning of humanized scFV of anti-MUC1* extracellular domain antibodies

[00283] Humanized E6 scFV cloning:

[00284] pSEC Tag2 was digested with SfiI and PmeI (New England Biolabs) and purified. Humanized E6 scFV gBLOCKS were synthesized by our request by IDT, IA (SEQ ID NOS: 604-605). Both, gBLOCKS and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00285] Humanized E6 scFV cDNA was amplified by polymerase chain reaction (PCR) using the following primers: 5'- ACTGTCATATGGAGGTGCAGCTGGTGGAGTCTG-3' (SEQ ID NO:632) and 5'- ACTGTCTCGAGTTTAATTTCCACTTTGGTGCCGCTGC-3' (SEQ ID NO:633). After digestion with NdeI and XhoI restriction enzymes (New England Biolabs), the purified fragment was cloned into the pET21b vector (Novagen) digested with the same restriction enzymes. Humanized E6 scFV cDNA was cloned 5' of the Histidine Tag for protein purification.

[00286] Humanized E6 scFV cDNA was amplified by polymerase chain reaction (PCR) using the following primers: 5'- ACTGTCATATGGAGGTGCAGCTGGTGGAGTCTG-3' (SEQ ID NO:634) and 5'- ACTGTACCGGTTTTAATTTCCACTTTGGTGCCGCTGC-3' (SEQ ID NO:635). After digestion with NdeI and AgeI restriction enzymes (New England Biolabs), the purified fragment was cloned into a modified pET21b vector (Novagen) digested with the same restriction enzymes. The vector was modified to include the StrepTag2 sequence followed by 2 stop codons 5' of the Histidine Tag. Humanized E6 scFV cDNA was cloned 5' of the StrepTag2 for protein purification.

[00287] Humanized E6, C2, C3 and C8 scFV-Fc cloning

[00288] Humanized E6 IgG1 construct (pSECTag2) was digested with SfiI and SacII (New England Biolabs) to remove the E6 heavy chain variable region and part of the IgG1 heavy chain constant region. The vector without humanized E6 heavy chain variable region was purified. Humanized E6, C2, C3 and C8 scFV gBLOCKS were synthesized by our request by IDT, IA (SEQ ID NO:258-259, 262-263, 266-267 and 270-271). E6, C2, C3 and C8 gBLOCKS and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs) to assemble the corresponding scFV in frame of the human IgG1 Fc region.

[00289] Humanized E6 scFV-Fc Y407R cloning

[00290] Humanized E6 scFV-Fc tyrosine 407 was mutated to an arginine (Y407R) by site directed mutagenesis. The Q5 site directed mutagenesis kit (NEB) was used with the following primers: 5'- CTTCTTCCTCAGGAGCAAGCTCACCGTGG-3' (SEQ ID NO:636) and 5'- GAGCCGTCGGAGTCCAGC-3' (SEQ ID NO:637)

[00291] Humanized E6 scFV-Fc hingeless cloning

[00292] Hinge region of humanized E6 scFV-Fc was removed by site directed mutagenesis. The Q5 site directed mutagenesis kit (NEB) was used with the following primers: 5'-GCACCTGAACTCCTGGGG-3' (SEQ ID NO:638) and 5'-TTTAATTTCCACTTTGGTGCCG-3' (SEQ ID NO:639)

[00293] **EXAMPLE 4 – Cloning of CAR-T of anti-MUC1* extracellular domain antibodies**

[00294] **CAR E6 CD28/4-1BB/CD3z cloning :**

[00295] pCDNA 3.1 V5 was digested with KpnI and PmeI (New England Biolabs) and purified. Full CAR-T E6 (CD8/CD28/4-1BB/CD3z) gBLOCK was synthesized by our request by IDT, IA (SEQ ID NO:305). Both, gBLOCK and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00296] **CAR E6 CD3z cloning :**

[00297] pCDNA 3.1 V5 CAR-T E6 CD8/CD28/4-1BB/CD3z was digested with EcoRV and PmeI (New England Biolabs) to remove cytoplasmic domains. The vector without cytoplasmic domains was purified. CAR-T E6 CD8/CD3z gBLOCK was synthesized by our request by IDT, IA (SEQ ID NO:296). Both, gBLOCK and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00298] **CAR E6 CD28/CD3z cloning :**

[00299] pCDNA 3.1 V5 CAR-T E6 CD8/CD28/4-1BB/CD3z was digested with EcoRV and PmeI (New England Biolabs) to remove cytoplasmic domains. The vector without cytoplasmic domains was purified. CAR-T E6 CD8/CD28/CD3z gBLOCK was synthesized by our request by IDT, IA (SEQ ID NO:299). Both, gBLOCK and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00300] **CAR E6 4-1BB/CD3z cloning :**

[00301] pCDNA 3.1 V5 CAR-T E6 CD8/CD28/4-1BB/CD3z was digested with EcoRV and PmeI (New England Biolabs) to remove cytoplasmic domains. The vector without cytoplasmic domains was purified. CAR-T E6 CD8/4-1BB/CD3z gBLOCK was synthesized by our request by IDT, IA (SEQ ID NO:302). Both, gBLOCK and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00302] **CAR C2 CD28/4-1BB/CD3z cloning :**

[00303] pCDNA 3.1 V5 CAR-T E6 CD8/CD28/4-1BB/CD3z was digested with KpnI and EcoRV (New England Biolabs) E6 scFV. The vector without E6 scFV was purified. CAR-T C2 gBLOCKs were synthesized by our request by IDT, IA (SEQ ID NOS: 308-309). Both, gBLOCKs and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00304] CAR sub-cloning into lentiviral vectors:

[00305] All pcDNA 3.1 V5 CAR cDNAs were amplified by polymerase chain reaction (PCR) using the following primers: 5-CGCGGCTAGCTTAAGCTTGGTACCGAGGGCCA-3' (SEQ ID NO:640) and 5'-CGCGGCGGCCGCCTGATCAGCGGGTTTAACTTATC-3' (SEQ ID NO:641). After digestion with NheI and NotI restriction enzymes (New England Biolabs), the purified fragments were cloned into lentiviral vectors (pCDH-EF1-MCS-IRES GFP and pCDH-CMV-MCS-EF1-copGFP+puro, SBI) digested with the same restriction enzymes.

[00306] CAR-E6- Fc/8/41BB/CD3z cloning :

[00307] pCDH-CMV-MCS-EF1-copGFP+puro (SBI) was digested with NheI and NotI (New England Biolabs) and the vector was purified. gBLOCKs were synthesized by our request by IDT, IA (Seq ID NOS: 312, 313 and 314). The gBLOCKs and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00308] CAR-E6- FcH/8/41BB/CD3z cloning :

[00309] pCDH-CMV-MCS-EF1-copGFP+puro (SBI) was digested with NheI and NotI (New England Biolabs) and the vector was purified. gBLOCKs were synthesized by our request by IDT, IA (Seq ID NOS: 312, 317 and 314). The gBLOCKs and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00310] CAR-E6-Fc-4-41BB-CD3z cloning :

[00311] pCDH-CMV-MCS-EF1-copGFP+puro (SBI) was digested with NheI and NotI (New England Biolabs) and the vector was purified. gBLOCKs were synthesized by our request by IDT, IA (Seq ID NOS: 312, 313 and 320). The gBLOCKs and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00312] CAR-E6 FcH/4/41BB/CD3z cloning :

[00313] pCDH-CMV-MCS-EF1-copGFP+puro (SBI) was digested with NheI and NotI (New England Biolabs) and the vector was purified. gBLOCKs were synthesized by our request by IDT, IA (Seq ID NOS: 312, 317 and 320). The gBLOCKs and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00314] CAR-E6 IgD/8/41BB/CD3z cloning :

[00315] pCDH-CMV-MCS-EF1-copGFP+puro (SBI) was digested with NheI and NotI (New England Biolabs) and the vector was purified. gBLOCKs were synthesized by our request by IDT, IA (Seq ID NOS: 312, 325 and 326). The gBLOCKs and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00316] CAR-E6 IgD/4/41BB/CD3z cloning :

[00317] pCDH-CMV-MCS-EF1-copGFP+puro (SBI) was digested with NheI and NotI (New England Biolabs) and the vector was purified. gBLOCKs were synthesized by our request by IDT, IA (Seq ID NOS: 312, 329 and 326). The gBLOCKs and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00318] **CAR-E6 X4/8/41BB/CD3z cloning :**

[00319] pCDH-CMV-MCS-EF1-copGFP+puro (SBI) was digested with NheI and NotI (New England Biolabs) and the vector was purified. gBLOCKs were synthesized by our request by IDT, IA (Seq ID NOS: 312, 332 and 326). The gBLOCKs and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00320] **CAR-E6 X4/4/41BB/CD3z cloning :**

[00321] pCDH-CMV-MCS-EF1-copGFP+puro (SBI) was digested with NheI and NotI (New England Biolabs) and the vector was purified. gBLOCKs were synthesized by our request by IDT, IA (Seq ID NOS: 312, 335 and 326). The gBLOCKs and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00322] **CAR E6-8+4-4-41BB-CD3z cloning :**

[00323] pCDH-CMV-MCS-EF1-copGFP+puro (SBI) was digested with NheI and NotI (New England Biolabs) and the vector was purified. gBLOCKs were synthesized by our request by IDT, IA (Seq ID NOS: 312, 338 and 326). The gBLOCKs and the purified vector were ligated using the Gibson assembly cloning kit (New England Biolabs).

[00324] **EXAMPLE 5 - Lentivirus production**

[00325] HEK 293T cells (ATCC) were used to produce lentivirus. The day prior transfection plates (6well plate) were coated with poly-D-lysine and cells seeded so that cell density reaches 90-95% at the time of transfection and cultures in a 5% CO₂ atmosphere. The next day cells were transfected with Lipofectamine 3000 (life technologies) and Opti-MEM® I Reduced Serum Medium according to the manufacturer instructions (0.75ug of lentiviral expression vector and 2.25ug of pPACKH1 packaging mix was used). After 6h incubation, the media was changed and media containing lentivirus was harvested after 24 and 48 hours. Lentivirus was concentrated with Lenti-X concentrator (Clontech) and titer was calculated using the Lenti-X p@4 Rapid Titer Kit (Clontech). Lentivirus was store at -80C in single-use aliquots.

[00326] **EXAMPLE 6 - Lipofectamine Transient expression**

[00327] HEK 293T cells (ATCC) were used to test expression of humanized IgG. The night before transfection, cells were passed at 1/3 dilution (6well plate) and cultures in a 5% CO₂ atmosphere. The next day, 1 hour before transfection, the media was change to complete media

without antibiotics (DMEM high glucose from ATCC containing 10% fetal calf serum). For transfection, we used Lipofectamine 3000 (life technologies) and Opti-MEM® I Reduced Serum Medium according to the manufacturer instructions. 1.25ug of the heavy chain construct and 1.25ug of the light chain construct or 2,5 ug of Fc-fusion constructs was used. After 48h incubation, the media was collected, cleared by centrifugation and used in an ELISA assay to quantify the level of humanized IgG expression and binding to PSMGFR peptide.

[00328] EXAMPLE 7 - Polyethylenimine (PEI) large scale Transient expression

[00329] HEK 293T cells (ATCC) were used for large scale expression of Fc-fusion protein. The night before transfection, cells were passed (6.5×10^6 cells in 150mm dish) and cultures in a 5% CO₂ atmosphere. The next day, 1 hour before transfection, cell were washed once with PBS pH 7.4 and the media was change to complete media without antibiotics (DMEM high glucose from ATCC containing 10% ultra low IgG fetal calf serum). For transfection, we used Polyethylenimine "Max" (PEI "Max", Polysciences) and Opti-MEM® I Reduced Serum Medium (25 ug of Fc-fusion constructs + 250 ug of PEI). After 72h incubation, the media was collected and stored at -20°C or cleared by centrifugation/filtration for purification.

[00330] EXAMPLE 8 - Jurkat cells lentivirus transduction:

[00331] Protocol#1: A 50/50 solution (2mL) of lentivirus was prepared in fresh media, supplemented with 8ug/mL of polybrene and added to a well of a 6 well plate. Jurkat E6-1 cells (ATCC, TIB-152) were pelleted at 1200 rpm for 5 min at RT and resuspended in fresh media (RPMI containing 10% fetal calf serum and 1% penicillin/streptomycin/amphotericin b). Cells were counted and add 2×10^5 cells to the well containing the virus+Polybrene solution. Incubate for 24-48h and add fresh media and/or split the cells. After 72h, start growing cells with antibiotic selection (puromycin).

[00332] Protocol#2: Jurkat E6-1 cells (ATCC, TIB-152) were pelleted at 1200 rpm for 5 min at RT and resuspended in fresh media (RPMI containing 10% fetal calf serum and 1% penicillin/streptomycin/amphotericin b) at 2.5×10^5 cells/mL. Add 2mL of cells to a 15mL sterile conical tube, add 1X of Transdux infection reagent (1X, SBI) and lentivirus. Mix gently and incubate at RT for 20 min. Centrifuge cells at 1900 rpm for 30 min at 32°C, remove supernatant, resuspend cells in 2 mL of fresh media and transfer cells to a well of a 6 well plate. Inspect cells for GFP expression after 48h.

[00333] EXAMPLE 9 - T cells lentivirus transduction:

[00334] Highly purified T cells (AllCells) were pelleted at 200xg for 5min at RT and resuspended at 1×10^6 cells/mL in fresh media (RPMI1640 containing 10% fetal calf serum and 1%

penicillin/streptomycin). Add CD3/CD28 activator Dynabeads (Thermo Fisher, 25uL for 1×10^6 cells) and seed 24 well plate with 1mL of cells and add IL2 (Thermo Fisher). Monitor cells daily and split cells if needed. The day before the transduction coat a plate with Retronectin (Takara) and store it overnight at 4°C. The next, remove the Retronectin solution add add a blocking solution (2% BSA in PBS) and incubate 30 min at RT. Remove BSA solution add add PBS until cells are ready. Collect activated T cells and resuspend them at 0.5×10^6 cells/mL in fresh media. Add 1mL of cells to the retronectin treated plate, 1mL of lentivirus solution and IL2. Cells were spinoculated by centrifugation of the pate at 1000xg for 90 min at RT. The plate was return to the incubator overnight. Next, remove 1mL of media, add 1mL of virus and repeat spinoculation. Monitor cells and split them if necessary at a density of $0.5-1 \times 10^6$ cells/mL. T cells can be used for cytokine release assay or cytotoxicity assay 48h post transduction.

[00335] EXAMPLE 10 - IL2 detection

[00336] IL-2 secretion in media was measured using a human IL-2 ELISA kit (Thermo Fisher). Plates were coated with and anti-IL-2 antibody (coating antibody, 1/100 in PBS). After overnight incubation at 4°C, the plate was wash 3 times with PBS-T and a 4% BSA solution was added to block remaining binding site on the well. After 1h at RT the plate was washed once with PBS-T and conditioned media (CM) and IL-2 standard diluted in PBS + 4% BSA, was added. After 2h at RT the plate was washed 3x with PBS-T and anti-human IL-2 (detection antibody) diluted in PBS + 4% BSA (1/100), was added. After 2h at RT the plate was washed 5x with PBS-T and Streptavidin-HRP (1/400) was added. After 30min at RT, the plate was washed 7x with PBS-T (soak 1min each wash) and .substrate solution was added. The reaction was stopped after 20min by adding the stop solution and absorbance was read at 450nm (minus absorbance at 550nm) within 30 min of stopping.

[00337] EXAMPLE 11 - IFN- γ detection

[00338] IFN- γ secretion in media was measured using a human IFN- γ ELISA kit (Biolegend). Plates were coated with and anti- IFN- γ antibody (capture antibody, 1X in coating buffer). After overnight incubation at 4°C, the plate was washed 4 times with PBS-T and blocking solution was added to block remaining binding site on the well. After 1h at RT (shaking at 500rpm) the plate was washed 4 times with PBS-T and conditioned media (CM) and IFN- γ standard, was added. After 2h at RT with shaking, the plate was washed 4 times with PBS-T and detection antibody (1x), was added. After 1h at RT with shaking, the plate was washed 4 times with PBS-T and Avidin-HRP (1x) was added. After 30min at RT with shaking, the plate was washed 5 times with PBS-T (soak 1min each wash) and TMB substrate solution was added. The reaction was stopped after 20min by adding the

stop solution and absorbance was read at 450nm (minus absorbance at 570nm) within 15 min of stopping.

[00339] EXAMPLE 12 – CAR T Cytotoxicity assay

[00340] Human T cells were isolated from whole blood according to standard protocols. The T cells were then separately transduced twice with lenti virus bearing the CAR constructs, wherein the CAR constructs bear a GFP tag. Following 2-3 days of culture in RPMI 10% FBS and IL-2, the cells were stained with F(ab')₂ to label surface expression of MN-E6, MN-C2, MN-C3 and MN-C8. Cells were then sorted by flow cytometry for Fab-positive, GFP-positive cells. That means that the double positive population had a CAR inserted and that the CAR exposed the correct antibody fragment. The CAR T cells were then ready to be mixed with the MUC1* negative control cells or the target MUC1* positive cancer cells.

[00341] The target cells were prepared as follows: Harvest target cells and resuspend cells in serum-free medium containing 15uM of CMTMr dye (Cell Tracker Orange, 5-and-6-4-chloromethyl benzoyl amino tetramethylrhodamine, Thermo Fisher) at $1-1.5 \times 10^6$ cells/mL. Incubate 30 min under growth conditions appropriate to particular cell type. Wash in culture media and transfer stained cells to a new tube and incubate the cells 60 min in media. Wash 2 more times in culture media to get rid of all excess dye. Set up the assay in 24 well plates with 0.5 ml media total volume. Resuspend the target cells (and control target cells) so that there are always 20,000 cells per well (20,000 cells/250ul). Plate 250ul in each well. Add 250ul of the T cells so that the ratio of T cell: target cells = 20:1, 10:1, 5:1 or 1:1. . Analyse cells after 24h and 72h. For suspension target cells, take off the 0.5ml media from the well and place in tube, wash the well with 0.5ml media or PBS. For adherent target cells, take off the 0.5ml media from the well and place in tube, wash the well with 0.5ml PBS. Add the PBS to the same tube and add 120ul trypsin to the well. Incubate for 4 min then add 0.5ml media to neutralize trypsin and place that in the tube as well. Spin cells and resuspend pellet in 100ul FACS buffer. Spin cells again. Resuspend cells in 100ul buffer + 5ul anti-CD3 antibody, for 30 min on ice (to stain T cells). After 30 min, wash stained cells 2x with FACS buffer and resuspend in 250ul buffer. Run the cells through the filter cap of the FACS tube. 10 min prior to analysis, add 10ul 7AAD dye to each tube and analyze with Fortessa under the Cytotoxicity template.

[00342] EXAMPLE 13 - ELISA expression level of humanized IgG

[00343] Goat Anti-human Fc specific antibody was diluted to 5ug/mL in 0.1M carbonate/bicarbonate buffer pH 9.6 and 50uL was added to each well of a 96 well plate. After overnight incubation at 4°C, the plate was wash twice with PBS-T and a 3% BSA solution was

added to block remaining binding site on the well. After 1h at RT the plate was washed twice with PBS-T and conditioned media (CM), diluted in PBS-T + 1% BSA, was added at different concentrations. Also, purified human IgG (life technologies), diluted in PBS-T + 1% BSA, was added at different concentrations to make a standard curve for determination of the expression level of the humanized IgG or Fc-fusion protein. After 1h at RT the plate was washed 3x with PBS-T and anti-human (H+L) HRP (life technologies) diluted in PBS-T + 1% BSA, was added at 1/2500. After 1h at RT the plate was washed 3x with PBS-T and binding of human IgG and humanized IgG was measured at 415nm using a ABTS solution (ThermoFisher) (Figure 9 (MN-E6) and Figure 11 (MN-C2)).

[00344] EXAMPLE 14 - ELISA humanized IgG binding to PSMGFR peptide of the MUC1* extracellular domain

[00345] A synthetic peptide of sequence PSMGFR was covalently coupled to BSA using Inject Maleimide activated BSA kit (Thermo Fisher). PSMGFR coupled BSA was diluted to 7.5ug/mL in 0.1M carbonate/bicarbonate buffer pH 9.6 and 50uL was added to each well of a 96 well plate. After overnight incubation at 4°C, the plate was wash twice with PBS-T and a 3% BSA solution was added to block remaining binding site on the well. After 1h at RT the plate was washed twice with PBS-T and conditioned media (CM), diluted in PBS-T + 1% BSA, was added at different concentrations. At the same time corresponding mouse IgG was diluted in PBS-T + 1% BSA and added at different concentrations as binding control. After 1h at RT the plate was washed 3x with PBS-T and anti-human (H+L) HRP (life technologies) diluted in PBS-T + 1% BSA, was added at 1/5000 to detect binding of humanized IgG. Anti-Mouse HRP (life technologies) diluted in PBS-T + 1% BSA, was added at 1/2500 to detect binding of mouse IgG. After 1h at RT the plate was washed 3x with PBS-T and binding was measured at 415nm using a ABTS solution (ThermoFisher) (Figure 8 (MN-E6) and Figure 10 (MN-C2)).

[00346] EXAMPLE 15 - Stable cell lines generation

[00347] CHO-K1 cells (ATCC) were used to create stable cell lines expressing high level of humanized IgG. HEK293 cells (ATCC) were used to create stable cell lines expressing high level of Fc-fusion proteins. The night before transfection, cells were passed at 1/3 dilution (6well plate) and cultures in a 5% CO₂ atmosphere. The next day, 1 hour before transfection, the media was change to complete media without antibiotics (F12K or DMEM containing 10% fetal calf serum). For transfection, we used Lipofectamine 3000 (life technologies) and Opti-MEM® I Reduced Serum Medium according to the manufacturer instructions. 1.25ug of the heavy chain construct and 1.25ug of the light chain construct or 2.5 ug of Fc-fusion constructs was used. After 24h, cells were

trypsinized and plated into a T75 flask (in F12K or DMEM containing 10% fetal calf serum). After 24h, cells were trypsinized, diluted to 100 cells/mL and 1000 cells/mL in F12K or DMEM containing 10% FCS and selection agent (Zeocin for pSECTag2 or G418 for pCDNA 3.1 V5), plated in 96 well plate (100 uL per well) and cultures in a 5% CO₂ atmosphere. After 2-3 weeks, the culture media from single clones were collected, cleared by centrifugation and used in an ELISA assay to quantify the level of humanized IgG expression and binding to PSMGFR peptide. The clones with the highest expression and PSMGFR binding were expanded for large scale expression.

[00348] EXAMPLE 16 - scFV expression:

[00349] pET21b E6 scFV plasmid (with HisTag or StrepTagII) was transformed into Shuffle T7 express competent cells (NEB). TB broth (Terrific broth) was inoculated with 1/100 of an overnight culture (LB broth-30°C-200rpm) and cultured at 30°C/200rpm. When OD₆₀₀ reached ~1, temperature was reduced to 20°C and growth was continued. After 2h, recombinant protein expression was induced with 0.2mM Isopropyl-β-D-thio-galactoside (IPTG, Gold Biotechnology) and culture was stopped after 22h. After harvesting the cells by centrifugation (6000 rpm for 10 min at 4°C), cell pellet was resuspended with running buffer. For Histag protein buffer was: 50mM Tris pH8.0, 300 mM NaCl and 5 mM imidazole. For StrepTagII protein buffer was 100mM Tris pH 8.0 and 150 mM NaCl.

[00350] EXAMPLE 17 - HisTag EscFV purification:

[00351] MgCl₂ (0.5mM), DNase (0.5 ug/mL, Sigma), PMSF (1mM, Gold Biotechnology) and BugBuster (1X, Novagen) was added. Cell suspension was incubated on a rotating platform for 20 min at RT. Insoluble cell debris was removed by centrifugation (20000 rpm for 30 min at 4°C). The cleared lysate was then applied to a Ni-NTA column (Qiagen) equilibrated with the running buffer. The column was washed before eluting the protein off the column with the running buffer supplemented with 495 mM imidazole. The protein was further purified by size exclusion chromatography (Superdex 200). The fractions containing the protein were pooled, aliquoted and stored at -80°C.

[00352] EXAMPLE 18 - StrepTagII EscFV purification:

[00353] MgCl₂ (0.5mM), DNase (0.5 ug/mL, Sigma), PMSF (1mM, Gold Biotechnology) and BugBuster (1X, Novagen) was added. Cell suspension was incubated on a rotating platform for 20 min at RT. Insoluble cell debris was removed by centrifugation (20000 rpm for 30 min at 4°C). The cleared lysate was then applied to a Strep-Tactin column (IBA) equilibrated with the running buffer. The column was washed before eluting the protein off the column with the running buffer supplemented with 5 mM d-Desthiobiotin. The protein was further purified by size exclusion

chromatography (Superdex 200). The fractions containing the protein were pooled, aliquoted and stored at -80°C

[00354] EXAMPLE 19 - Humanize IgG/Fc-fusion purification:

[00355] Condition media (from transient transfection or stable cell line) was collected, cleared by centrifugation and filtered (0.2 μ m). The media was then loaded on a protein A (Genscript) or CaptureSelect FcXL (Thermo Fisher) and the protein purified according to manufacturer instructions using acid condition for elution. The eluted protein was then dialyzed against PBS pH 7.4 and further purified by size exclusion chromatography (Superdex 200). The fractions containing the protein were pooled, aliquoted and stored at -80°C.

[00356] EXAMPLE 20 - Immunohistochemistry

[00357] Human tissue specimens were purchased from Biomax. The tissues were either normal or cancerous as determined by a board certified pathologist. Tissues were anonymized but were labeled with a number, tissue type, stage of cancer and if available, a TNM tumor grading designation. TNM grading is as follows: T is primary tumor. Tx is primary tumor cannot be assessed. T0 is no evidence of a tumor. This is carcinoma in situ, intraepithelial or invasion of lamina propria. T1 is tumor invades submucosa. T2 is tumor invades muscularis propria. T3 is tumor invades through muscularis propria into subserosa or into non-peritonealized pericolic or perirectal tissues. T4 is tumor directly invades other organs or structures and/or perforate visceral peritoneum. N is regional lymph nodes. N0 is no regional lymph node metastasis. N1 is metastasis in 1 to 3 regional lymph nodes. N2 is metastatic in 4 or more regional lymph nodes. M is for distant metastasis. M0 means no distant metastasis. M1 is distant metastasis.

[00358] Tissues were stained with a primary anti-MUC1* antibody mouse monoclonal MN-C2, MN-E6, humanized MN-E6 scFv-Fc, or humanized MN-E6 scFv-Fc-biotin. If the primary were a mouse monoclonal antibody, then the secondary antibody used was a rabbit anti-mouse HRP-conjugated antibody. If the primary were a humanized antibody, then the secondary was a goat-anti-human HRP conjugated antibody. If the primary were a biotinylated antibody, then the secondary was a streptavidin HRP conjugated antibody.

[00359] Tissue specimens were de-paraffinized using xylene and ethanol according to standard protocols. An antigen retrieval procedure was used for some tissues which involved 10mM Sodium Citrate-0.05% Tween pH 6 buffer (pre boil buffer, keep warm) boil 10', cool down 20' in rice cooker, then rinse cold tap water 5 minutes then two 5 min. washes in TBS. Tissues were blocked for 1 hr at RT in 10%NGS plus 5% BSA in TBS. If the primary antibody used was humanized MN-E6scFv, which was conjugated to biotin so that it could be visualized by a secondary antibody, the tissues

were pre-blocked with an avidin solution then a biotin solution. Primary antibodies were incubated with tissues overnight at 4 degrees C in 1% BSA-TBS with gentle orbital rotation. Tissues were rinsed with TBS-T for 5 minutes with gentle rocking. For HRP-conjugate detection only, mounted tissues were incubated in 3% H₂O₂ in TBS for 15 minutes at RT. For tissues incubated with biotinylated primary antibodies, they were then bathed in StreptAvidin for 10 min with Streptavidin-HRP label (Biocare Cat#: HP604 G, H, L), then washed 3 times for 5 minutes at RT in TBS-T with gentle rocking. They were then developed with chromogen (DAB - 1mL diluent; 1 drop DAB substrate) for 5 minutes at RT, then rinsed with running tap water for 5 minutes. They were then counterstained for 1 second hematoxylin then brief dip in 0.08% NH₄OH 'bluing reagent' followed by 5 minutes in running water. Tissues were then dehydrated and mounted with Cytoseal XYL (1 drop/section) and coverslipped.

[00360] All of the references cited herein are incorporated by reference in their entirety.

* * * * *

[00361] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention specifically described herein.

Claims:

1. A human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein that binds to a region on extracellular domain of MUC1 isoform or cleavage product that is devoid of the tandem repeat domains.
2. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein of claim 1, which specifically binds to
 - (i) PSMGFR region of MUC1;
 - (ii) PSMGFR peptide;
 - (iii) a peptide having amino acid sequence of
SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620);
 - (iv) a peptide having amino acid sequence of
SVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621);
 - (v) a peptide having amino acid sequence of VQLTLAFREGTINVHDTVETQFNQY (SEQ ID NO:622); or
 - (vi) a peptide having amino acid sequence of SNIKFRPGSVVVQLTLAFREGTIN (SEQ ID NO:623).
3. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein of claim 1, wherein the human or humanized antibody is IgG1, IgG2, IgG3, IgG4 or IgM.
4. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein of claim 1, wherein the human or humanized antibody fragment or antibody-like protein is scFv or scFv-Fc.
5. The human or humanized antibody, antibody fragment or antibody-like protein according to claim 1, which comprises a heavy chain variable region and light chain variable region which is derived from mouse monoclonal MN-E6 antibody, and has at least 80%, 90% or 95% or 98% sequence identity to the mouse monoclonal MN-E6 antibody.
6. The human or humanized antibody, antibody fragment or antibody-like protein according to claim 5, wherein the heavy chain variable region has at least 90% or 95% or 98% sequence identity

to SEQ ID NO:13 and the light chain variable region has at least 90% or 95% or 98% sequence identity to SEQ ID NO:66.

7. The human or humanized antibody, antibody fragment or antibody-like protein according to claim 5, comprising complementarity determining regions (CDRs) in the heavy chain variable region and light chain variable region having at least 90% or 95% or 98% sequence identity to CDR1, CDR2 or CDR3 regions having sequence as follows:

CDR1 heavy chain SEQ ID NO:17

CDR1 light chain SEQ ID NO:70,

CDR2 heavy chain SEQ ID NO:21

CDR2 light chain SEQ ID NO:74,

CDR3 heavy chain SEQ ID NO:25

CDR3 light chain SEQ ID NO:78.

8. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein of claim 5, wherein the human or humanized antibody is IgG1, IgG2, IgG3, IgG4 or IgM.

9. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein of claim 5, wherein the human or humanized antibody fragment or antibody-like protein is scFv or scFv-Fc.

10. The human or humanized antibody, antibody fragment or antibody-like protein according to claim 1, which comprises a heavy chain variable region and light chain variable region which is derived from mouse monoclonal MN-C2 antibody, and has at least 80%, 90% or 95% or 98% sequence identity to the mouse monoclonal MN-C2 antibody.

11. The human or humanized antibody, antibody fragment or antibody-like protein according to claim 10, wherein the heavy chain variable region has at least 90% or 95% or 98% sequence identity to SEQ ID NO:119 and the light chain variable region has at least 90% or 95% or 98% sequence identity to SEQ ID NO:169.

12. The antibody according to claim 10, comprising complementarity determining regions (CDRs) in the heavy chain variable region and light chain variable region having at least 90% or 95% or 98% sequence identity to CDR1, CDR2 or CDR3 regions having sequence as follows:

CDR1 heavy chain SEQ ID NO:123

CDR1 light chain SEQ ID NO:173,

CDR2 heavy chain SEQ ID NO:127

CDR2 light chain SEQ ID NO:177,

CDR3 heavy chain SEQ ID NO:131

CDR3 light chain SEQ ID NO:181.

13. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein of claim 10, wherein the human or humanized antibody is IgG1, IgG2, IgG3, IgG4 or IgM.

14. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein of claim 10, wherein the human or humanized antibody fragment or antibody-like protein is scFv or scFv-Fc.

15. The human or humanized antibody, antibody fragment or antibody-like protein according to claim 1, which comprises a heavy chain variable region and light chain variable region which is derived from mouse monoclonal MN-C3 antibody, and has at least 80%, 90% or 95% or 98% sequence identity to the mouse monoclonal MN-C3 antibody.

16. The human or humanized antibody, antibody fragment or antibody-like protein according to claim 15, wherein the heavy chain variable region has at least 90% or 95% or 98% sequence identity to SEQ ID NO:414 and the light chain variable region has at least 90% or 95% or 98% sequence identity to SEQ ID NO:459.

17. The antibody according to claim 15, comprising complementarity determining regions (CDRs) in the heavy chain variable region and light chain variable region having at least 90% or 95% or 98% sequence identity to CDR1, CDR2 or CDR3 regions having sequence as follows:

CDR1 heavy chain SEQ ID NO:418

CDR1 light chain SEQ ID NO:463,

CDR2 heavy chain SEQ ID NO:422

CDR2 light chain SEQ ID NO:467,
CDR3 heavy chain SEQ ID NO:426,
CDR3 light chain SEQ ID NO:471.

18. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein of claim 15, wherein the human or humanized antibody is IgG1, IgG2, IgG3, IgG4 or IgM.

19. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein of claim 15, wherein the human or humanized antibody fragment or antibody-like protein is scFv or scFv-Fc.

20. The human or humanized antibody, antibody fragment or antibody-like protein according to claim 1, which comprises a heavy chain variable region and light chain variable region which is derived from mouse monoclonal MN-C8 antibody, and has at least 80%, 90% or 95% or 98% sequence identity to the mouse monoclonal MN-C8 antibody.

21. The human or humanized antibody, antibody fragment or antibody-like protein according to claim 20, wherein the heavy chain variable region has at least 90% or 95% or 98% sequence identity to SEQ ID NO:506 and the light chain variable region has at least 90% or 95% or 98% sequence identity to SEQ ID NO:544.

22. The antibody according to claim 20, comprising complementarity determining regions (CDRs) in the heavy chain variable region and light chain variable region having at least 90% or 95% or 98% sequence identity to CDR1, CDR2 or CDR3 regions having sequence as follows:

CDR1 heavy chain SEQ ID NO:508
CDR1 light chain SEQ ID NO:546,
CDR2 heavy chain SEQ ID NO:510
CDR2 light chain SEQ ID NO:548,
CDR3 heavy chain SEQ ID NO:512,
CDR3 light chain SEQ ID NO:550.

23. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein of claim 20, wherein the human or humanized antibody is IgG1, IgG2, IgG3, IgG4 or IgM.

24. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein of claim 20, wherein the human or humanized antibody fragment or antibody-like protein is scFv or scFv-Fc.

25. An anti-MUC1* extracellular domain antibody comprised of sequences of a humanized MN-E6 represented by humanized IgG2 heavy chain, or humanized IgG1 heavy chain, paired with humanized Kappa light chain, or humanized Lambda light chain.

26. The antibody according to claim 25, wherein the humanized IgG2 heavy chain is SEQ ID NOS:53, humanized IgG1 heavy chain is SEQ ID NO:57, humanized Kappa light chain is SEQ ID NO:108, and humanized Lambda light chain is SEQ ID NO:112, or a sequence having 90%, 95% or 98% sequence identity thereof.

27. An anti-MUC1* extracellular domain antibody comprised of sequences of a humanized MN-C2 represented by humanized IgG1 heavy chain, humanized IgG2 heavy chain, paired with humanized Lambda light chain, and humanized Kappa light chain.

28. The antibody according to claim 27, wherein the humanized IgG1 heavy chain MN-C2 (SEQ ID NOS:159) or IgG2 heavy chain (SEQ ID NOS:164) paired with Lambda light chain (SEQ ID NO:219) or Kappa light chain (SEQ ID NO:213), or a sequence having 90%, 95% or 98% sequence identity thereof.

29. An anti-MUC1* extracellular domain antibody comprised of sequences of a humanized MN-C3 represented by humanized IgG1 heavy chain or humanized IgG2 heavy chain paired with humanized Lambda light chain or humanized Kappa light chain.

30. The antibody according to claim 29, wherein the humanized MN-C3 IgG1 heavy chain is SEQ ID NOS:454, IgG2 heavy chain is SEQ ID NOS:456, Lambda light chain is SEQ ID NO:501, and Kappa light chain is SEQ ID NO:503, or a sequence having 90%, 95% or 98% sequence identity thereof.

31. An anti-MUC1* extracellular domain antibody comprised of sequences of a humanized MN-C8 represented by humanized IgG1 heavy chain or humanized IgG2 heavy chain paired with humanized Lambda light chain or humanized Kappa light chain.
32. The antibody according to claim 31, wherein the humanized MN-C8 IgG1 heavy chain is SEQ ID NOS:540, IgG2 heavy chain is SEQ ID NOS:542, Lambda light chain is SEQ ID NO:580 and Kappa light chain is SEQ ID NO:582, or a sequence having 90%, 95% or 98% sequence identity thereof.
33. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein according to claim 1, which inhibits the binding of NME protein to MUC1*.
34. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein according to claim 33, wherein the NME is NME7 or NME1.
35. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein according to claim 33, wherein the NME is NME6 or NME8.
36. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein of claim 33, wherein the human or humanized antibody is IgG1, IgG2, IgG3, IgG4 or IgM.
37. The human or humanized anti-MUC1* antibody or antibody fragment or antibody-like protein of claim 33, wherein the human or humanized antibody fragment or antibody-like protein is scFv or scFv-Fc.
38. A single chain variable fragment (scFv) comprising a heavy and light chain variable regions connected via a linker, further comprising CDRs of antibodies that bind to MUC1* extracellular domain.
39. The scFv according to claim 38, wherein the CDRs of the anti-MUC1* antibody are derived from the MN-E6, MN-C2, MN-C3 or MN-C8 antibodies.

40. The scFv according to claim 39, wherein the CDRs of the anti-MUC1* antibody are derived from humanized MN-E6, MN-C2, MN-C3 or MN-C8 antibodies.
41. A scFv according to claim 38, selected from the group of SEQ ID NOS:233, 235 and 237 (E6).
42. A scFv according to claim 38, selected from the group of SEQ ID NOS:239, 241, and 243 (C2).
43. A scFv according to claim 38, selected from the group of SEQ ID NOS:245, 247, and 249 (C3).
44. A scFv according to claim 38, selected from the group of SEQ ID NOS:251, 253, and 255 (C8).
45. A chimeric antigen receptor (CAR) comprising a scFv or a humanized variable region that binds to the extracellular domain of a MUC1 that is devoid of tandem repeats, a linker molecule, a transmembrane domain and a cytoplasmic domain.
46. The CAR according to claim 45, wherein the single chain antibody fragment binds to
- (i) PSMGFR region of MUC1,
 - (ii) PSMGFR peptide,
 - (iii) a peptide having amino acid sequence
SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620);
 - (iv) a peptide having amino acid sequence of
SVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621);
 - (v) a peptide having amino acid sequence of VQLTLAFREGTINVHDTVETQFNQY (SEQ ID NO:622); or
 - (vi) a peptide having amino acid sequence of SNIKFRPGSVVVQLTLAFREGTIN (SEQ ID NO:623).
47. The CAR according to claim 46, comprising portions of any of the variable regions set forth in claims 25 to 32 or combination thereof in the extracellular domain, a transmembrane region and a cytoplasmic tail that comprises sequence motifs that signal immune system activation.

48. The CAR as in Claim 45, in which the extracellular domain is comprised of humanized single chain antibody fragments of an MN-E6 scFv, MN-C2 scFv, MN-C3 scFv or MN-C8 scFv.
49. The CAR as in Claim 48, in which the extracellular domain is comprised of humanized single chain antibody fragments of an MN-E6 scFv set forth as SEQ ID NOS: 233, 235, or 237), MN-C2 scFv (SEQ ID NOS:239, 241, or 243), MN-C3 scFv (SEQ ID NOS: 245, 247, or 249) or MN-C8 scFv (SEQ ID NOS:251, 253, or 255).
50. The CAR as in Claim 45, in which the cytoplasmic tail is comprised of one or more of signaling sequence motifs CD3-zeta, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICAM-1, LFA-1, ICOS, CD2, CD5, or CD7.
51. The CAR as in Claim 45, wherein its sequence is CARMN-E6 CD3z (SEQ ID NOS:295), CARMN-E6 CD28/CD3z (SEQ ID NOS:298); CARMN-E6 4-1BB/CD3z (SEQ ID NOS:301); CARMN-E6 OX40/CD3z (SEQ ID NOS:617); CARMN-E6 CD28/4-1BB/CD3z (SEQ ID NOS:304); CARMN-E6 CD28/OX40/CD3z (SEQ ID NOS:619); CAR MN-C2 CD3z (SEQ ID NOS:607); CAR MN-C2 CD28/CD3z (SEQ ID NOS:609); CAR MN-C2 4-1BB/CD3z (SEQ ID NOS:611); CAR MN-C2 OX40/CD3z (SEQ ID NOS:613); CAR MN-C2 CD28/4-1BB/CD3z (SEQ ID NOS:307); or CAR MN-C2 CD28/OX40/CD3z (SEQ ID NOS:615).
52. A cell comprising a CAR with an extracellular domain that binds to MUC1* transfected or transduced cell.
53. The cell according to claim 52, wherein the cell comprising the CAR is immune system cell.
54. The cell according to claim 53, wherein the immune system cell comprising the CAR is T cell.
55. The cell according to claim 53, wherein the immune system cell comprising the CAR is dendritic cell.
56. The cell according to claim 53, wherein the immune system cell comprising the CAR is mast cell.

57. A CAR molecule in which the extracellular domain unit recognizes a peptide.
58. The CAR molecule according to claim 57, wherein the peptide is PSMGFR (SEQ ID NO:2).
59. The CAR molecule according to claim 57, wherein the peptide is a peptide derived from NME7.
60. The CAR molecule according to claim 59, wherein the peptide is
NME7A peptide 1 (A domain): MLSRKEALDFHVDHQS (SEQ ID NO:7);
NME7A peptide 2 (A domain): SGVARTDASES (SEQ ID NO:8);
NME7B peptide 1 (B domain): DAGFEISAMQMFNMDRVNVE (SEQ ID NO:9);
NME7B peptide 2 (B domain): EVYKGVVTEYHDMVTE (SEQ ID NO:10); or
NME7B peptide 3 (B domain): AIFGKTKIQNAVHCTDLPEDGLLEVQYFF (SEQ ID NO:11).
61. A composition comprising at least two CARs with different extracellular domain units transfected into the same cell.
62. A composition comprising at least two CARs with different extracellular domain units transfected into the same cell, wherein one CAR does not have a targeting recognition unit and the other CAR does have a targeting recognition unit.
63. The composition comprising at least two CARs according to claim 61, wherein one of the extracellular domain recognition units binds to MUC1* extracellular domain.
64. The composition comprising at least two CARs according to claim 61, wherein one of the extracellular domain recognition units binds PD-1.
65. The composition comprising at least two CARs according to claim 61, wherein one of the extracellular domain recognition units is an antibody fragment and the other is a peptide.
66. The composition comprising at least two CARs according to claim 61, wherein one is an anti-MUC1* scFv chosen from the group consisting of scFv of MN-E6 antibody, scFv of MN-C2

antibody, scFv of MN-C3 antibody or scFv of MN-C8 antibody and the other is a peptide derived from NME7 or chosen from the group consisting of

NME7A peptide 1 (A domain): MLSRKEALDFHVDHQS (SEQ ID NO:7);

NME7A peptide 2 (A domain): SGVARTDASES (SEQ ID NO:8);

NME7B peptide 1 (B domain): DAGFEISAMQMFNMDRVNVE (SEQ ID NO:9);

NME7B peptide 2 (B domain): EVYKGVVTEYHDMVTE (SEQ ID NO:10); and

NME7B peptide 3 (B domain): AIFGKTKIQNAVHCTDLPEDGLLEVQYFF (SEQ ID NO:11).

67. The antibody according to claim 1, which is an engineered antibody-like protein.

68. A method of screening a library of antibodies or antibody fragments that are human, for those that bind to

(i) PSMGFR peptide;

(ii) a peptide having amino acid sequence

SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620);

(iii) a peptide having amino acid sequence of

SVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621);

(iv) a peptide having amino acid sequence of VQLTLAFREGTINVHDTVETQFNQY (SEQ ID NO:622);

(v) a peptide having amino acid sequence of SNIKFRPGSVVVQLTLAFREGTIN (SEQ ID NO:623);

(vi) NME7 protein; or

(vii) a peptide fragment of NME7 protein.

69. A method for treating a disease in a subject comprising administering an antibody according to any claim above, to a person suffering from the disease, wherein the subject expresses MUC1 aberrantly.

70. The method according to claim 69, wherein the disease is cancer.

71. A method for treating a disease in a subject comprising administering an NME peptide, to a person suffering from the disease, wherein the subject expresses MUC1 aberrantly.

72. A method of proliferating or expanding stem cell population comprising contacting the cells with the antibody according to any claim above.
73. A method of facilitating stem cell attachment to a surface comprising coating the surface with a humanized MN-C3 or MN-C8 antibody, antibody fragment or single chain antibody thereof and contacting stem cell to the surface.
74. A method of delivering stem cell *in vitro* or *in vivo* comprising the steps of coating a surface with a humanized MN-C3 or MN-C8 antibody, antibody fragment or single chain antibody thereof, contacting the stem cell to the surface and delivering the stem cell to a specific location.
75. A method of isolating stem cell comprising the steps of coating a surface with a humanized MN-C3 or MN-C8 antibody, antibody fragment or single chain antibody thereof, and contacting a mixed population of cells to the surface and isolating stem cell.
76. A scFv comprising variable domain fragments derived from an antibody that binds to a extracellular domain of MUC1 isoform or cleavage product that is devoid of the tandem repeat domains.
77. The scFv of claim 75, wherein the variable domain fragments are derived from mouse monoclonal antibody MN-E6 (SEQ ID NO:13 and 66) or from the humanized MN-E6 (SEQ ID NO: 39 and 94), or from MN-E6 scFv (SEQ ID NO: 233, 235 and 237).
78. The scFv of claim 75, wherein the variable domain fragments are derived from mouse monoclonal antibody MN-C2 (SEQ ID NO: 119 and 169) or from the humanized MN-C2 (SEQ ID NO: 145 and 195), or from MN-C2 scFv (SEQ ID NO: 239, 241 and 243).
79. The scFv of claim 75, wherein the variable domain fragments are derived from mouse monoclonal antibody MN-C3 (SEQ ID NO: 414 and 459) or from the humanized MN-C3 (SEQ ID NO: 440 and 487), or from MN-C3 scFv (SEQ ID NO: 245, 247 and 249).

80. The scFv of claim 75, wherein the variable domain fragments are derived from mouse monoclonal antibody MN-C8 (SEQ ID NO: 505 and 544) or from the humanized MN-C8 (SEQ ID NO: 526 and 566), or from MN-C8 scFv (SEQ ID NO: 251, 253, 255).
81. A method for the treatment of a person diagnosed with, suspected of having or at risk of developing a MUC1 Or MUC1* positive cancer involving administering to the person an effective amount of the scFv described in any of the Claims 76 to 80.
82. A scFv-Fc construct comprising the scFv according to claims 76 to 80.
83. The scFv-Fc construct according to claim 82, which is dimerized.
84. The scFv-Fc construct according to claim 82, wherein the Fc component is mutated so as that scFv-Fc is monomeric.
85. The scFv-Fc construct according to claim 84, wherein the mutation comprises mutating or deleting hinge region on Fc, making F405Q, Y407R, T366W/L368W, and T364R/L368R mutation or combinations thereof on the Fc represented by SEQ ID NO: 281, 279, 285 and 287.
86. A polypeptide comprising at least two different scFv sequences, wherein one of the scFv sequences is a sequence that binds to extracellular domain of MUC1 isoform or cleavage product that is devoid of the tandem repeat domains.
87. The polypeptide according to claim 86, wherein the polypeptide binds to
- (i) PSMGFR region of MUC1;
 - (ii) PSMGFR peptide;
 - (iii) peptide having amino acid sequence of
SNIKFRPGSVVVQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:620);
 - (iv) a peptide having amino acid sequence of
VQLTLAFREGTINVHDTVETQFNQYKTEAASRY (SEQ ID NO:621);
 - (v) a peptide having amino acid sequence of VQLTLAFREGTINVHDTVETQFNQY
(SEQ ID NO:622); or

(vi) a peptide having amino acid sequence of SNIKFRPGSVVVQLTLAFREGTIN (SEQ ID NO:623).

88. The polypeptide according to claim 86, wherein the polypeptide binds to a receptor on an immune cell.

89. The polypeptide according to claim 88, wherein the polypeptide binds to a receptor on a T-cell.

90. The polypeptide according to claim 89, wherein the polypeptide binds to CD3 on T-cell.

91. A method of detecting presence of a cell that expresses MUC1* aberrantly, comprising contacting a sample of cells with the scFv-Fc according to claim 82 and detecting for the presence of the binding of scFv-Fc to the cell.

92. The method according to claim 91, wherein the cell is cancer cell.

93. A method for testing a subject's cancer for suitability of treatment with a composition comprising portions of the variable regions of MN-E6, MN-C2, MN-C3 or MN-C8, comprising the steps of contacting a bodily specimen from the patient with the corresponding MN-E6 scFv-Fc, MN-C3 scFv-Fc, MN-C3 scFv-Fc or MN-C8 scFv-Fc.

94. A method of treating a subject suffering from a disease comprising,
exposing T cells from the subject to MUC1* peptides wherein through various rounds of maturation, T cells develop MUC1* specific receptors, creating adapted T cells,
expanding and administering the adapted T cells to the donor patient who is diagnosed with, suspected of having, or is at risk of developing a MUC1* positive cancer.

95. A method for treating a patient diagnosed with, suspected of having, or at risk of developing a MUC1 positive or MUC1* positive cancer, comprising administering an effective amount of immune cells that have been transduced with a MUC1* targeting CAR.

96. The method according to claim 95, wherein the immune cells are T cells isolated from a patient, which are then transduced with CARs wherein the targeting head of the CAR binds to MUC1*, and after expansion of transduced T cells, the CAR T cells are administered in an effective amount to the patient.

97. The method according to claim 95, wherein the immune cells are T cells isolated from a patient, which are then transduced with CARs wherein the targeting head of the CAR comprises portions of huMN-E6, huMN-C2, huMN-C3 or huMN-C8, and after optional expansion of transduced T cells, the CAR T cells are administered in an effective amount to the patient.

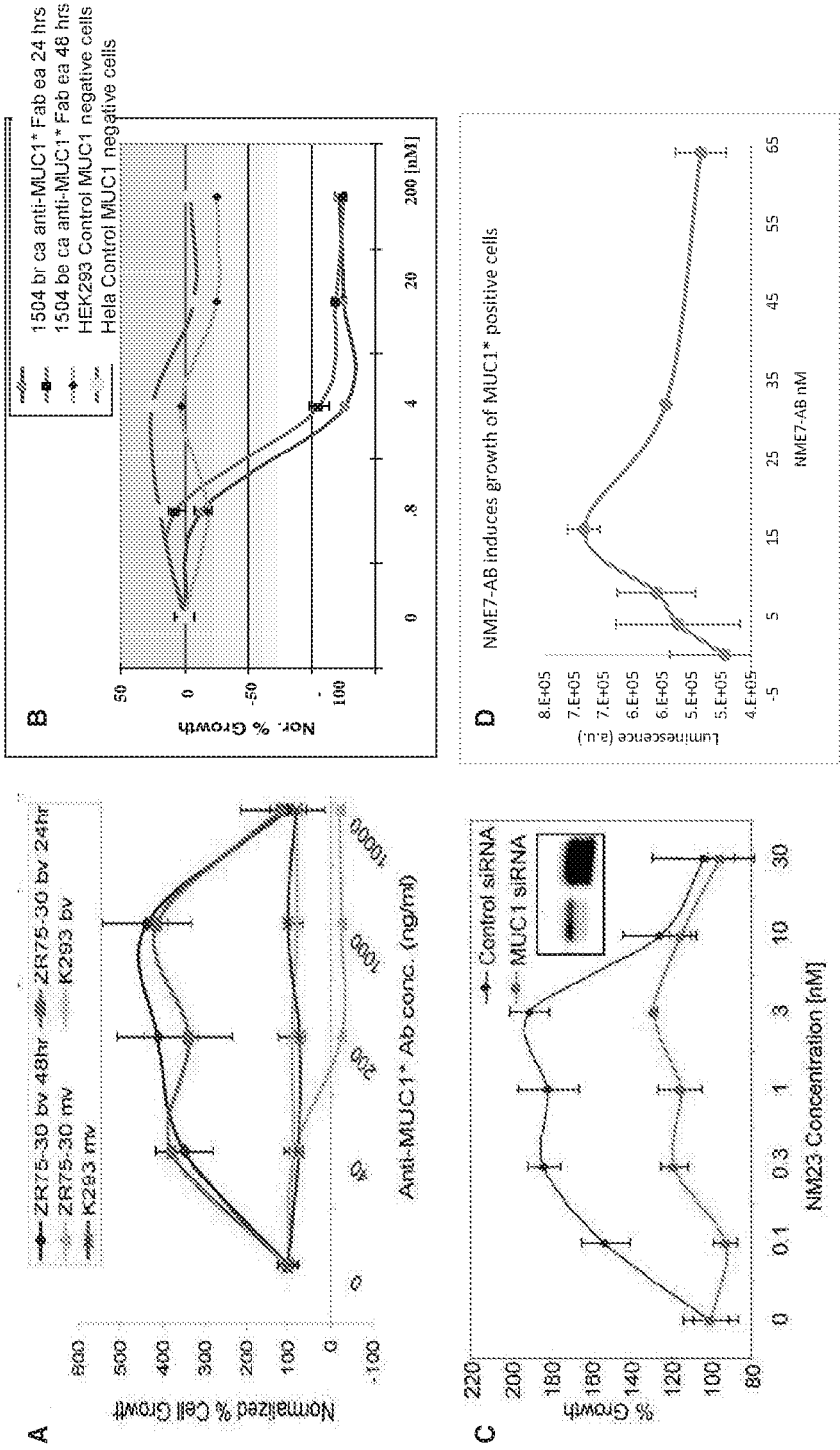
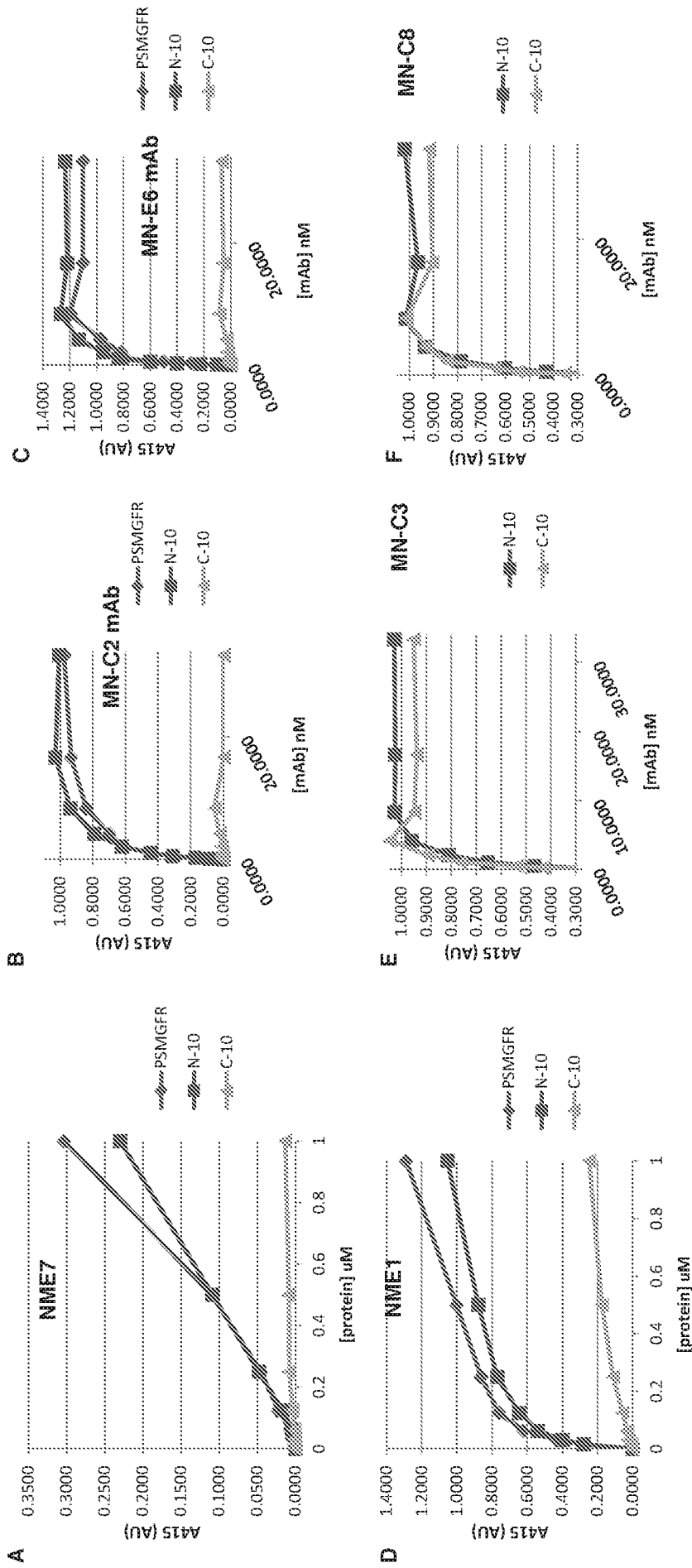


Figure 1



PSMGFR GTINVHDTVETQFNQYKTEAASRYNLTISDVSVSDVPFPFSAQSGA
PSMGFR N-10 QFNQYKTEAASRYNLTISDVSVSDVPFPFSAQSGA
PSMGFR C-10 GTINVHDTVETQFNQYKTEAASRYNLTISDVSVSDV

Figure 2

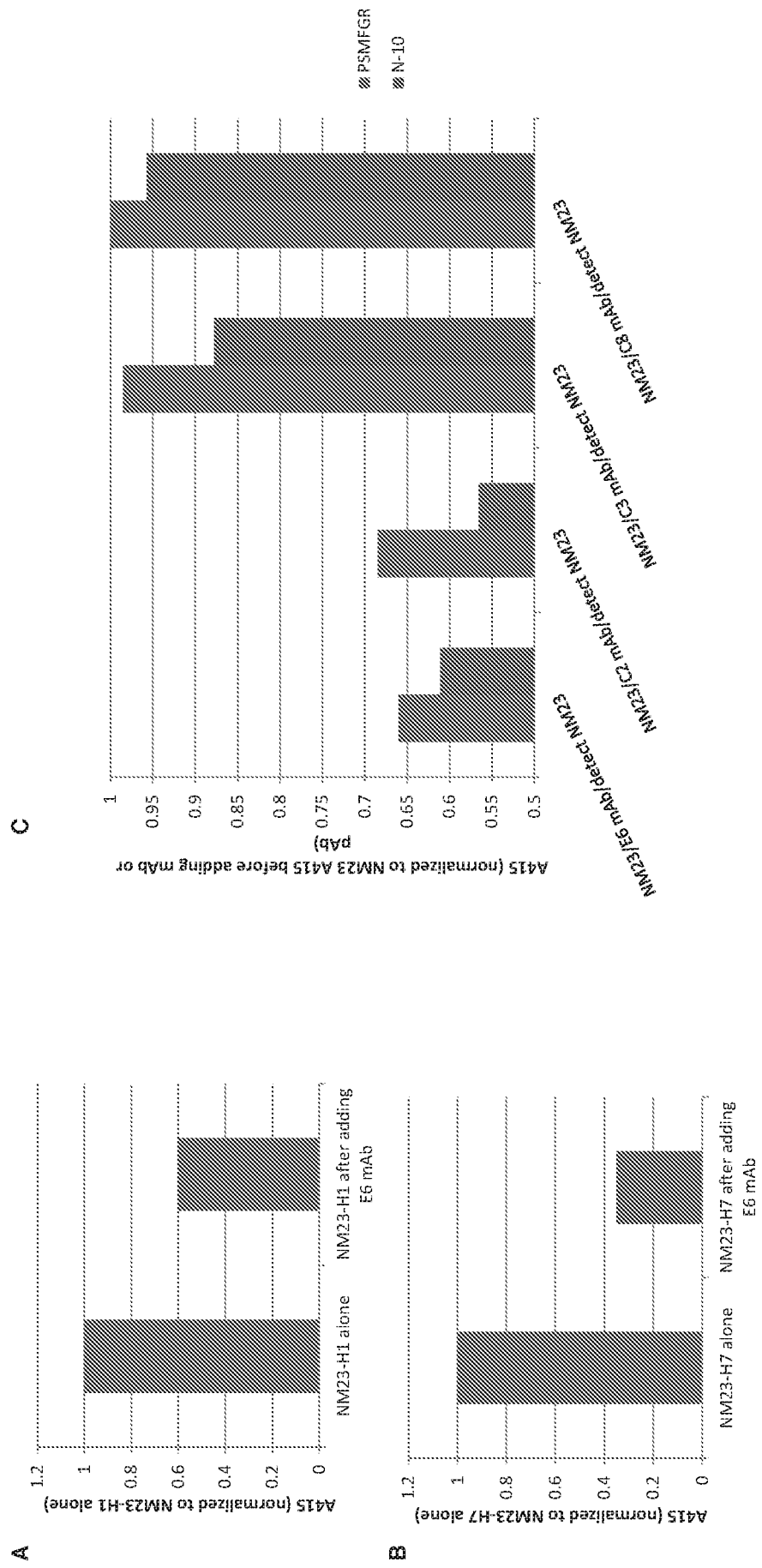


Figure 3

Humanized MN-C2 scFv binds to ZR-75-1, aka 1500, MUC1* positive breast cancer cells

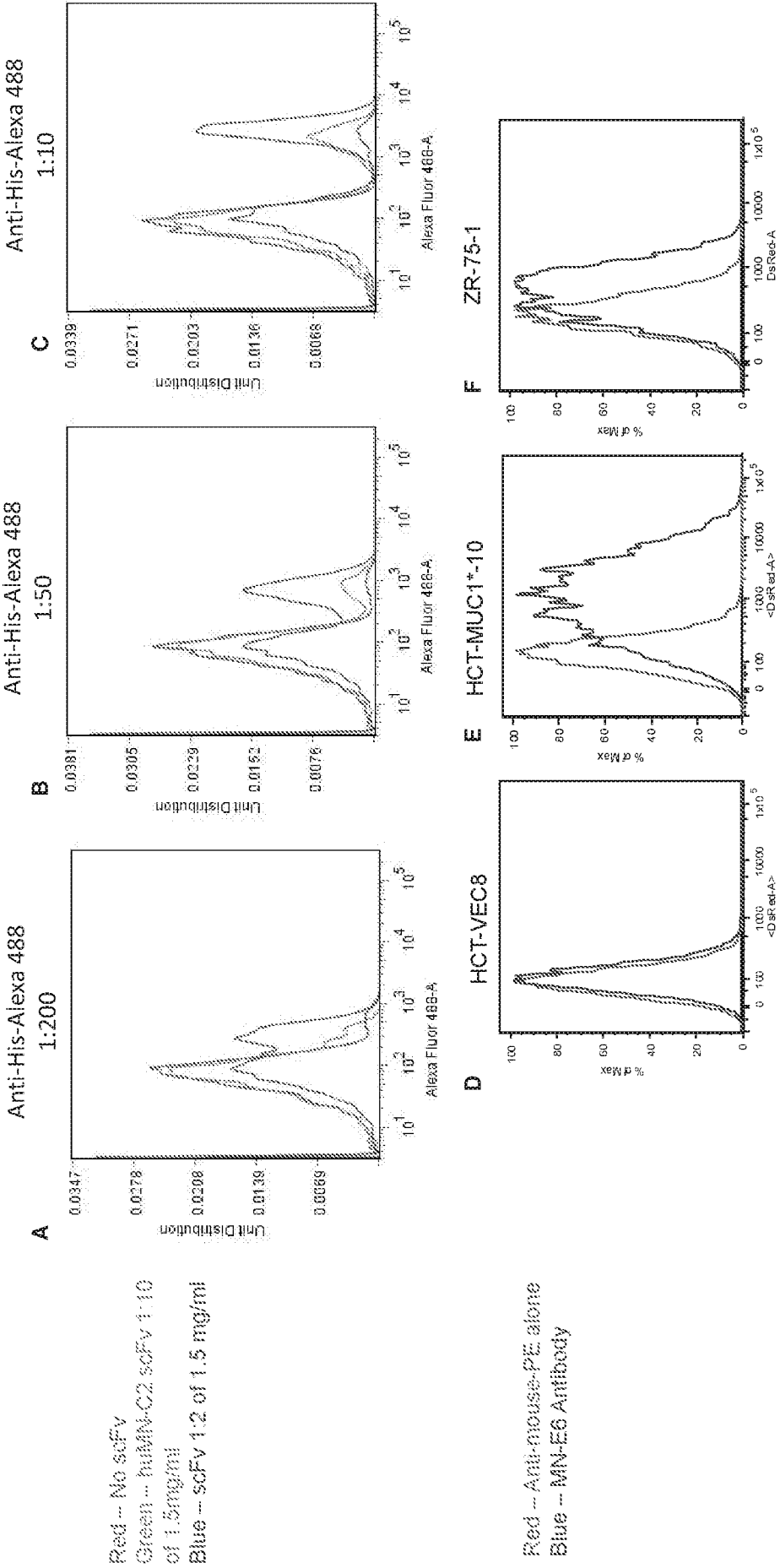


Figure 4

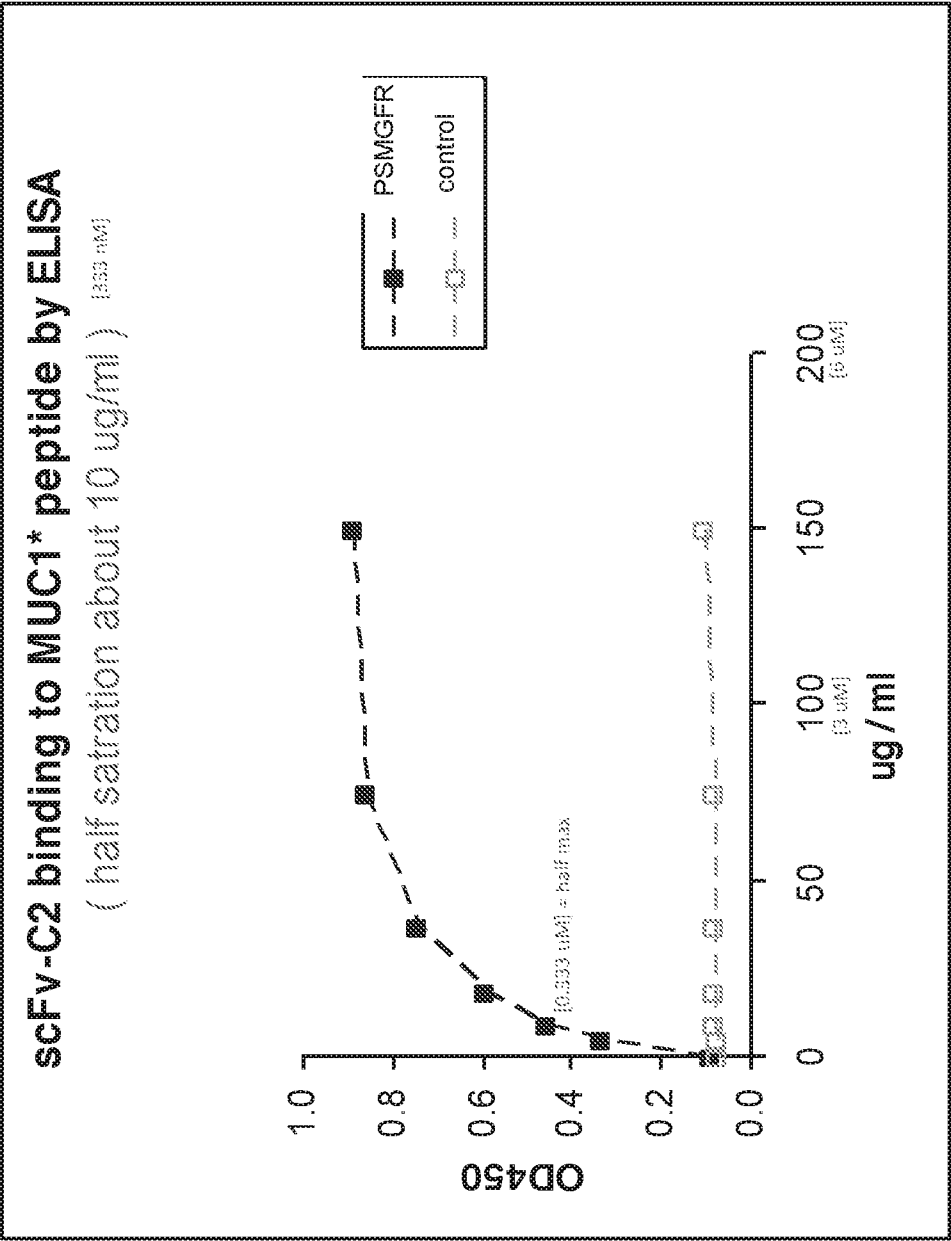


Figure 5

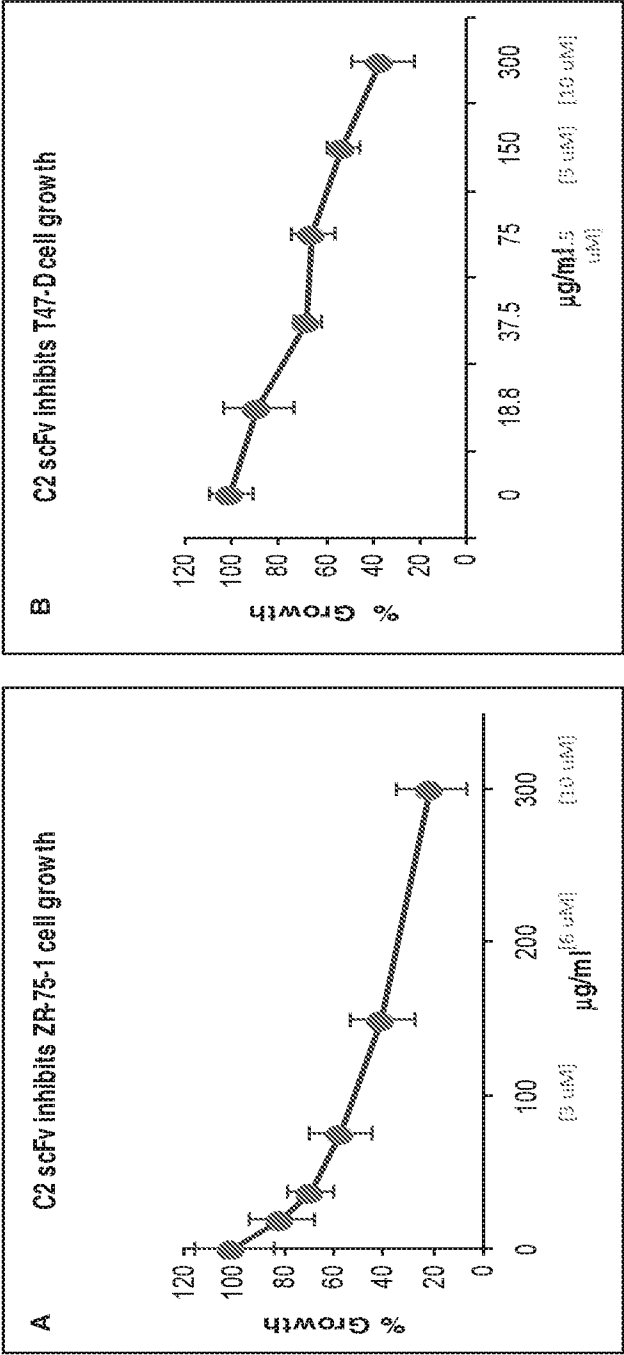


Figure 6

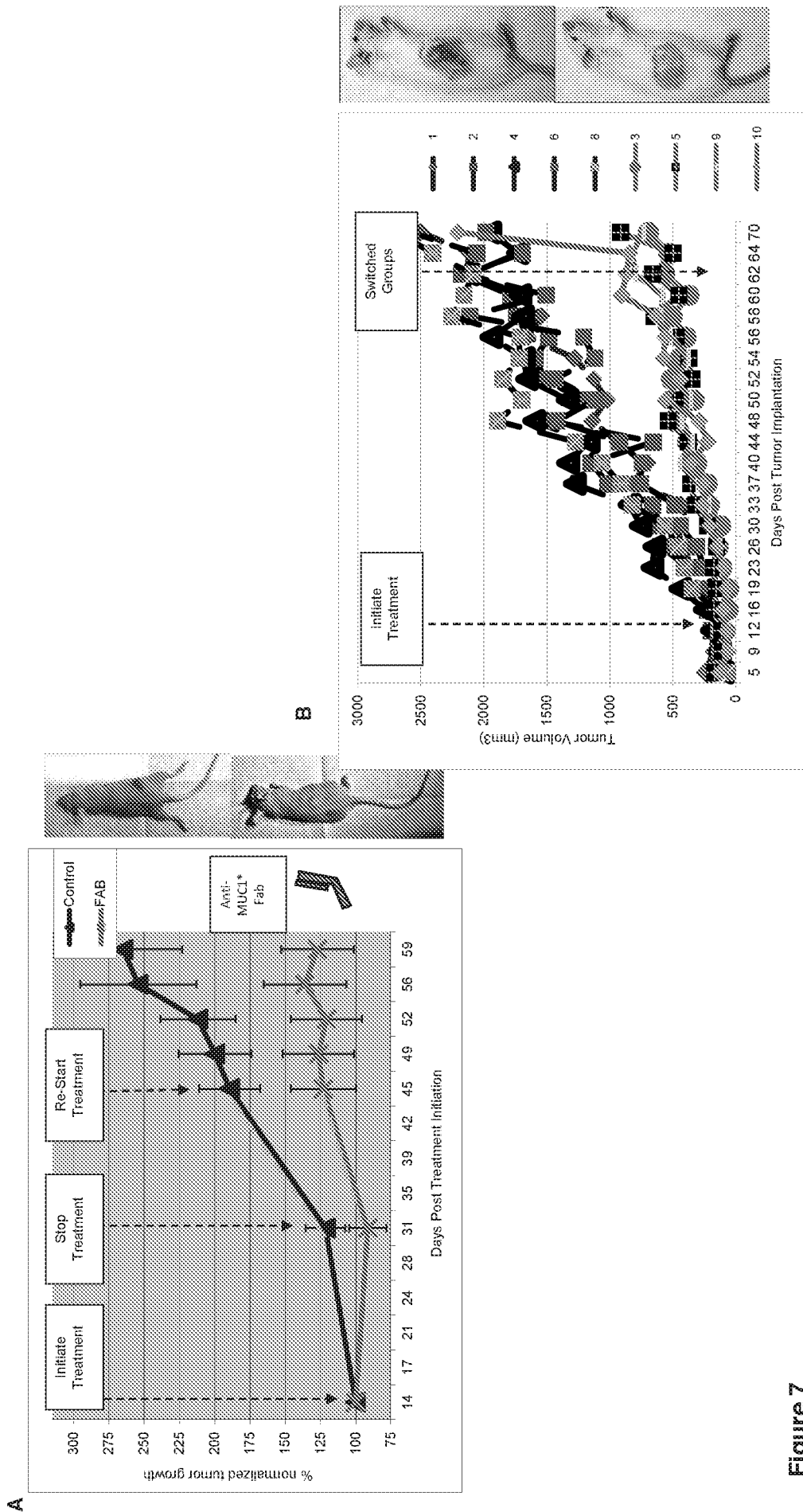


Figure 7

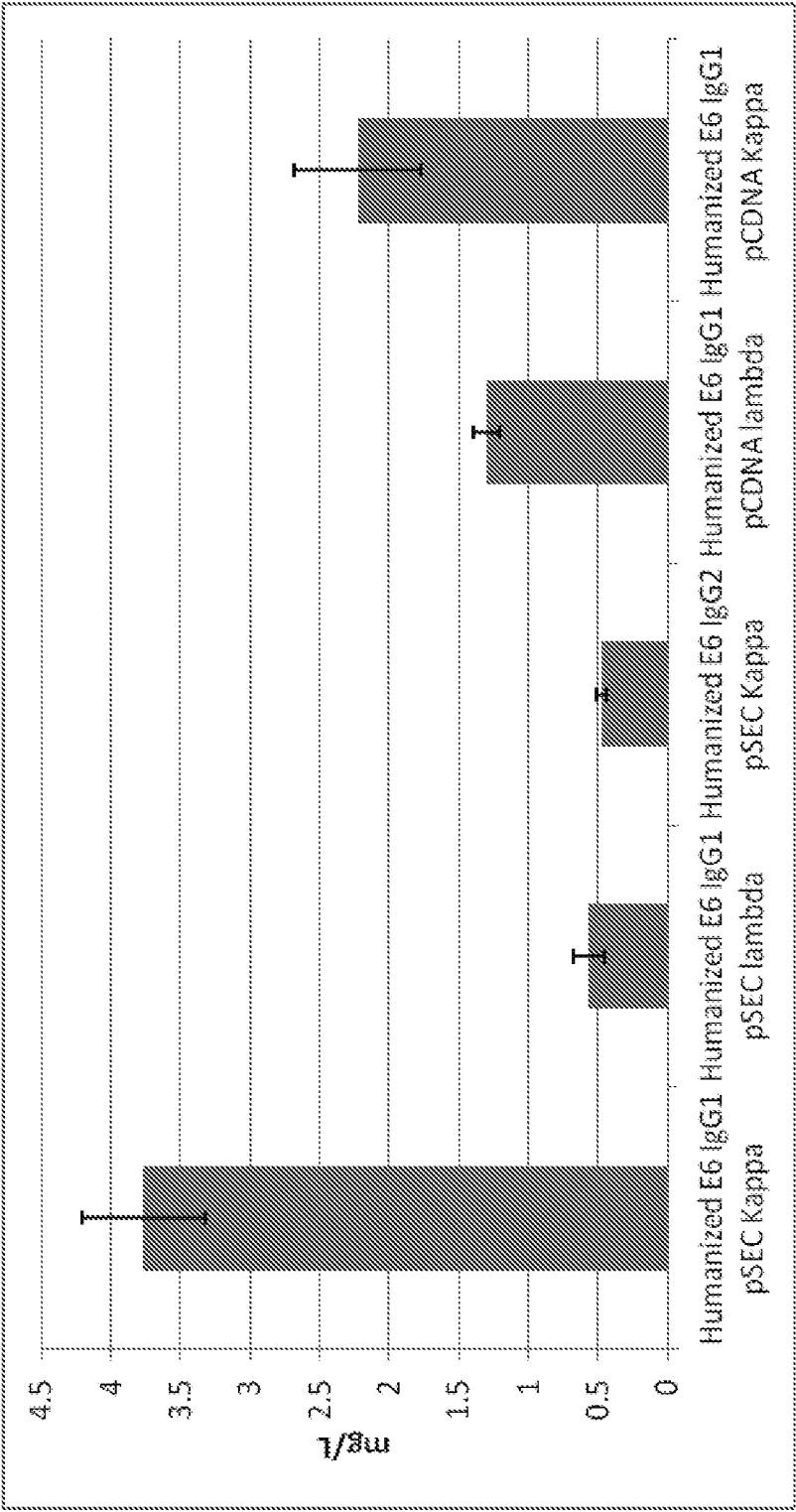


Figure 8

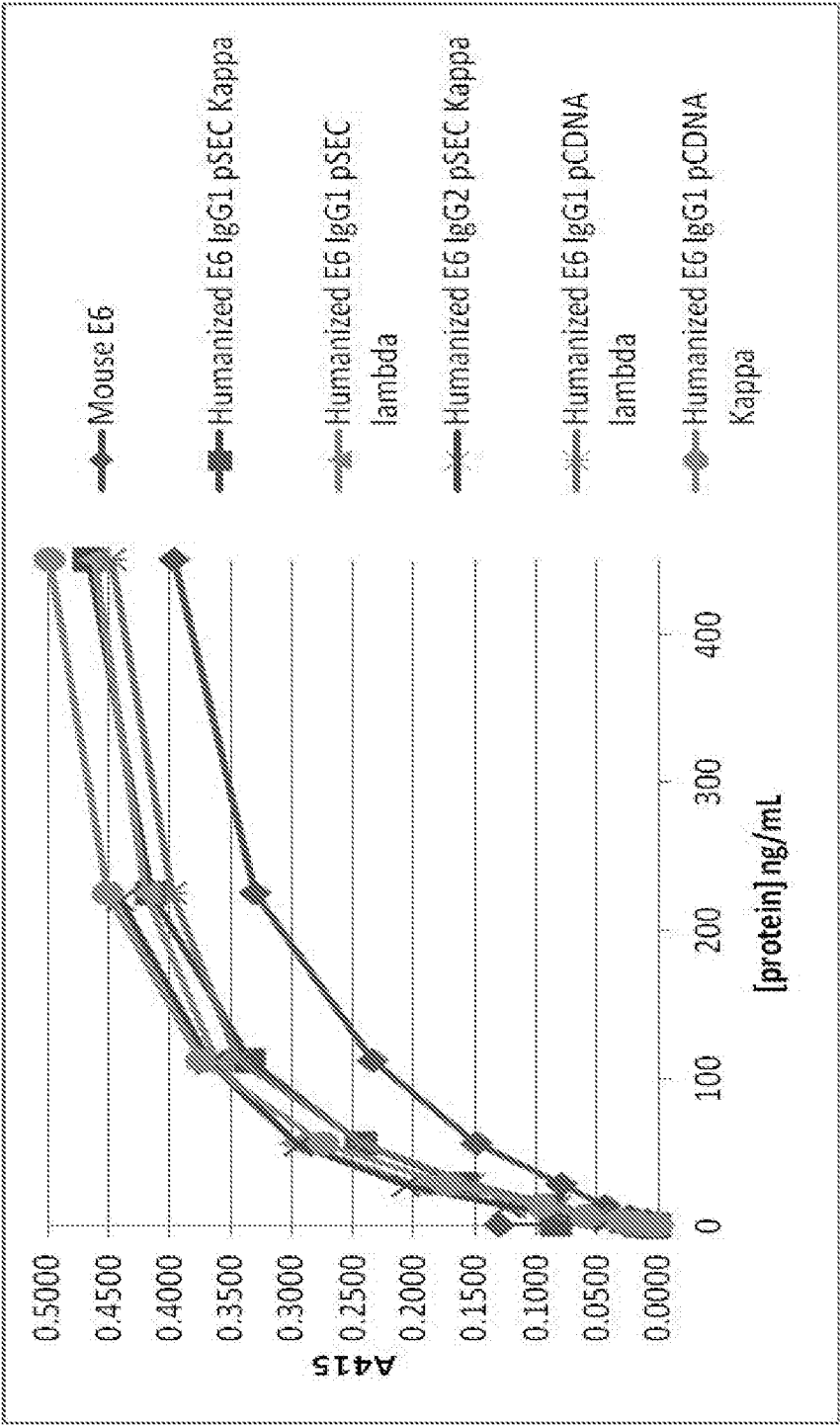


Figure 9

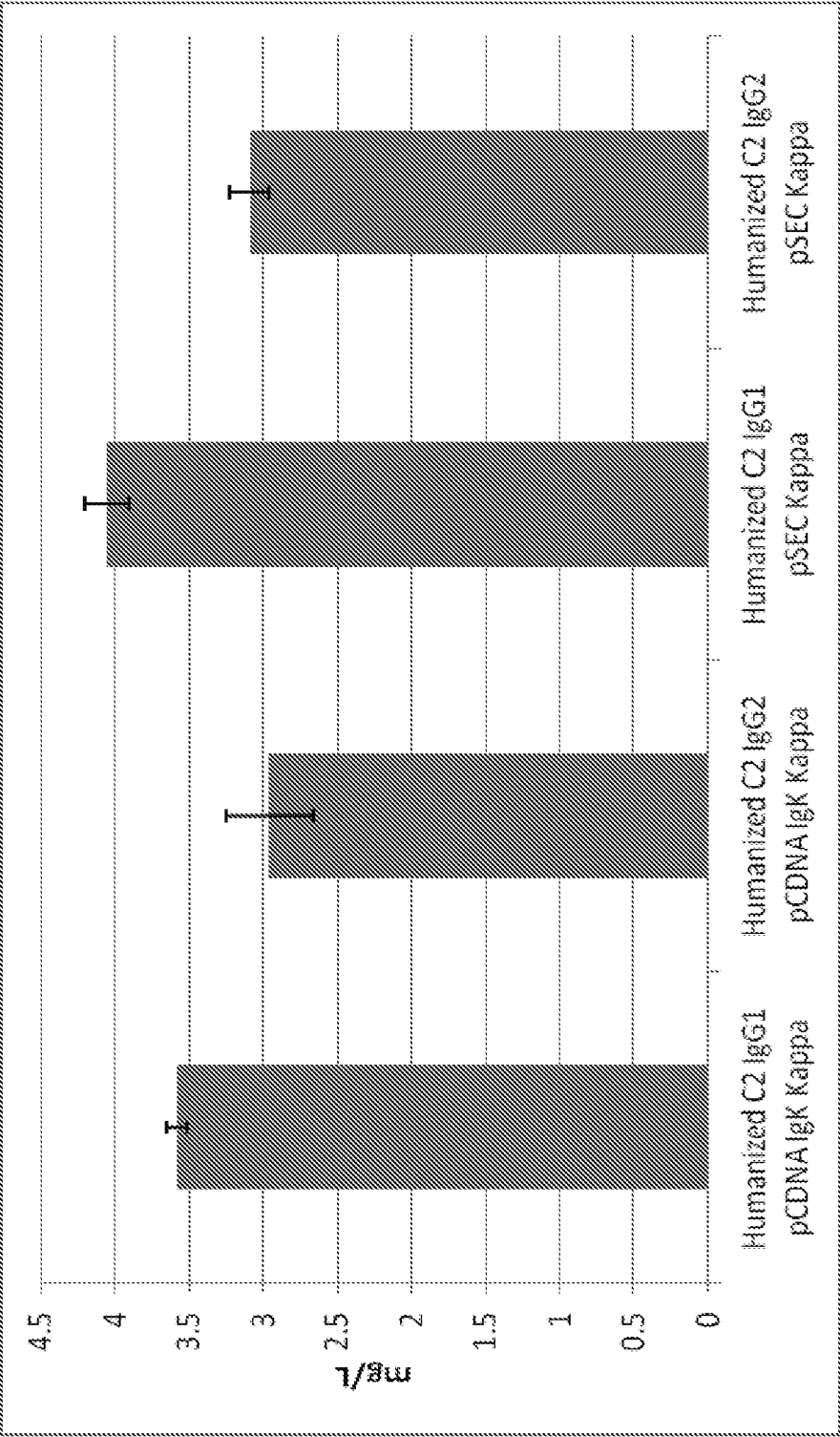


Figure 10

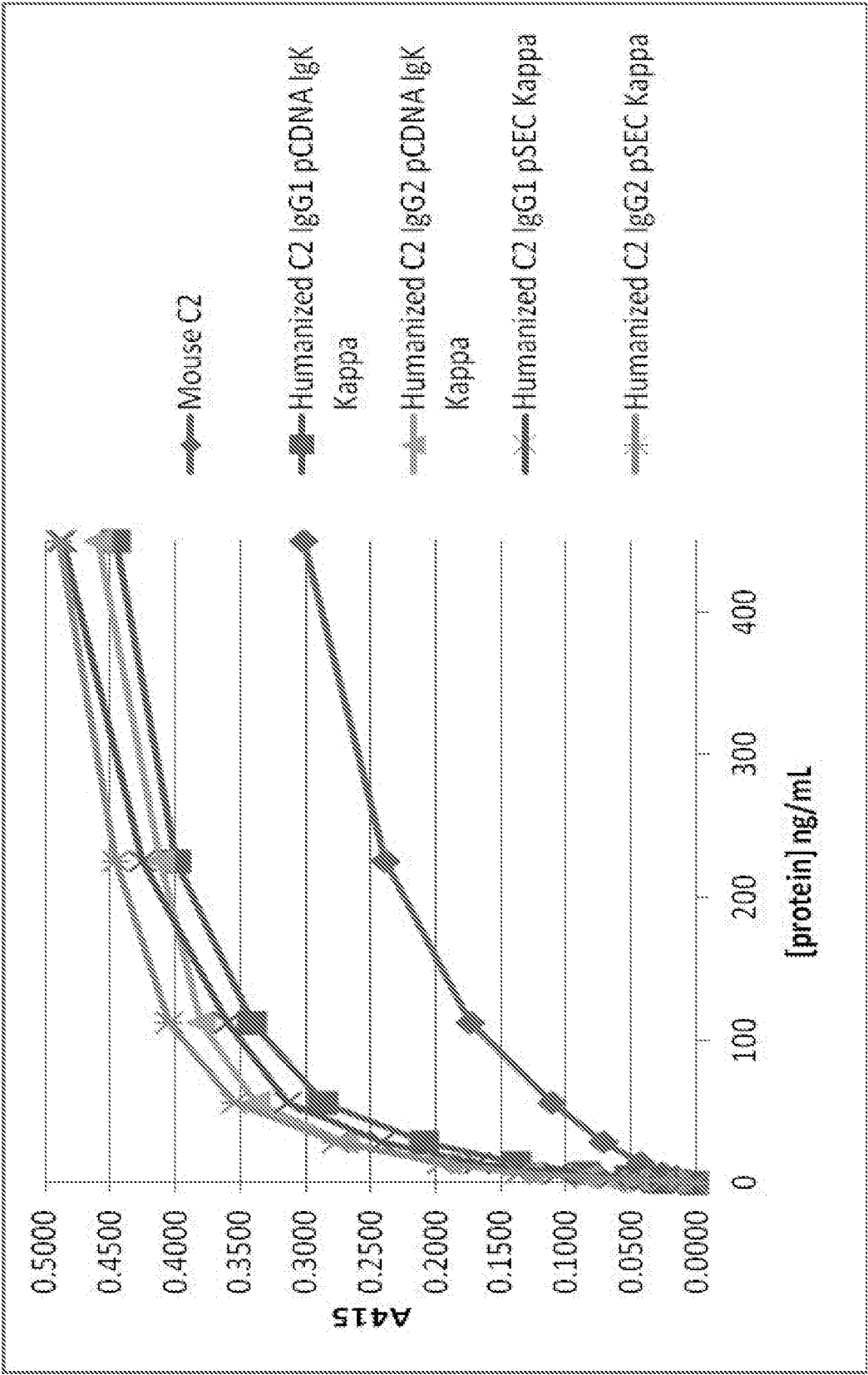


Figure 11

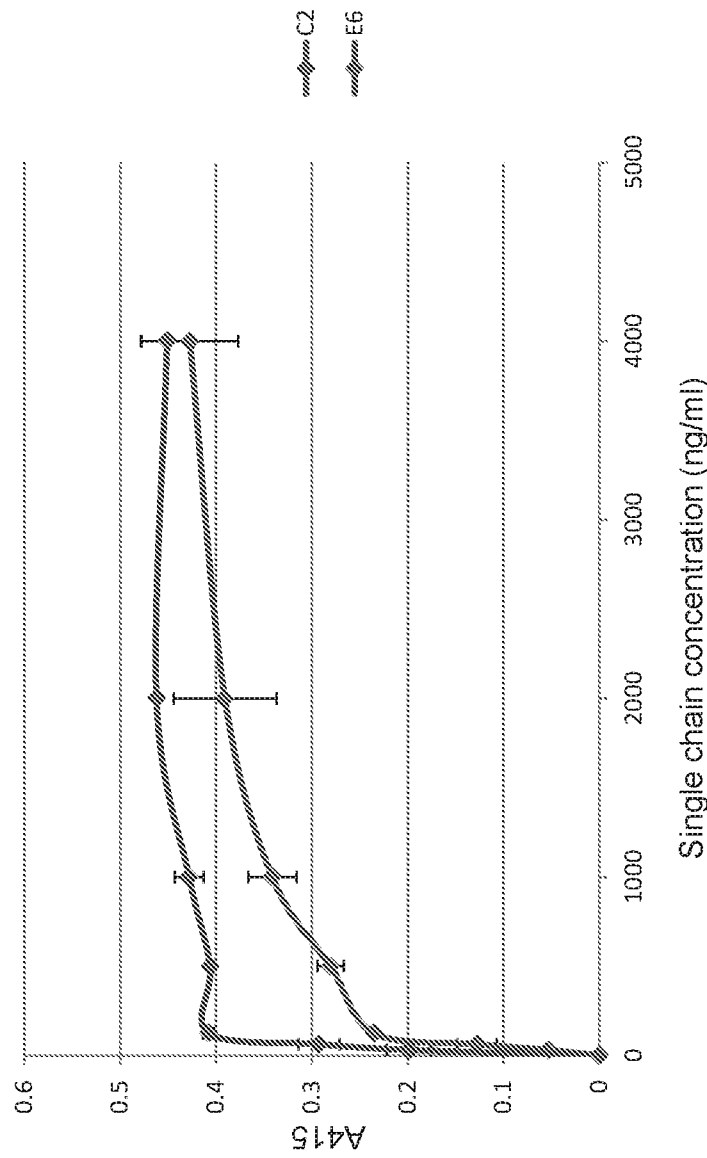


Figure 12

E6 scFv Fc-Fusion Protein A purification – Ultra low IgG FBS

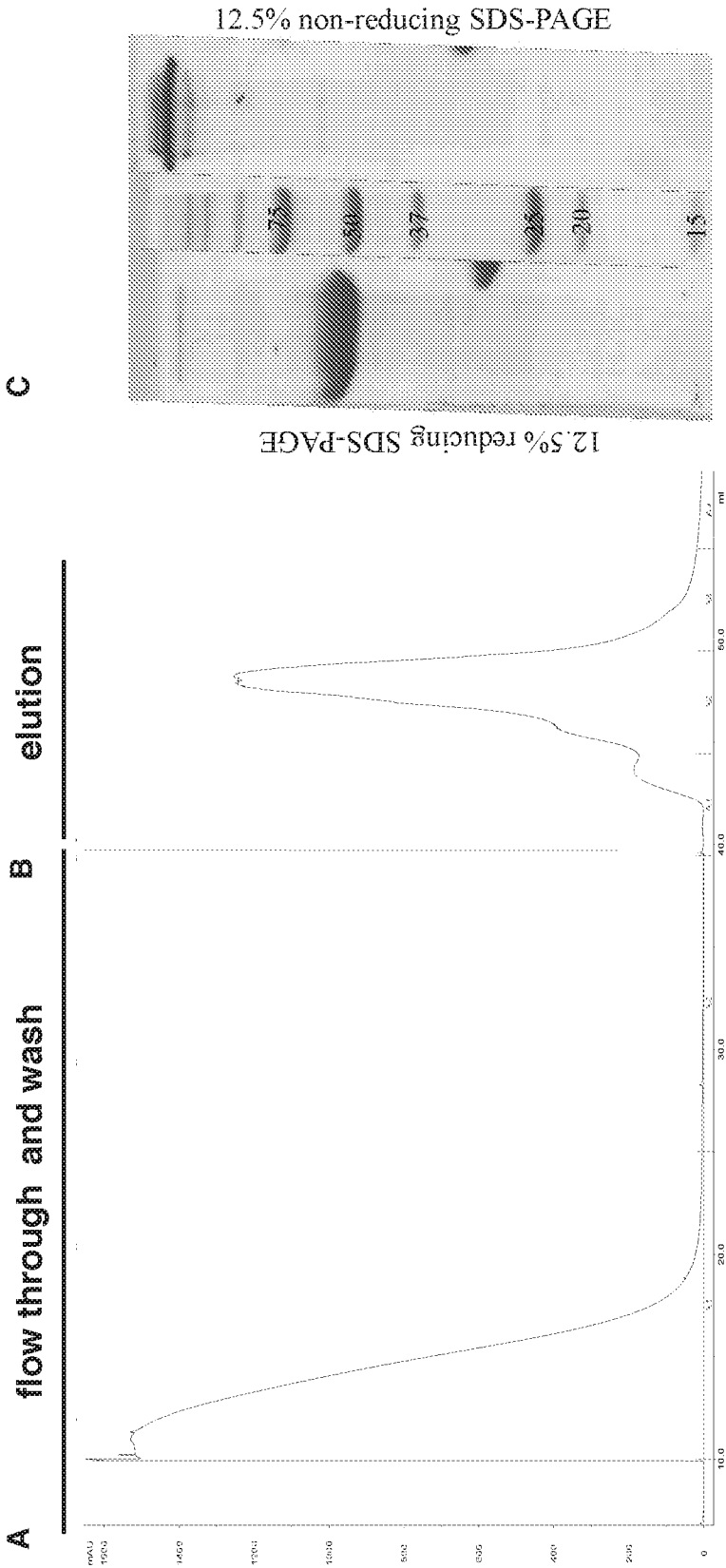


Figure 13

Purified E6 scFv-Fc with mutations in CH3 domain of Fc

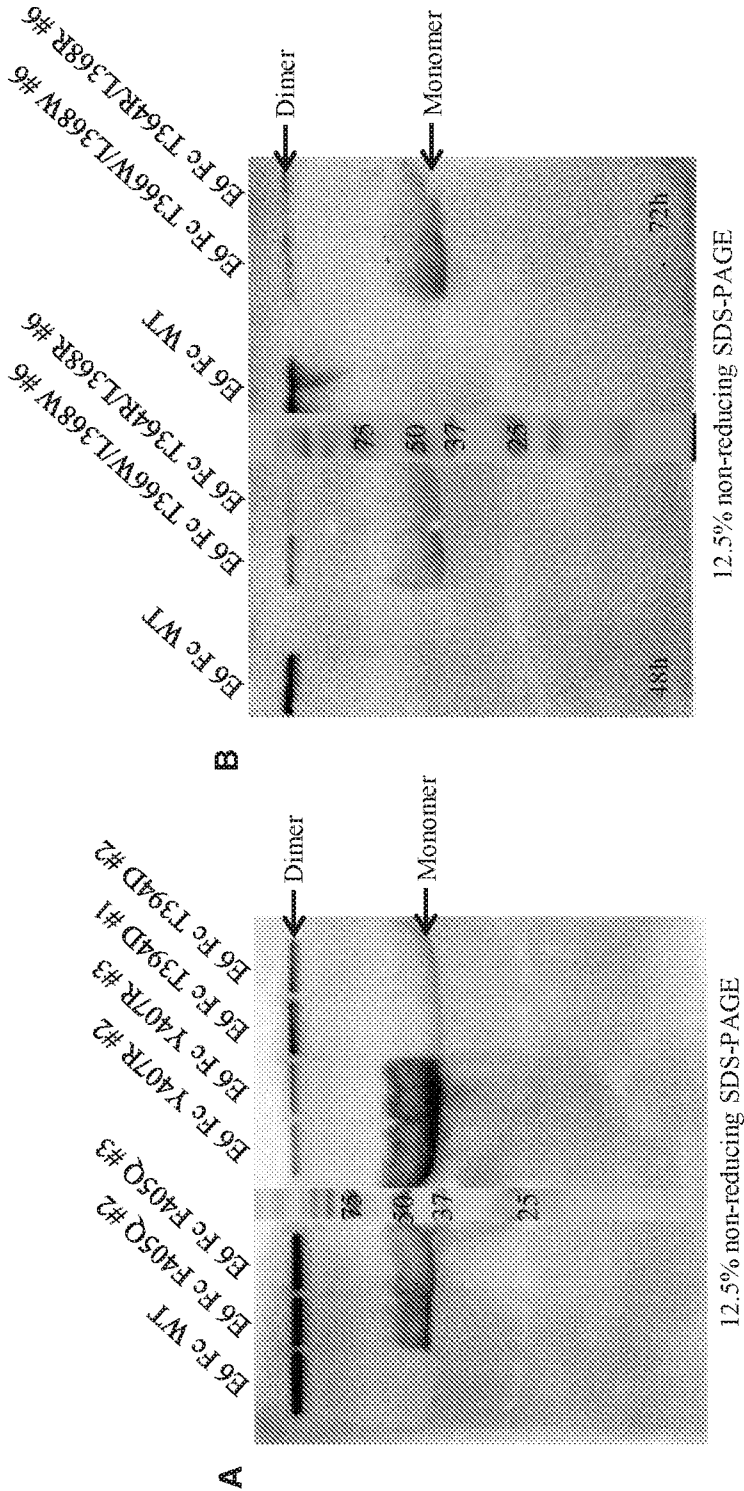


Figure 14

E6scFv-Fc Y407R Protein A purification (Ultra low IgG FBS)

flow through
A and wash B elution

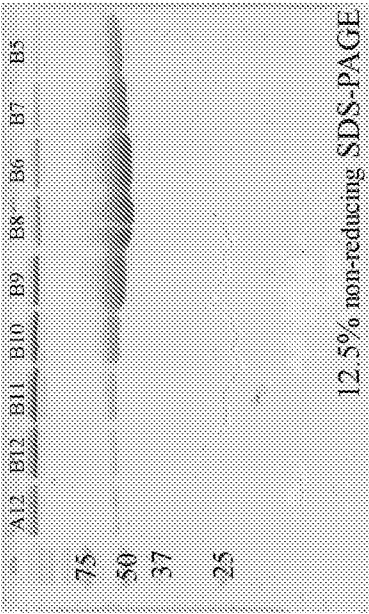
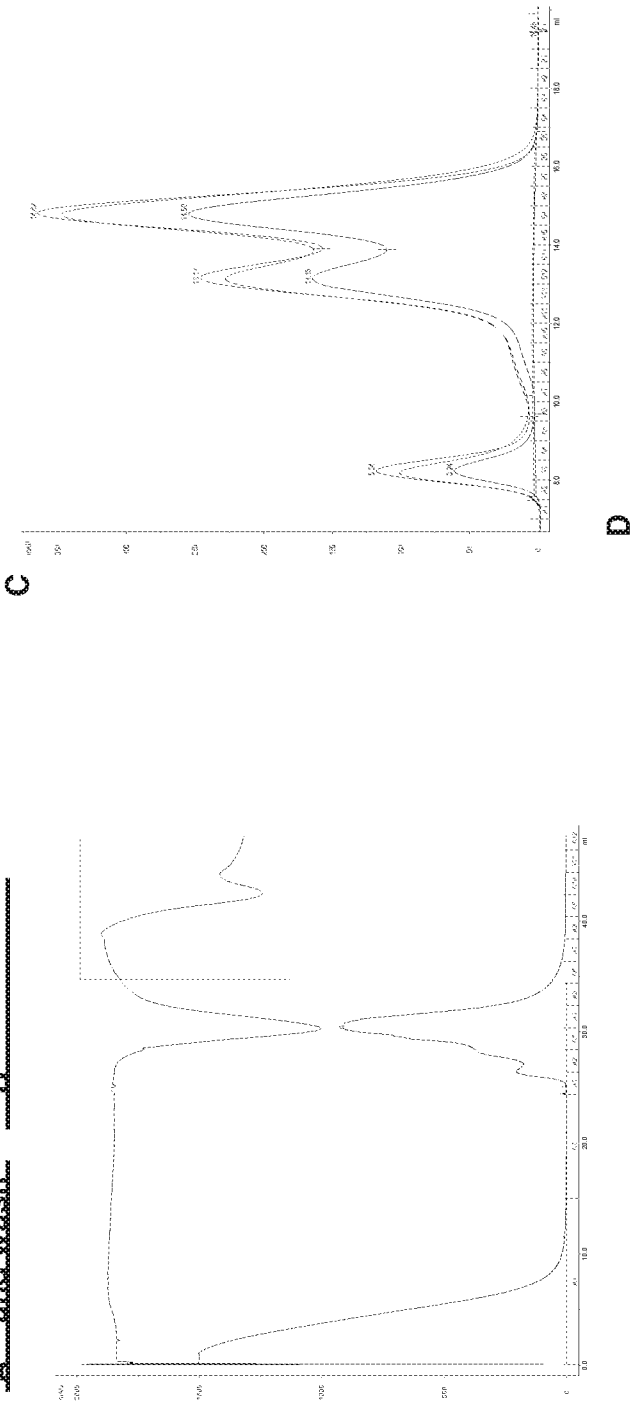
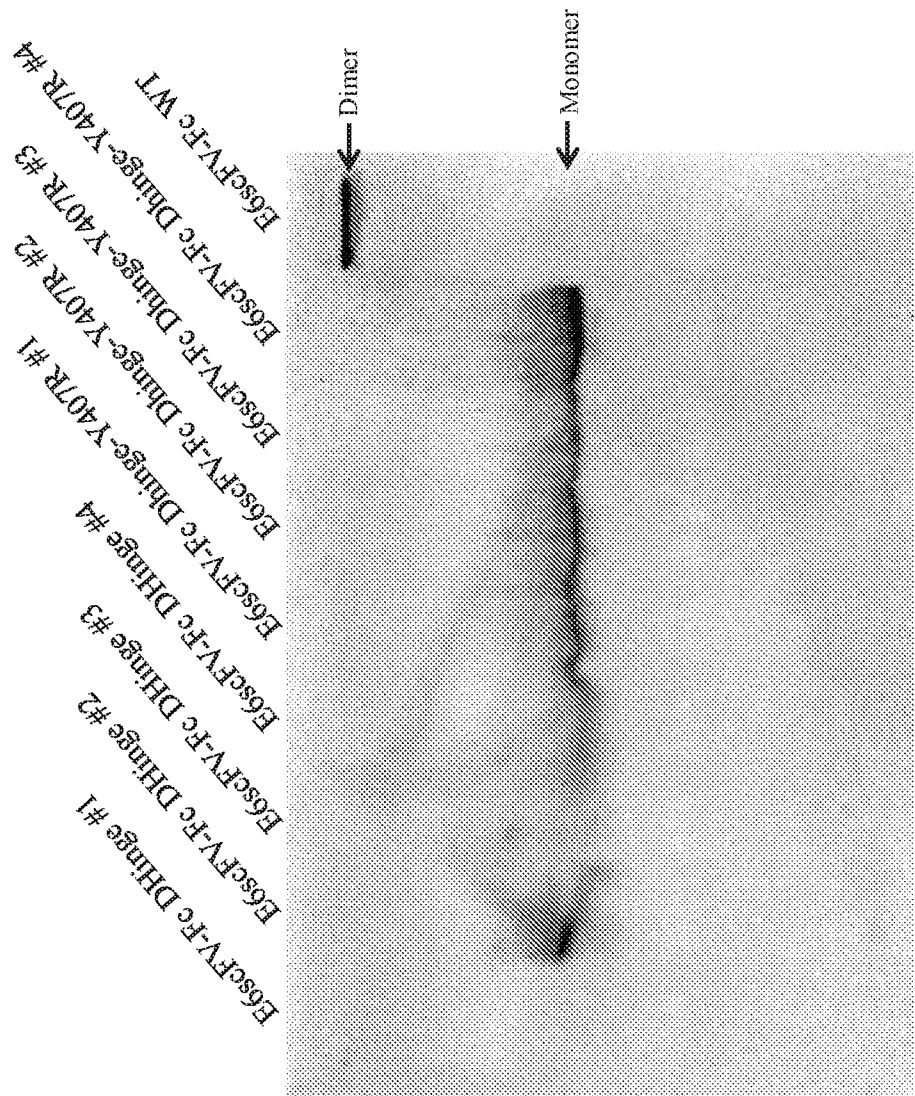


Figure 15

Western Blot of E6 scFv Fc-mutant (hingeless) transfection in HEK 293 T cells (48 hrs)



12.5% non-reducing SDS-PAGE

Figure 16

E6scFv-Fc Hingeless Capture Select FcXL purification

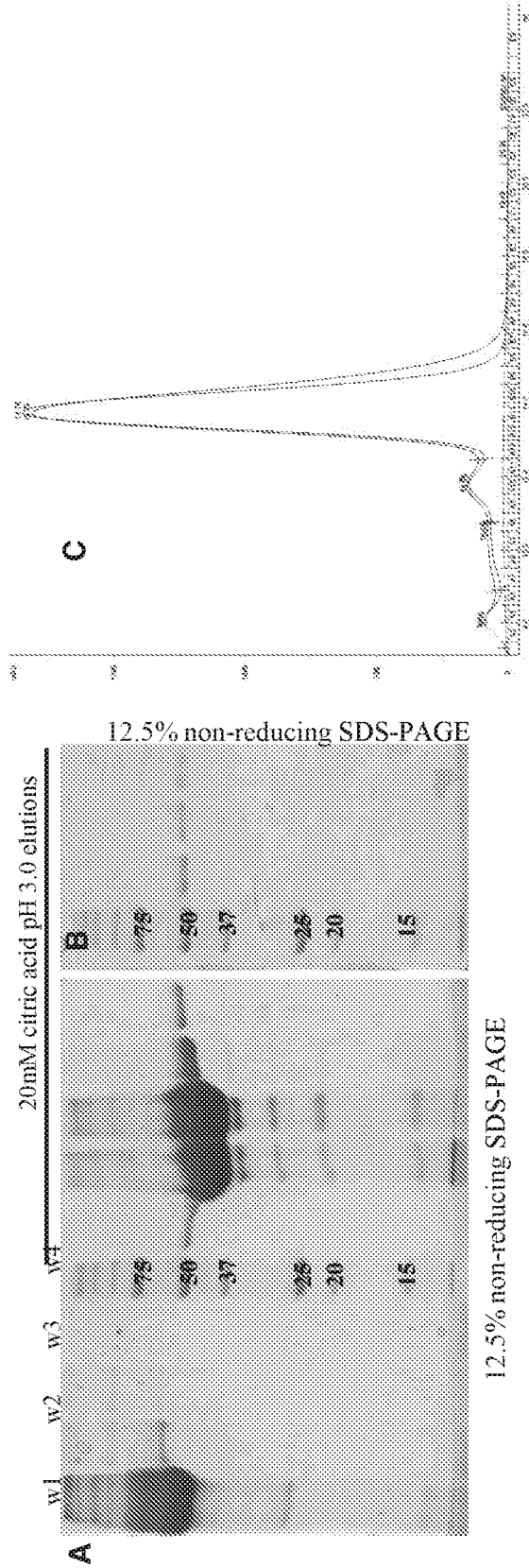


Figure 17

C3scFv-Fc Capture Select FcXL purification

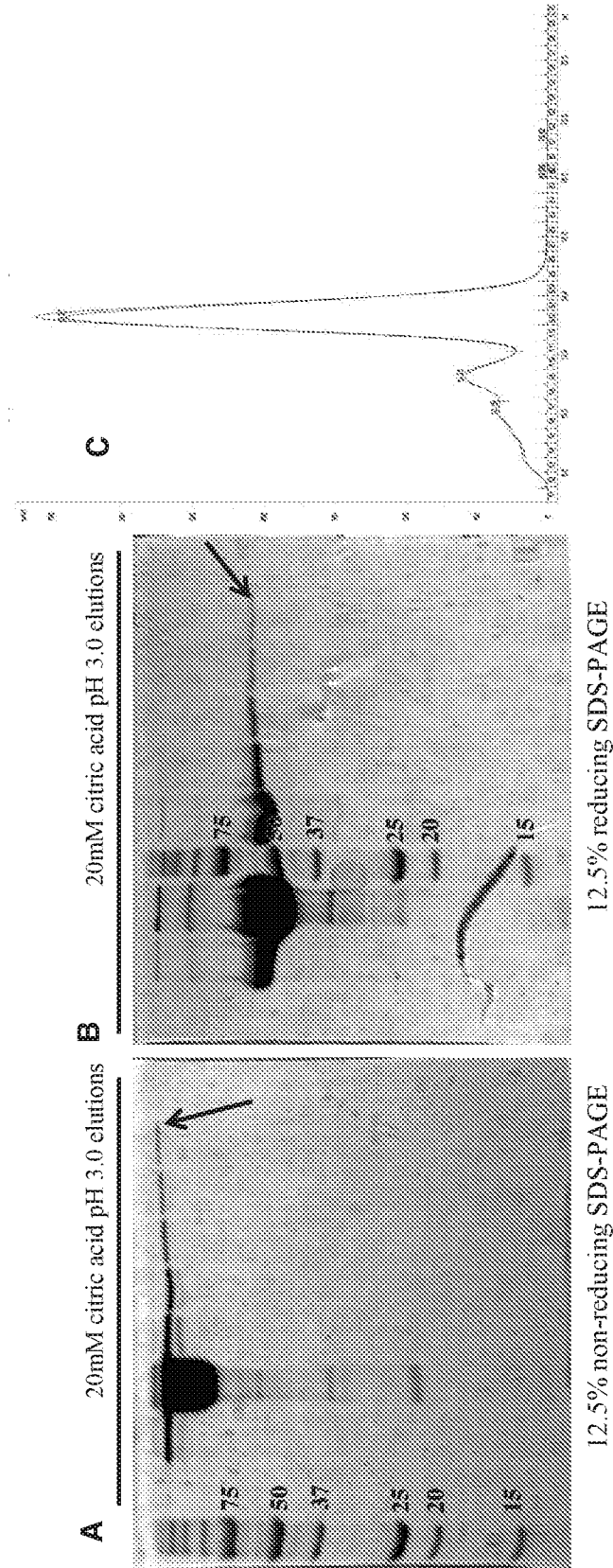


Figure 18

Native PAGE of humanized scFv-Fc fusion proteins

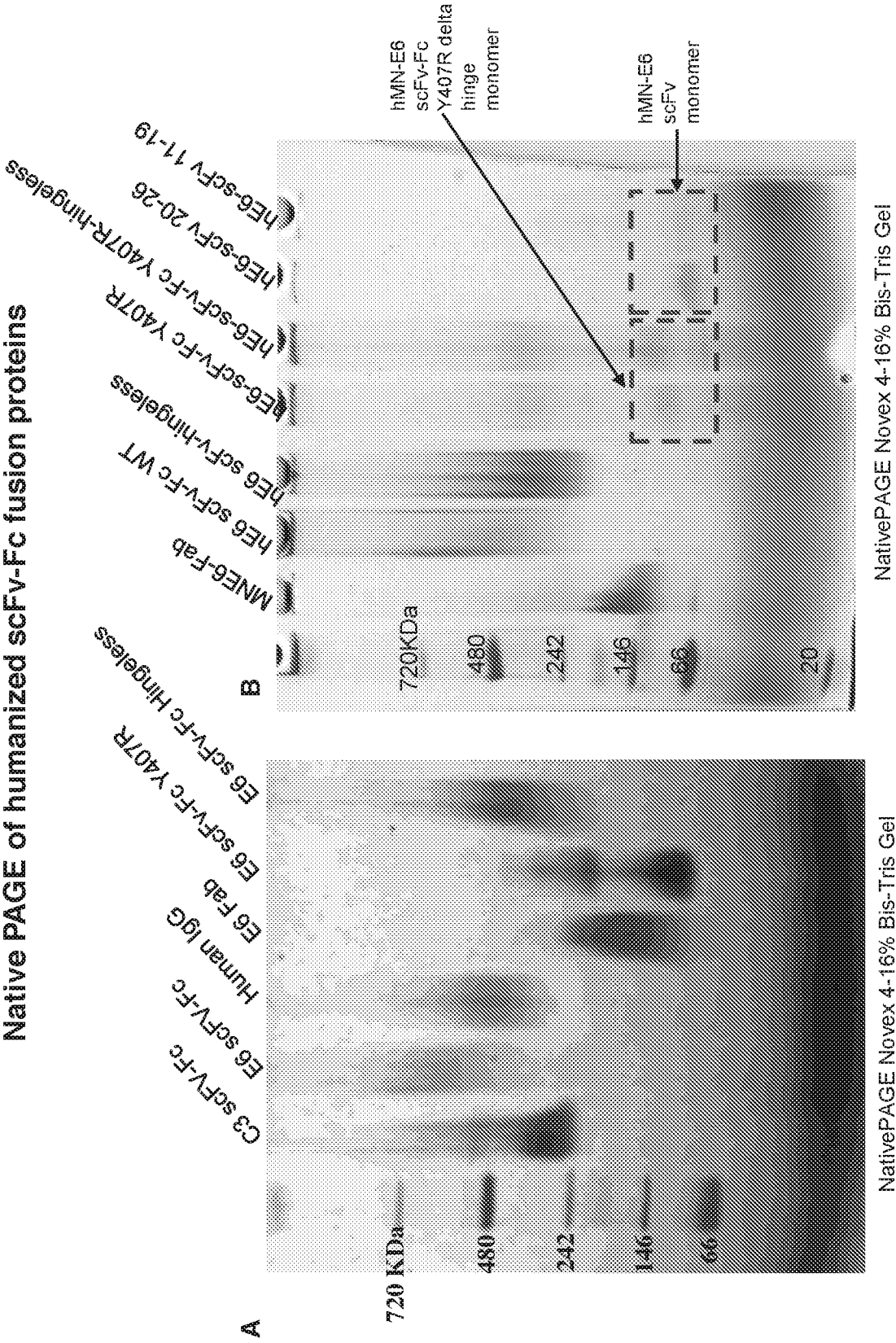


Figure 19

ELISA -- hu MN-E6 scFv-Fc binding to MUC1* peptides PSMGFR, PSMGFR N-10 and PSMGFR C-10

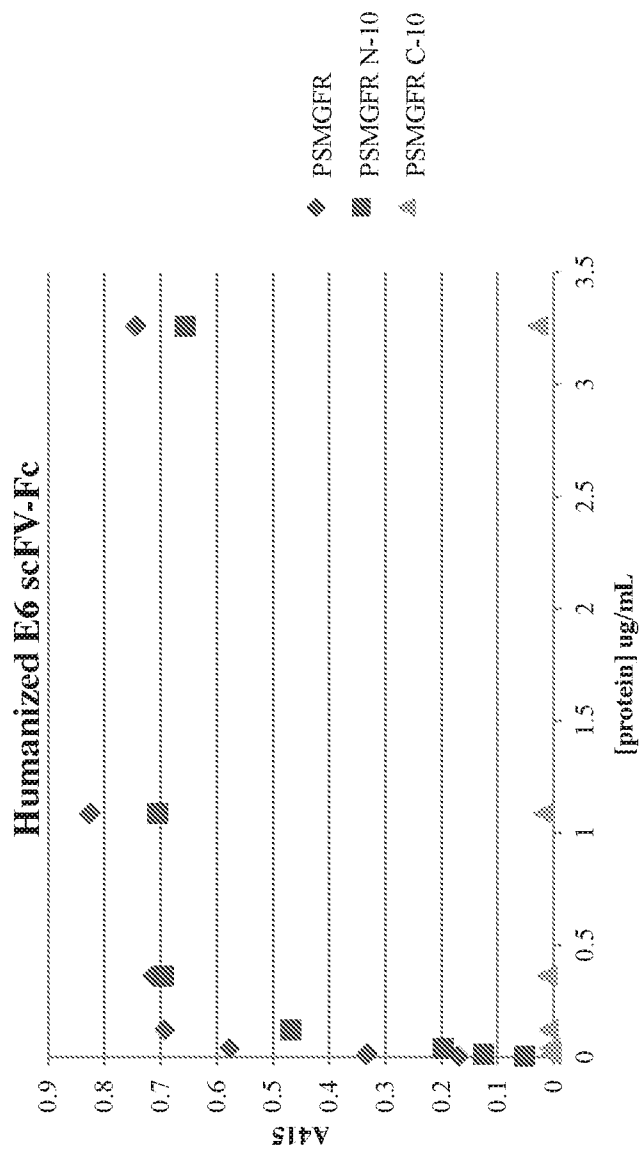


Figure 20

ELISA – binding of hu MN-E6 or MN-C3 scFv-Fc wild type or mutants to MUC1* PSMGFR peptide

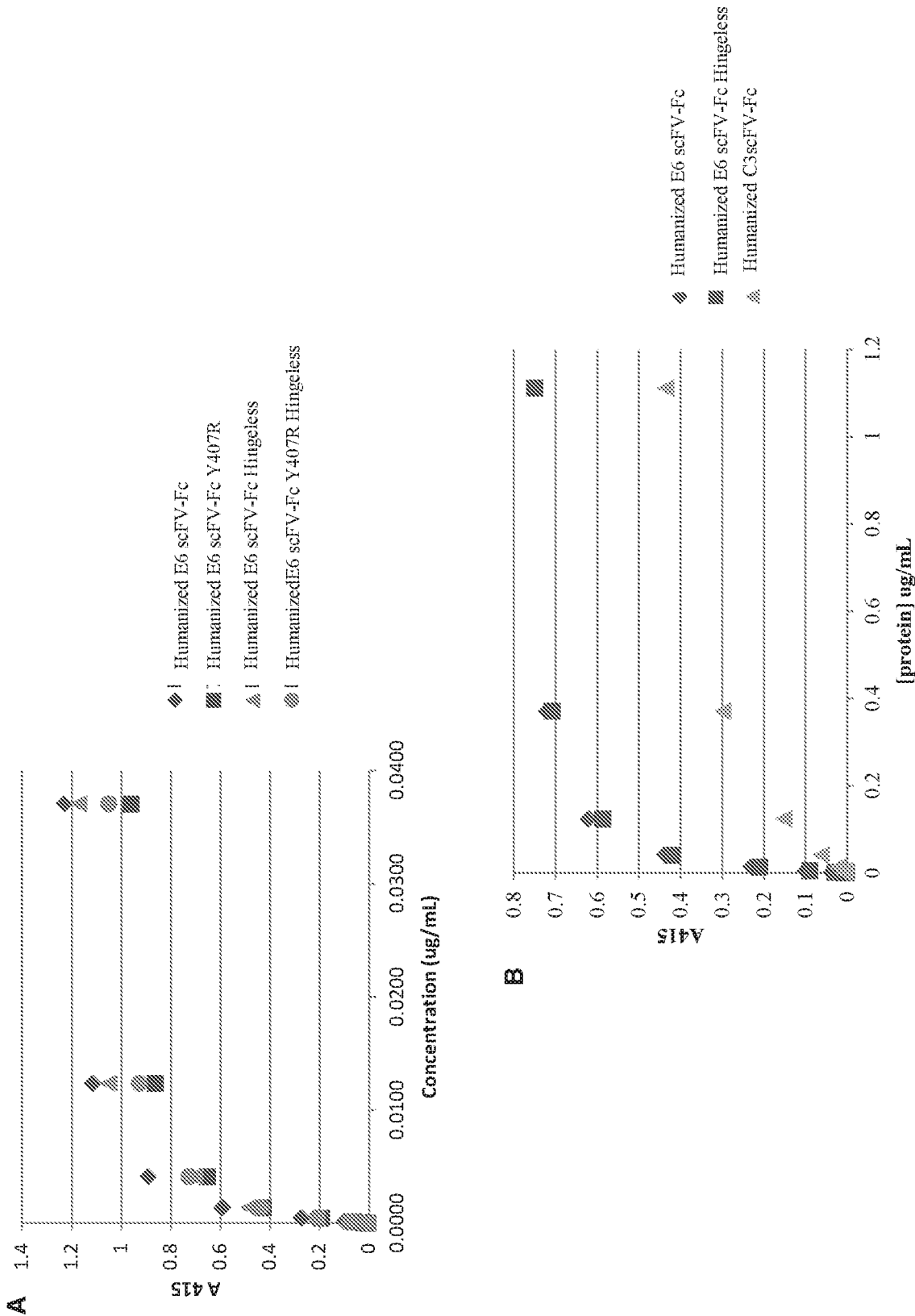


Figure 21

ELISA – mouse monoclonal MN-C3, humanized MN-C3 and humanized MN-C3 scFv-Fc binding to MUC1*
peptides PSMGFR, PSMGFR N-10 and PSMGFR C-10

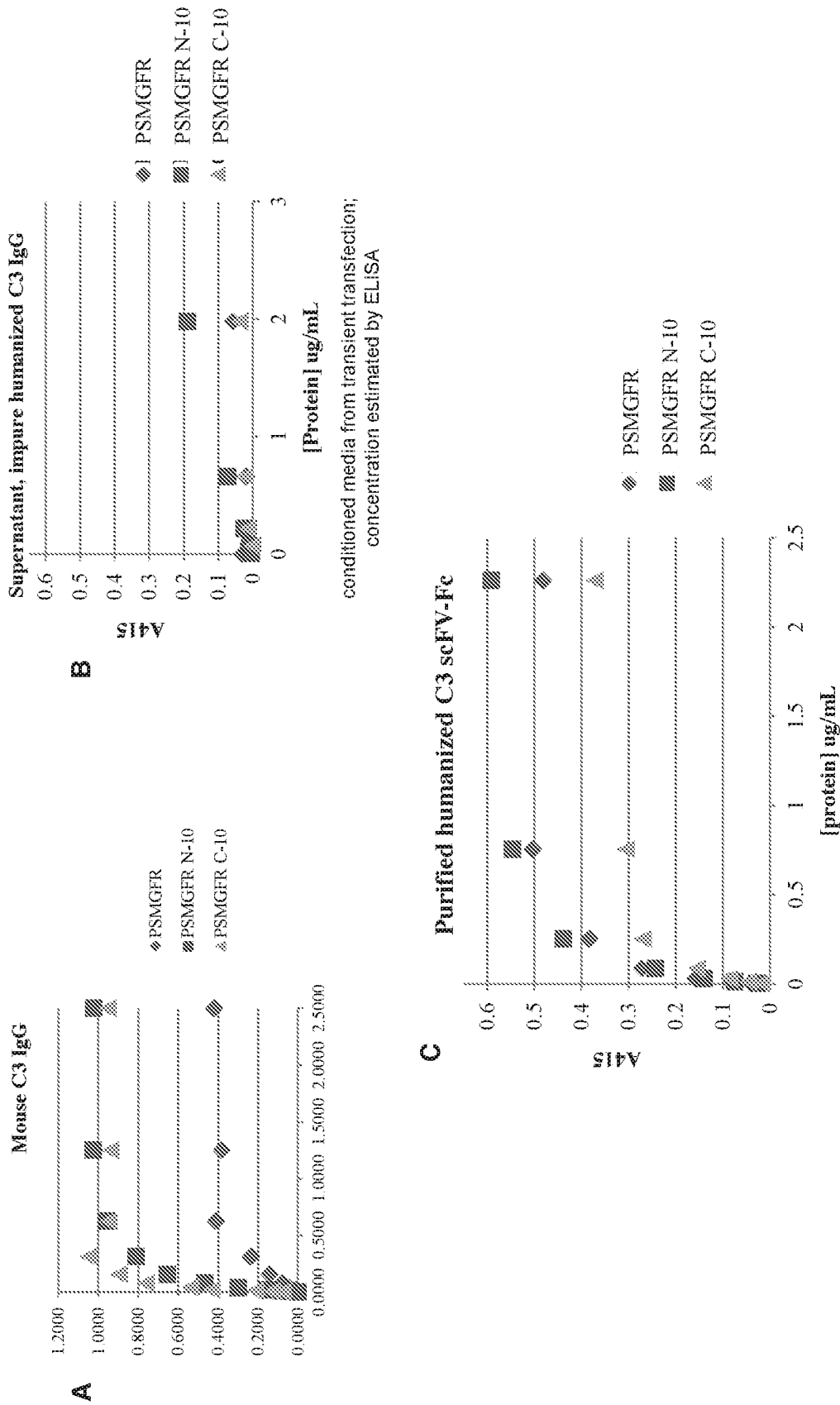


Figure 22

ELISA assay quantifies binding of humanized MN-E6 scFv-Fc-delta hinge, aka hingeless, and humanized MN-E6 scFv to the MUC1* peptide PSMGFR

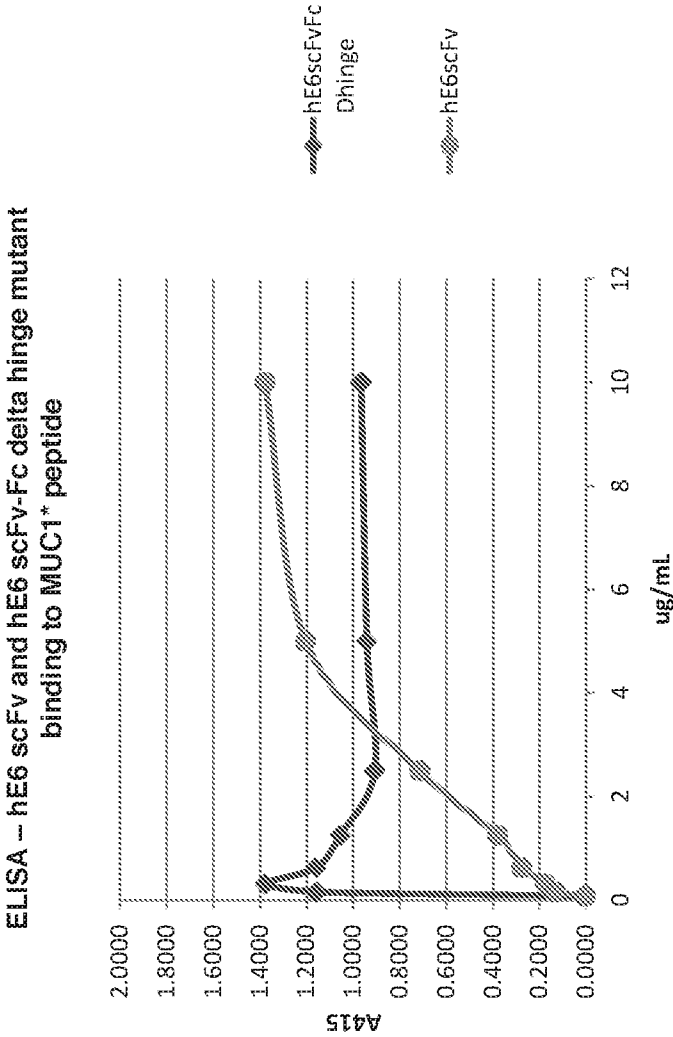
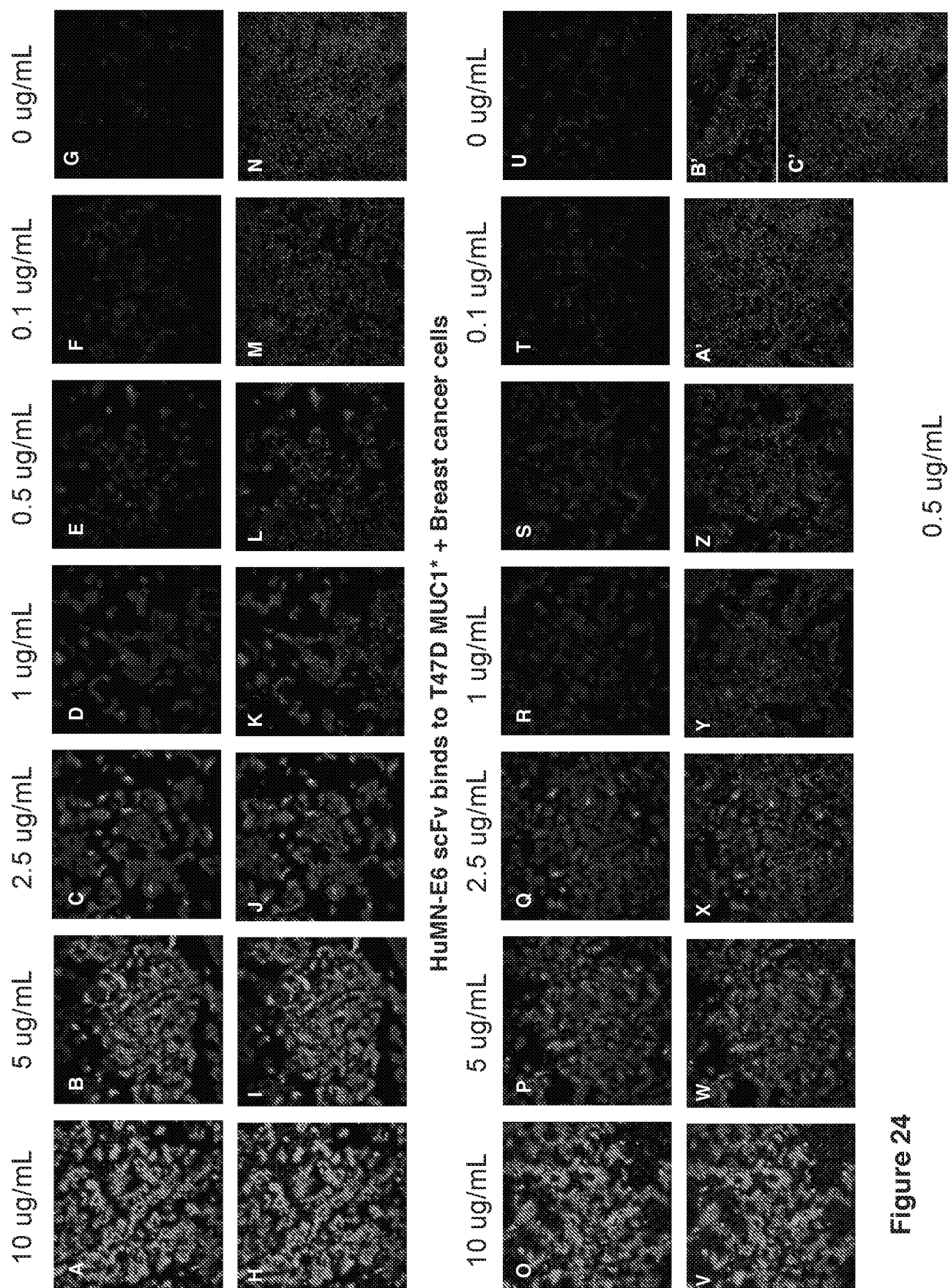


Figure 23



Humanized MN-E6 scFv inhibits growth of MUC1* positive cancer cells in a concentration dependent manner;
shown are 1500 MUC1* + breast cancer cells

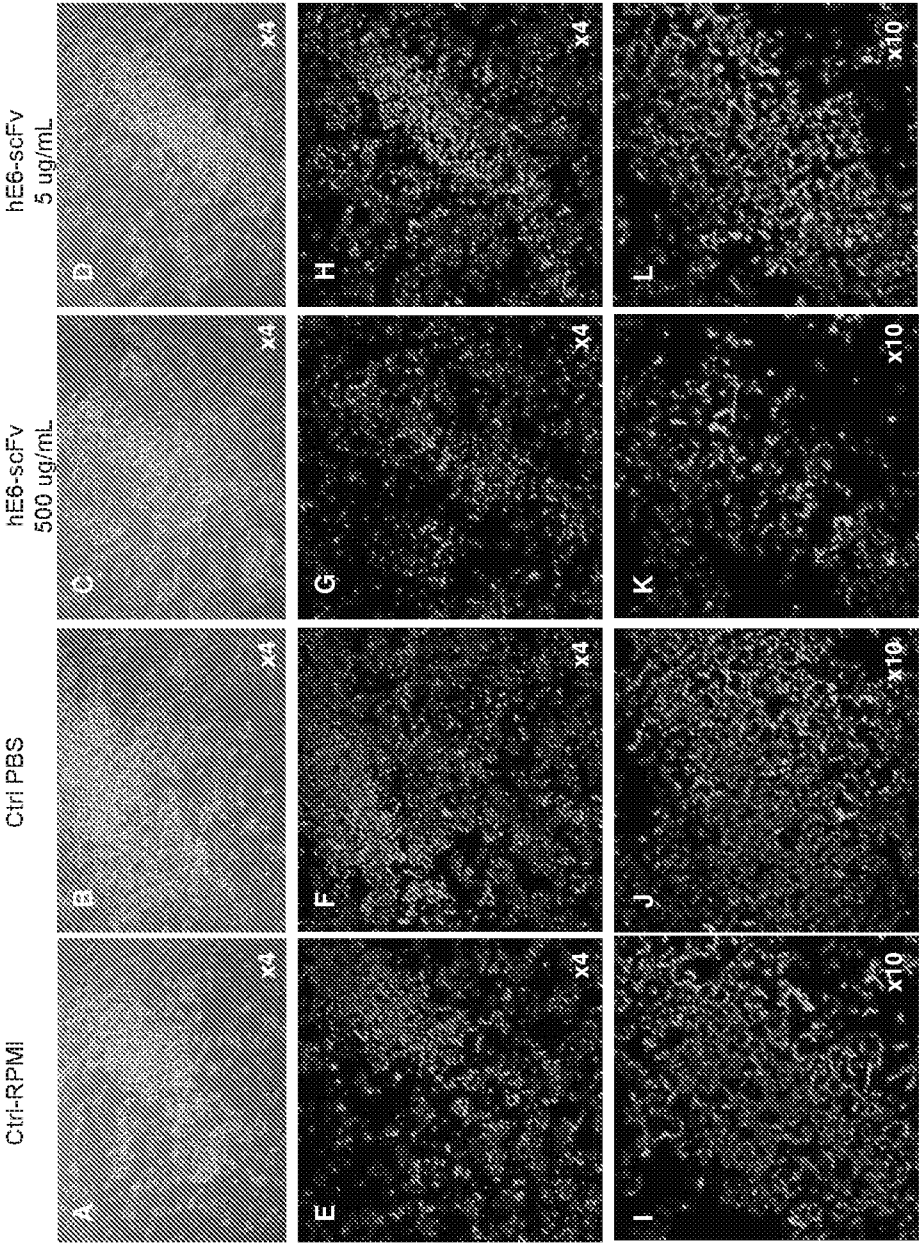


Figure 25

Humanized MN-E6 scFv-Fc mutants inhibit growth of MUC1* positive cancer cells in a concentration dependent manner; shown are 1500 MUC1* + breast cancer cells

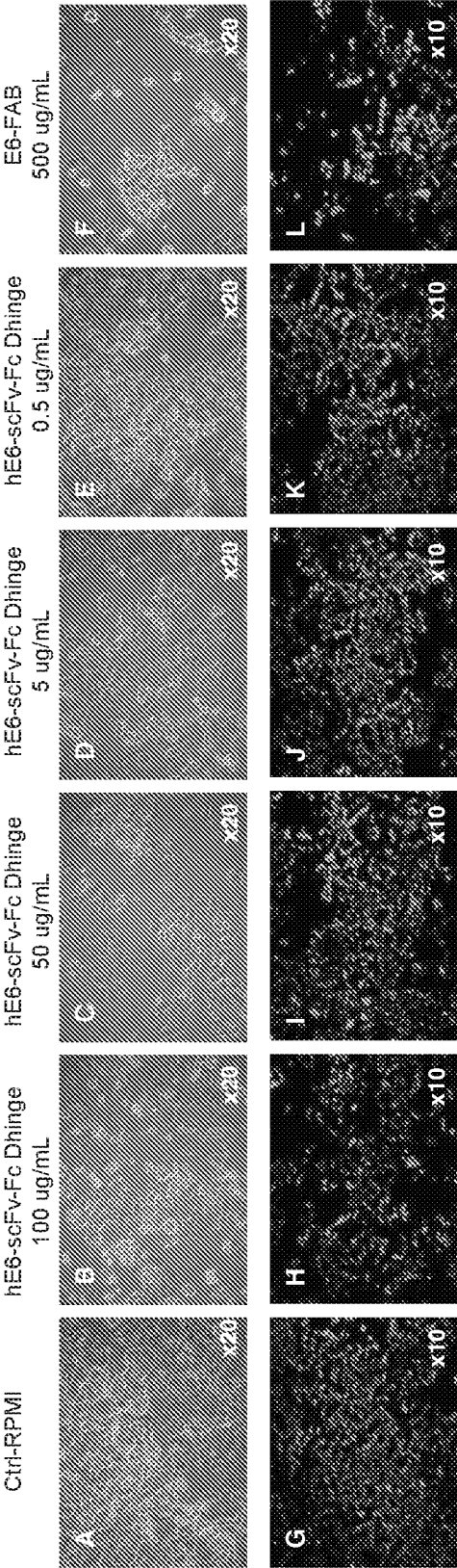


Figure 26

Image analysis that uses pixel counting/pixel fluorescence intensity quantifies growth inhibition of MUC1* positive cancers by MN-E6, humanized MN-E6 scFv-Fc-delta hinge, aka hingeless, and humanized MN-E6 scFv

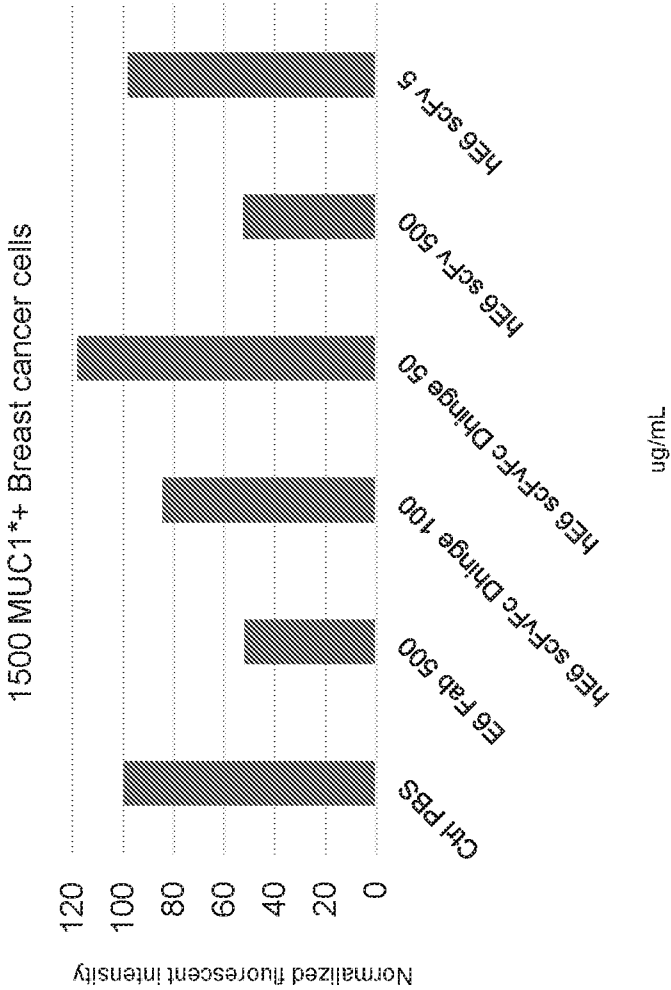


Figure 27

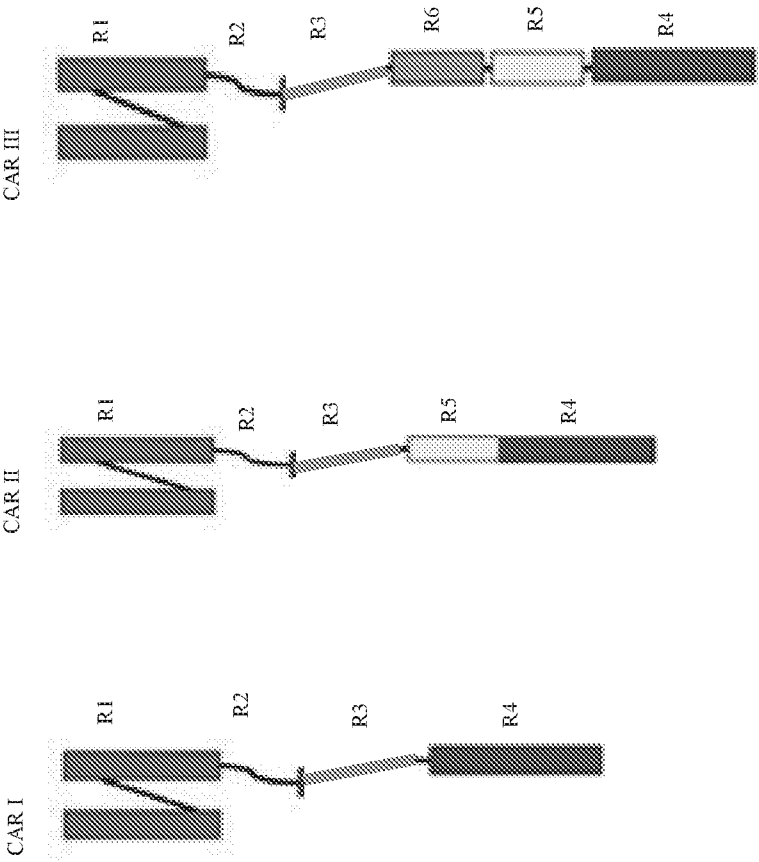


Figure 28

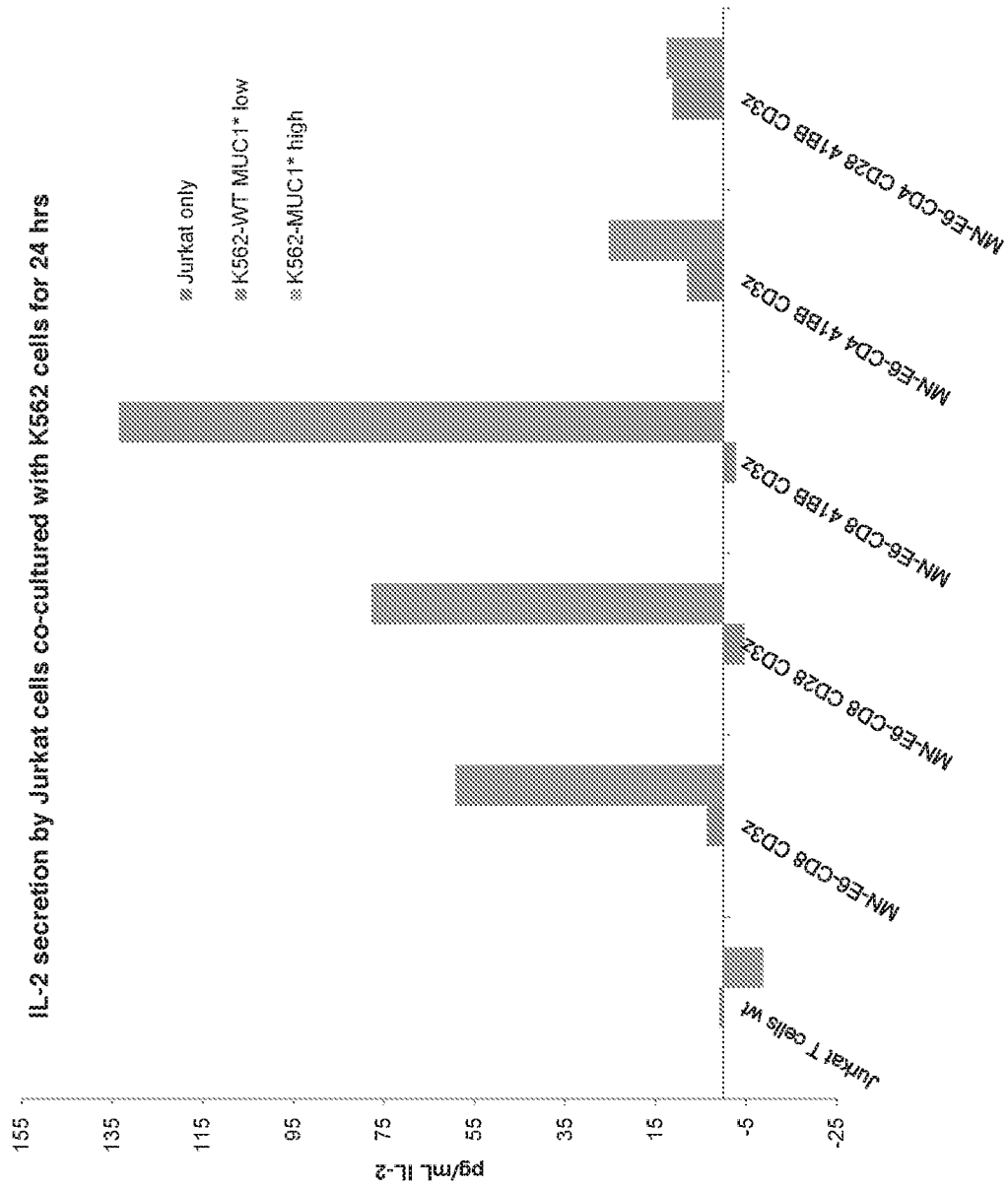


Figure 29

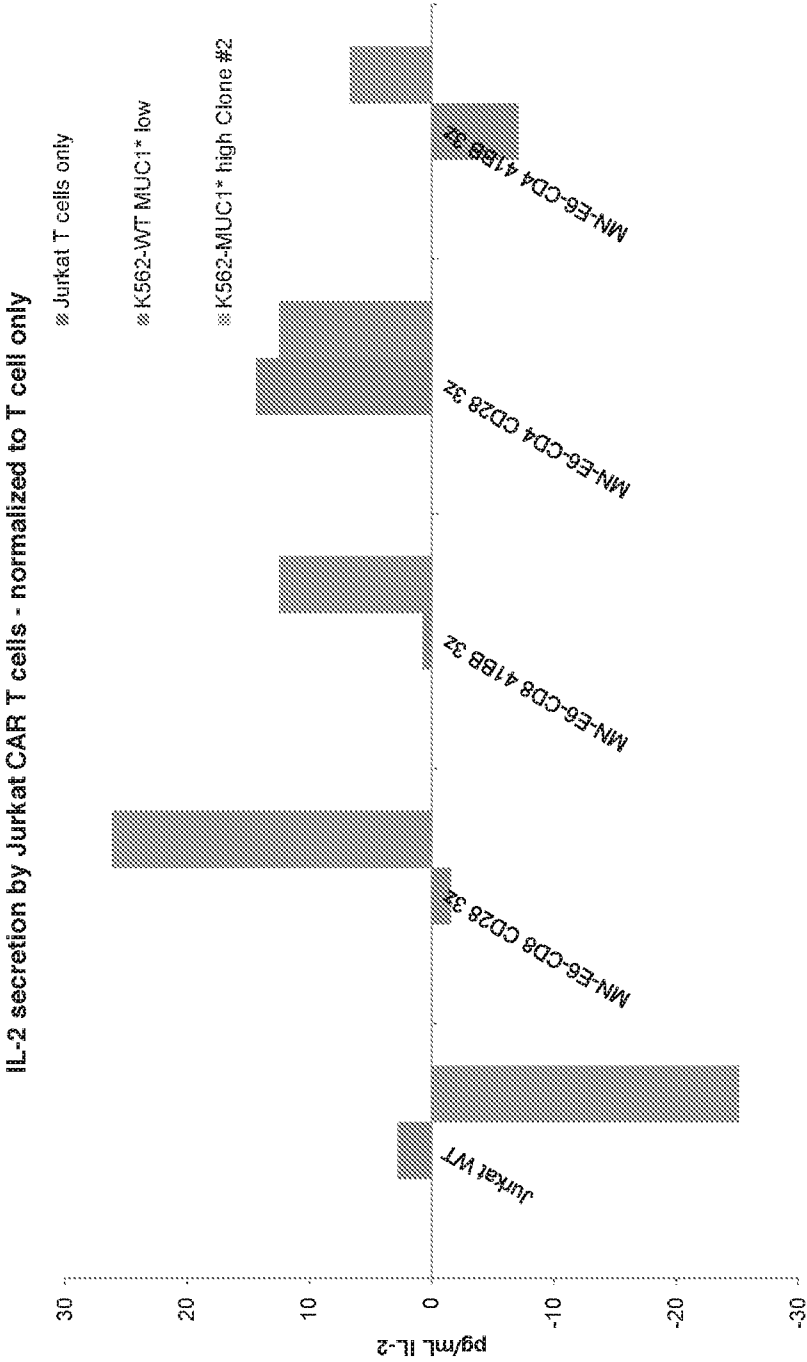


Figure 30

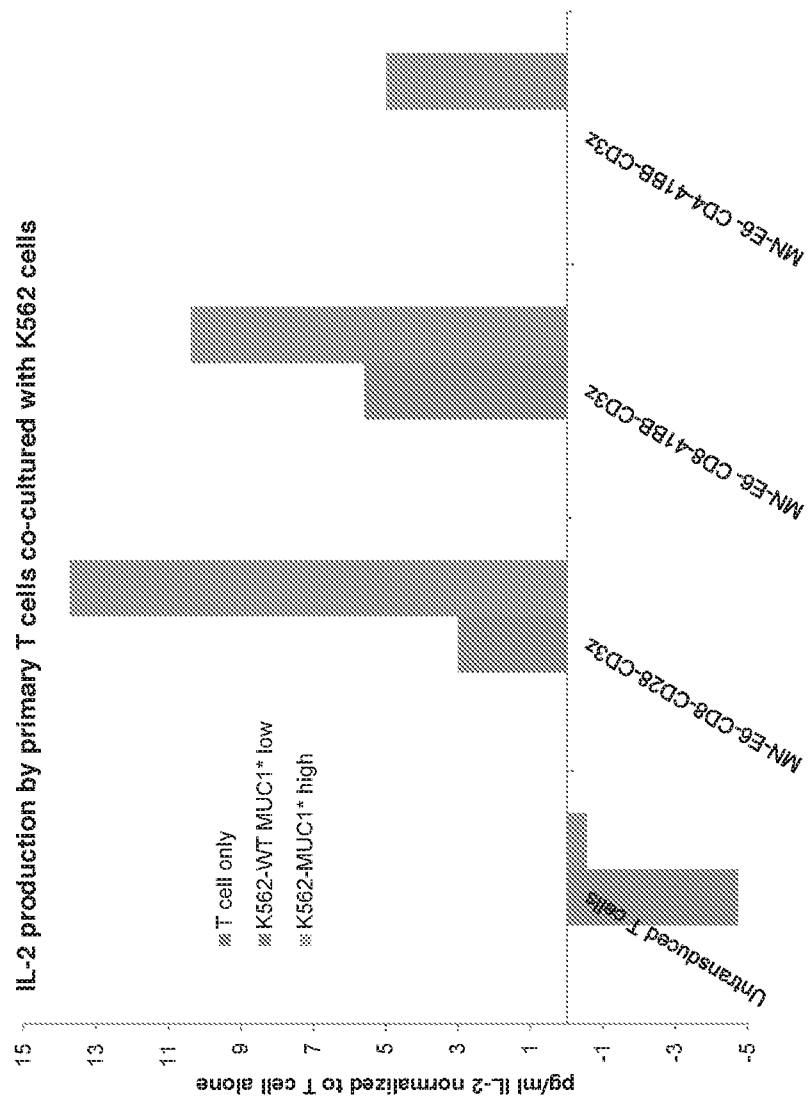


Figure 31

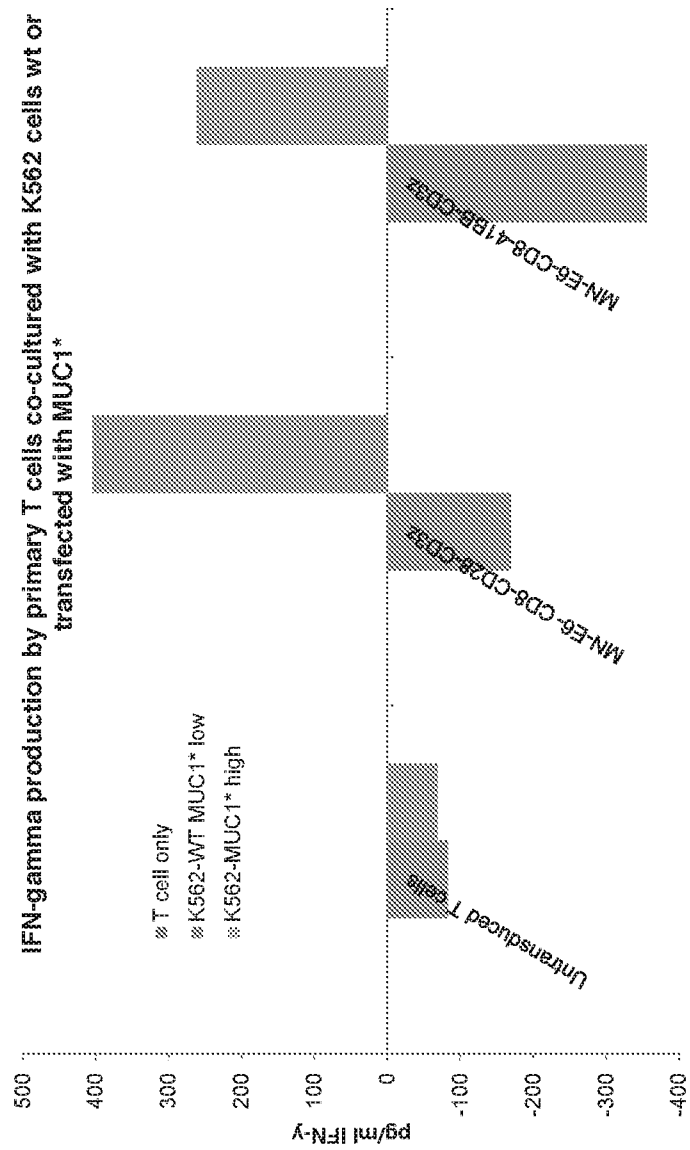


Figure 32

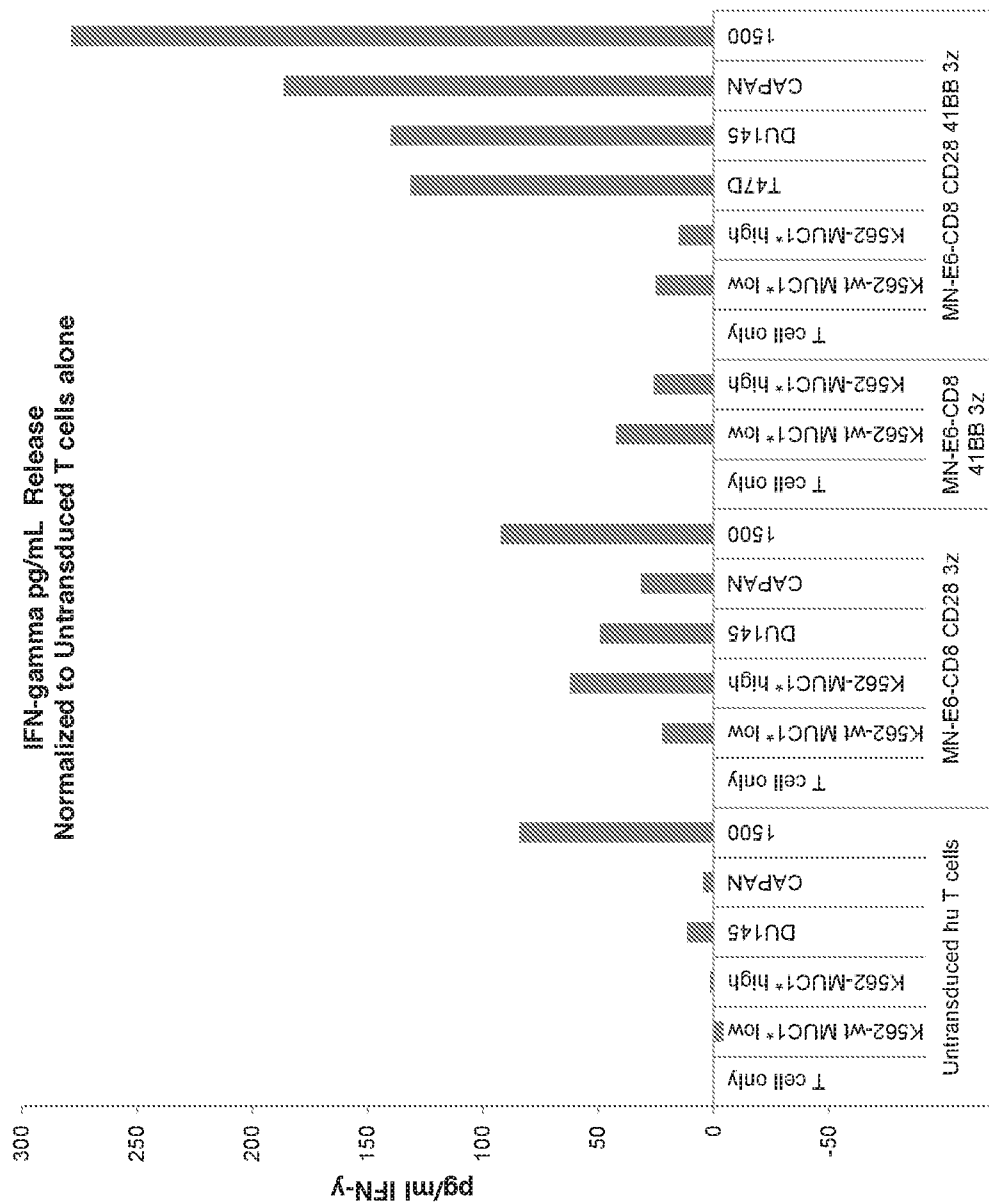


Figure 33

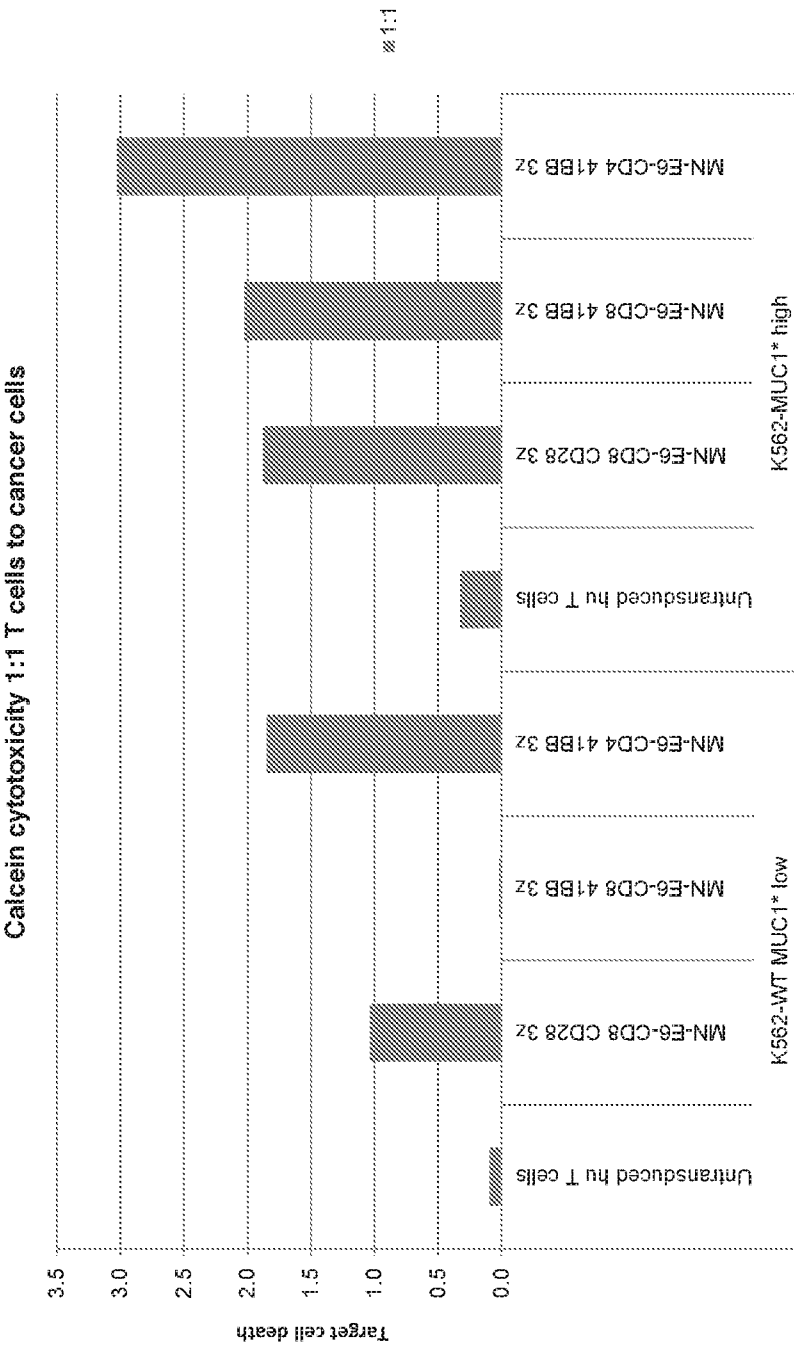


Figure 34

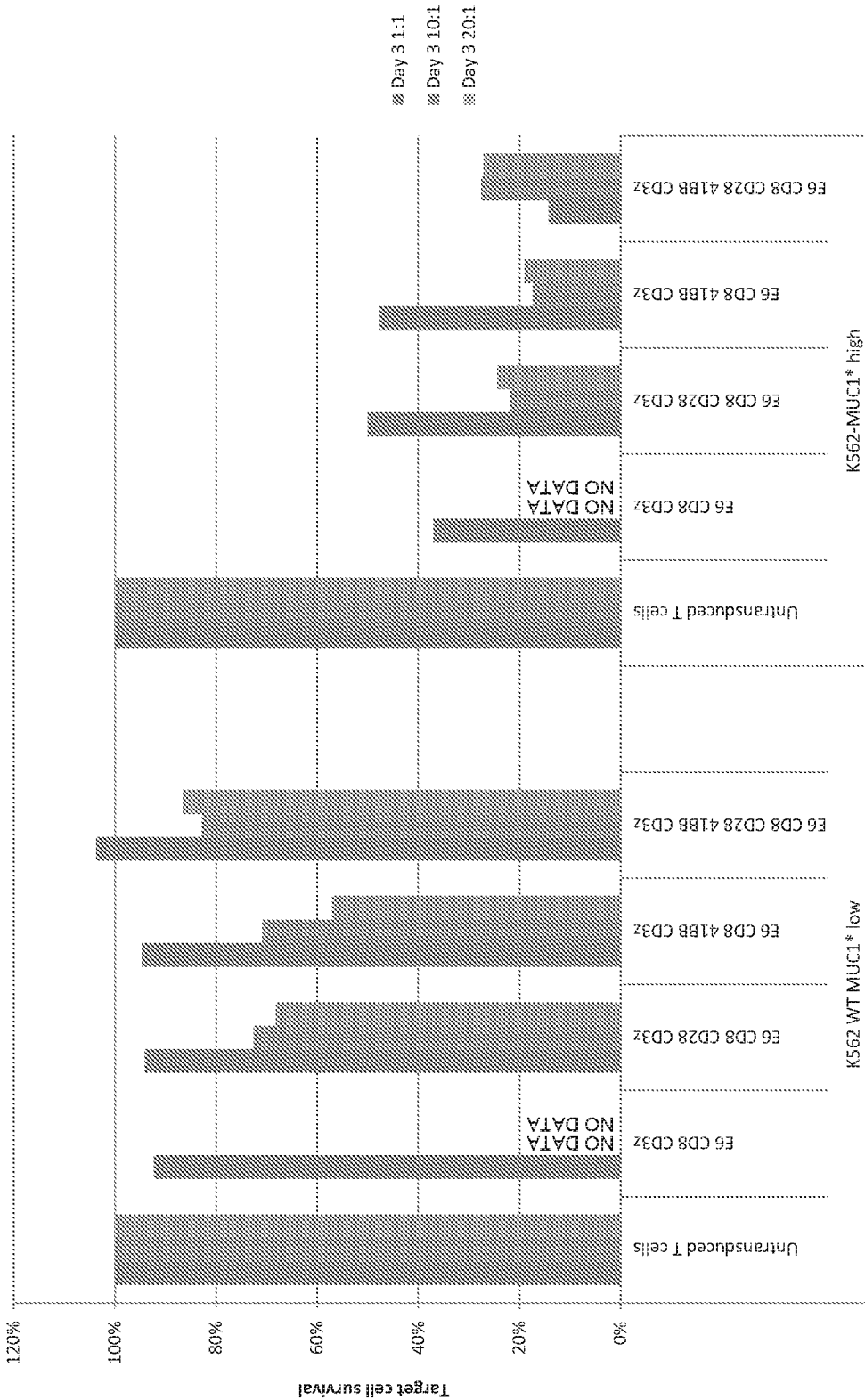


Figure 35

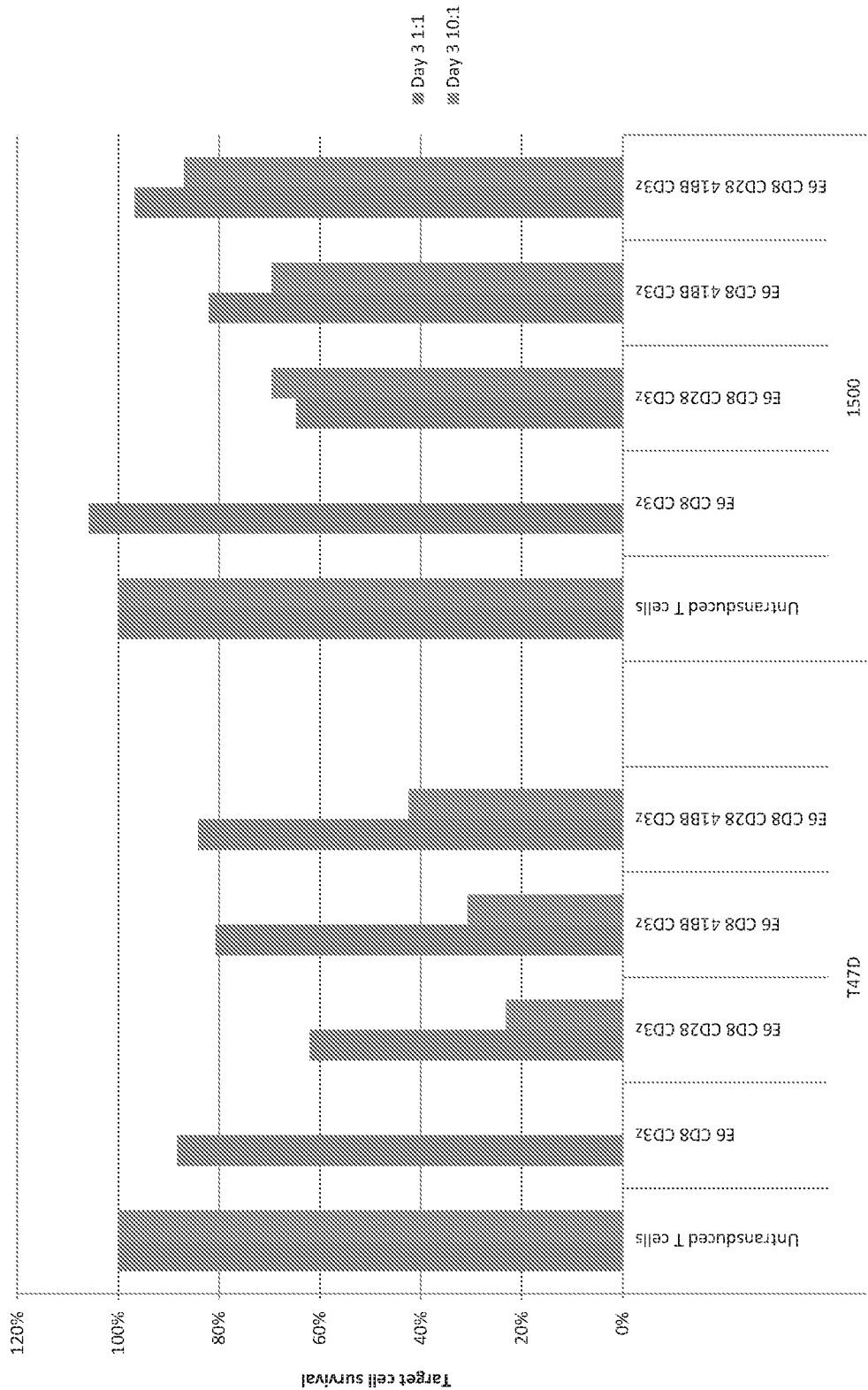


Figure 36

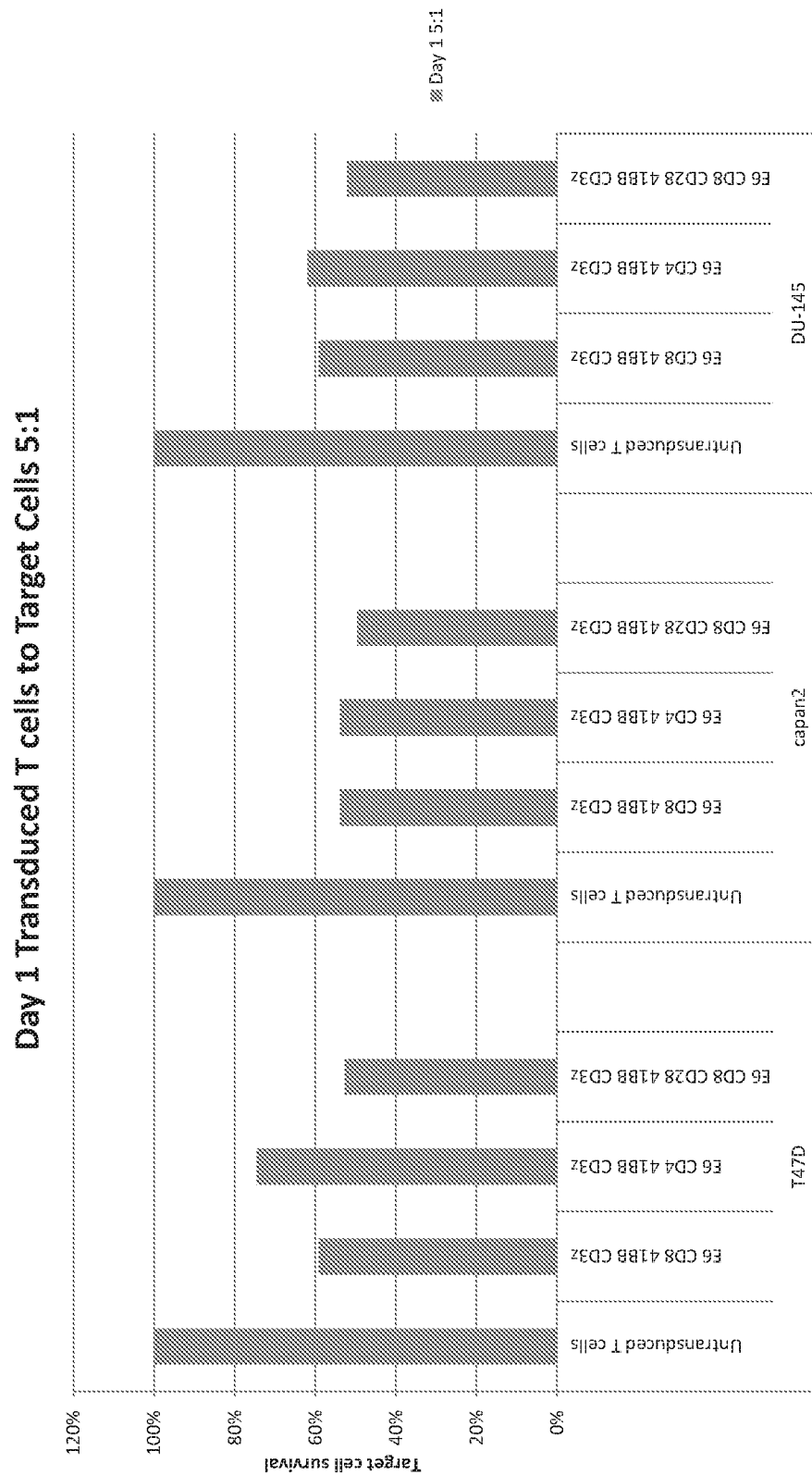


Figure 37

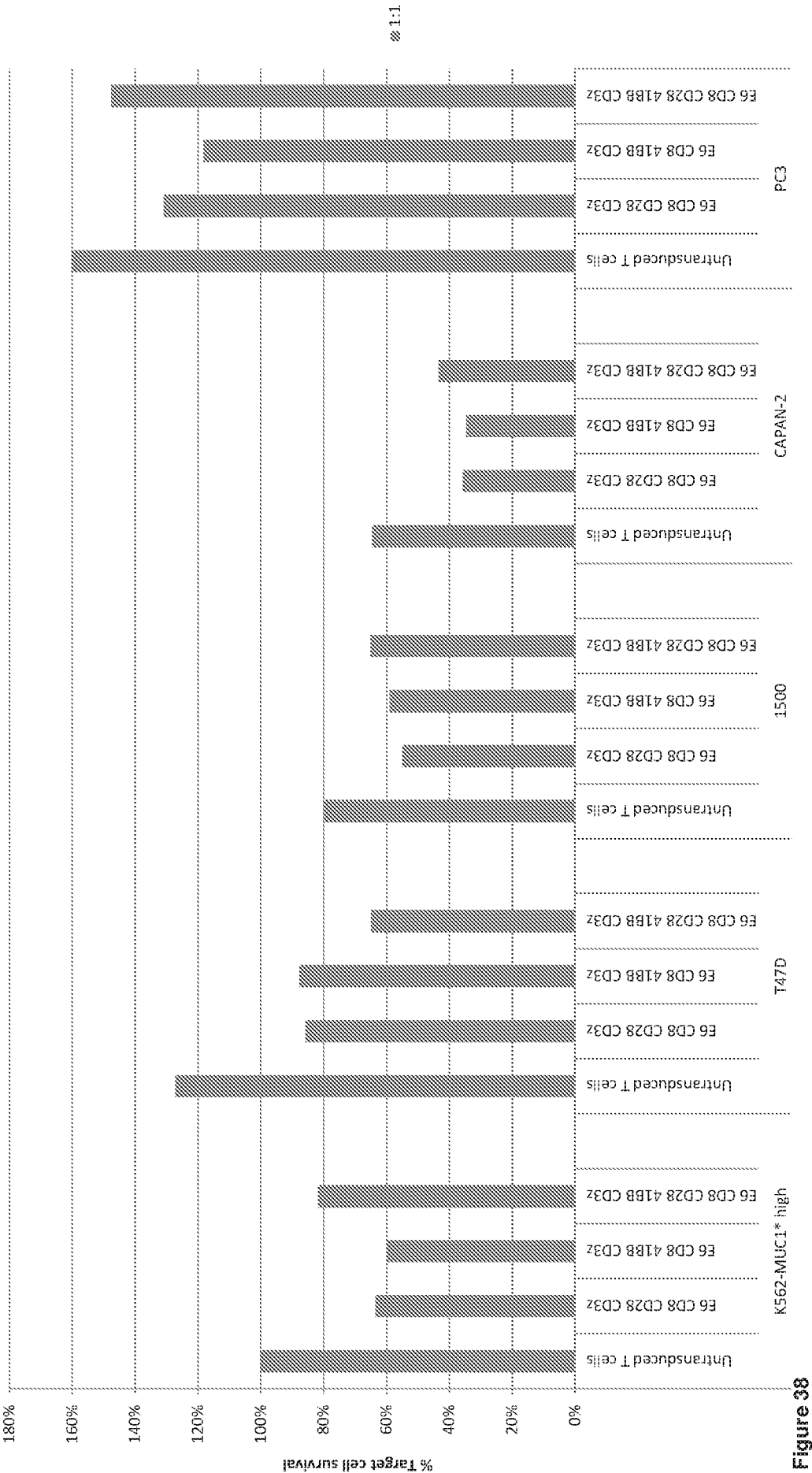


Figure 38

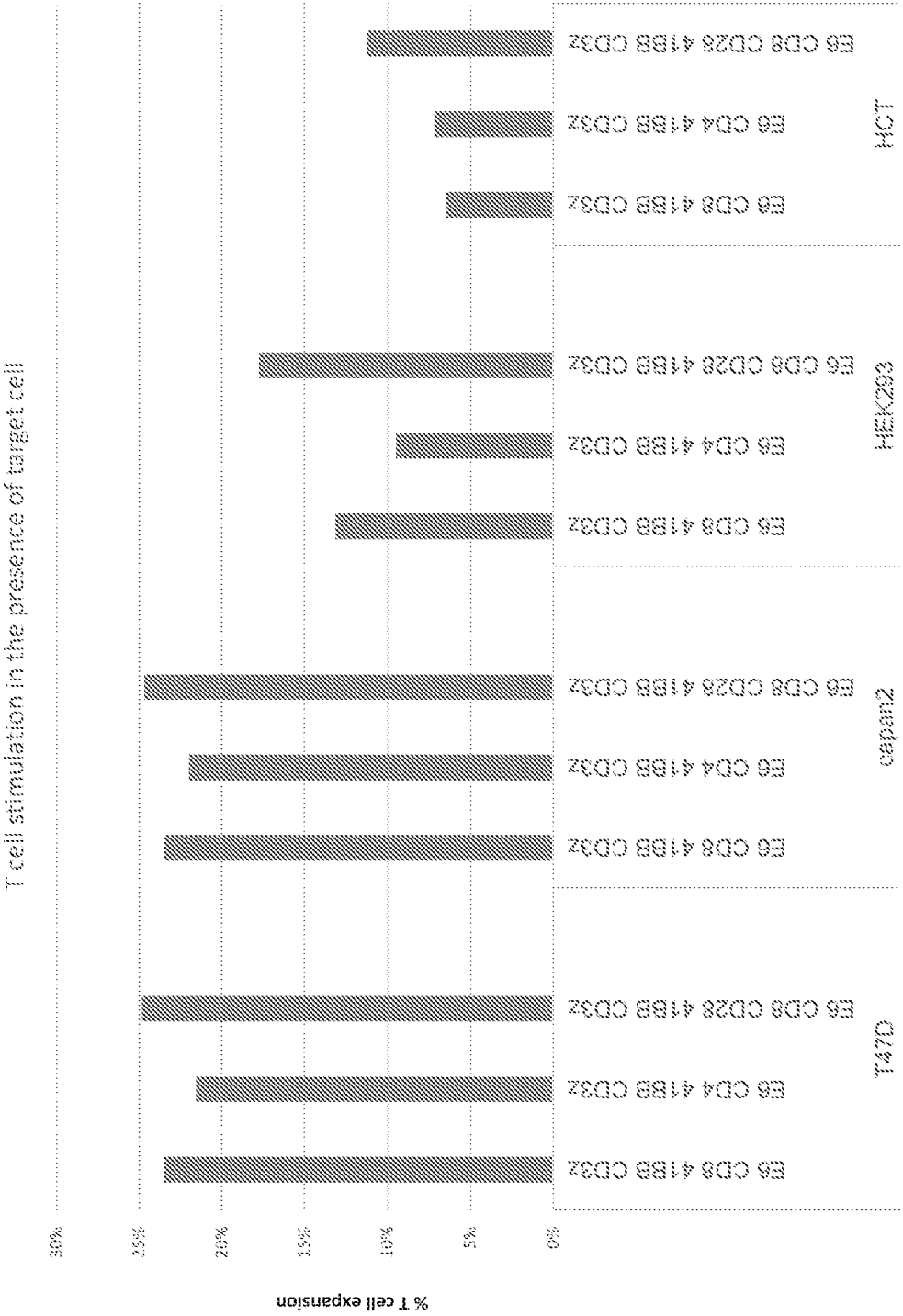


Figure 39

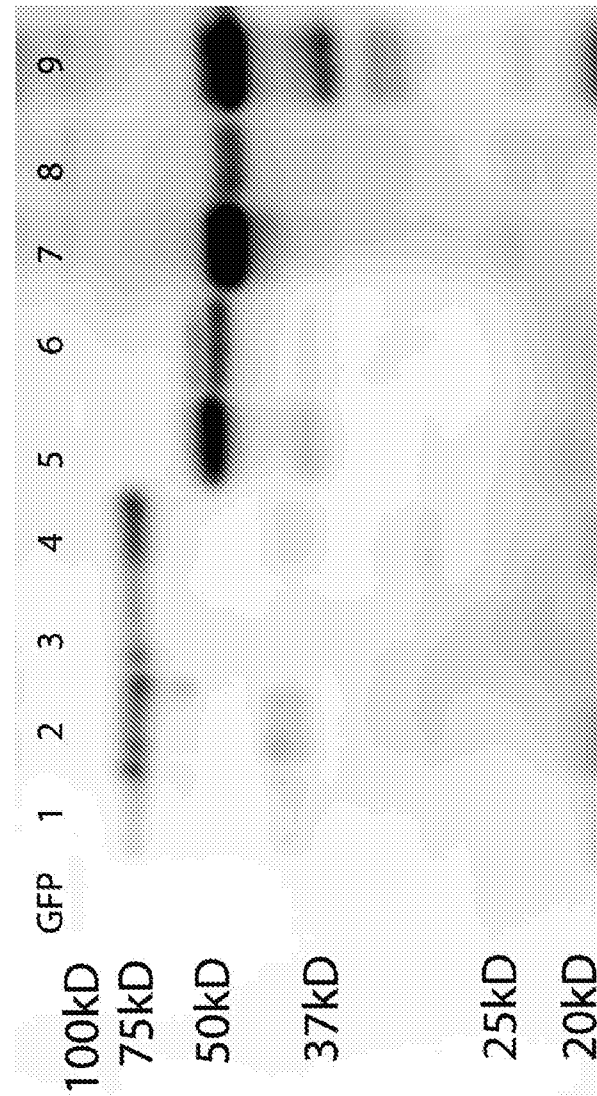
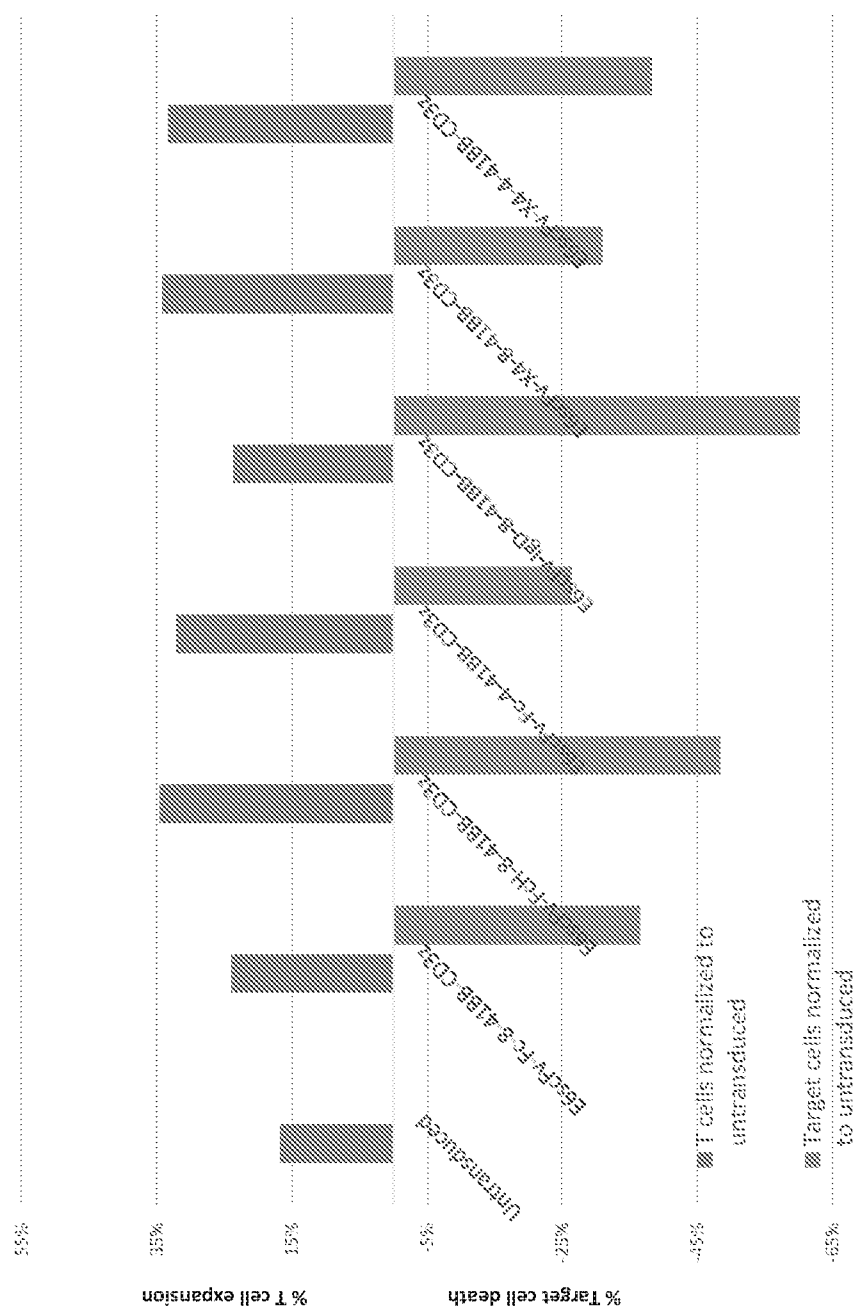


Figure 40



Page 41

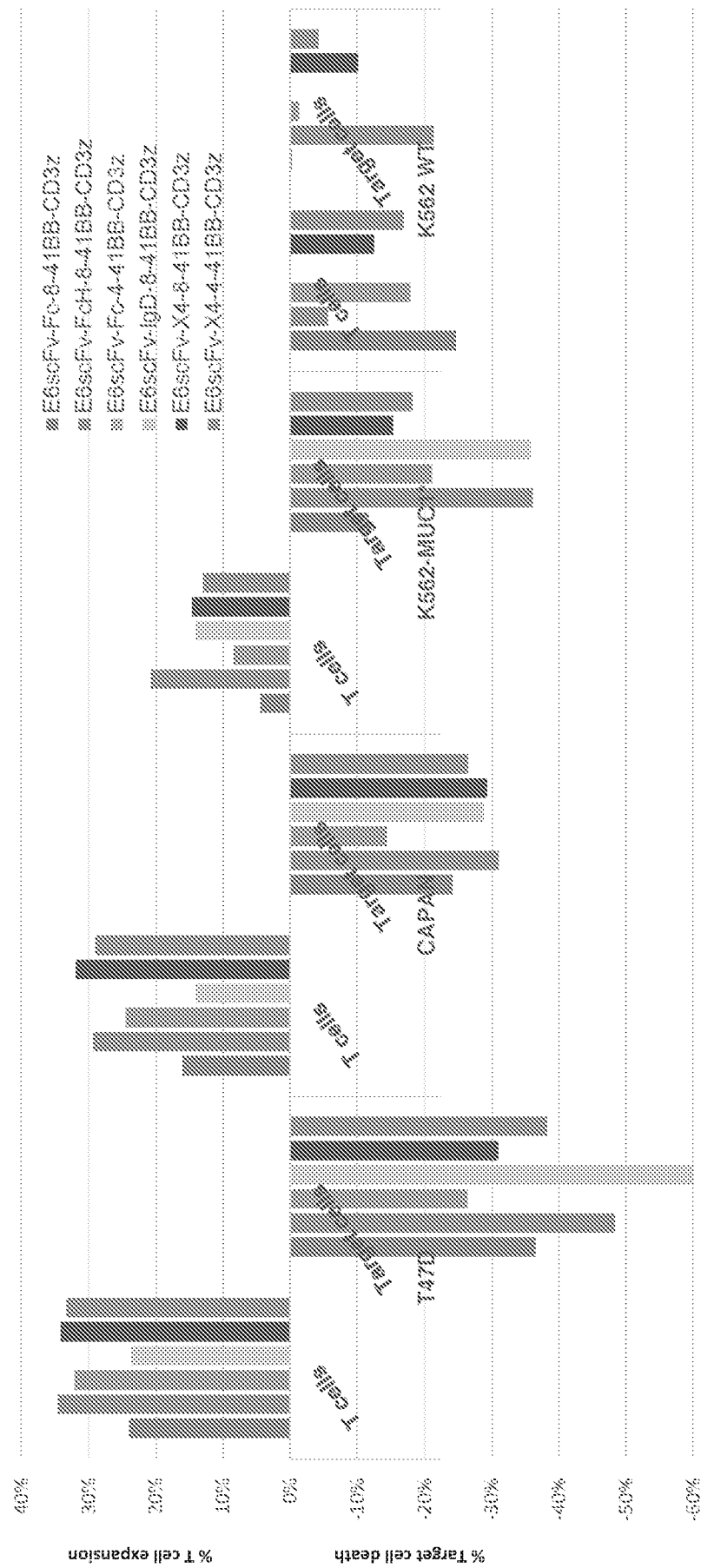


Figure 42

Tissue arrays comprising specimens from 240 breast cancer patients were stained with an antibody (VU4H5) that recognizes full-length MUC1 (left) or stained with an antibody that recognizes MUC1* (MN-C2). The data show that most or all (green boxes) of the MUC1 on cancerous tissue is MUC1* and not MUC1 full-length (MUC1-FL). The data further show that MN-C2 monoclonal antibody binds to cancerous tissue but not the healthy control tissue.

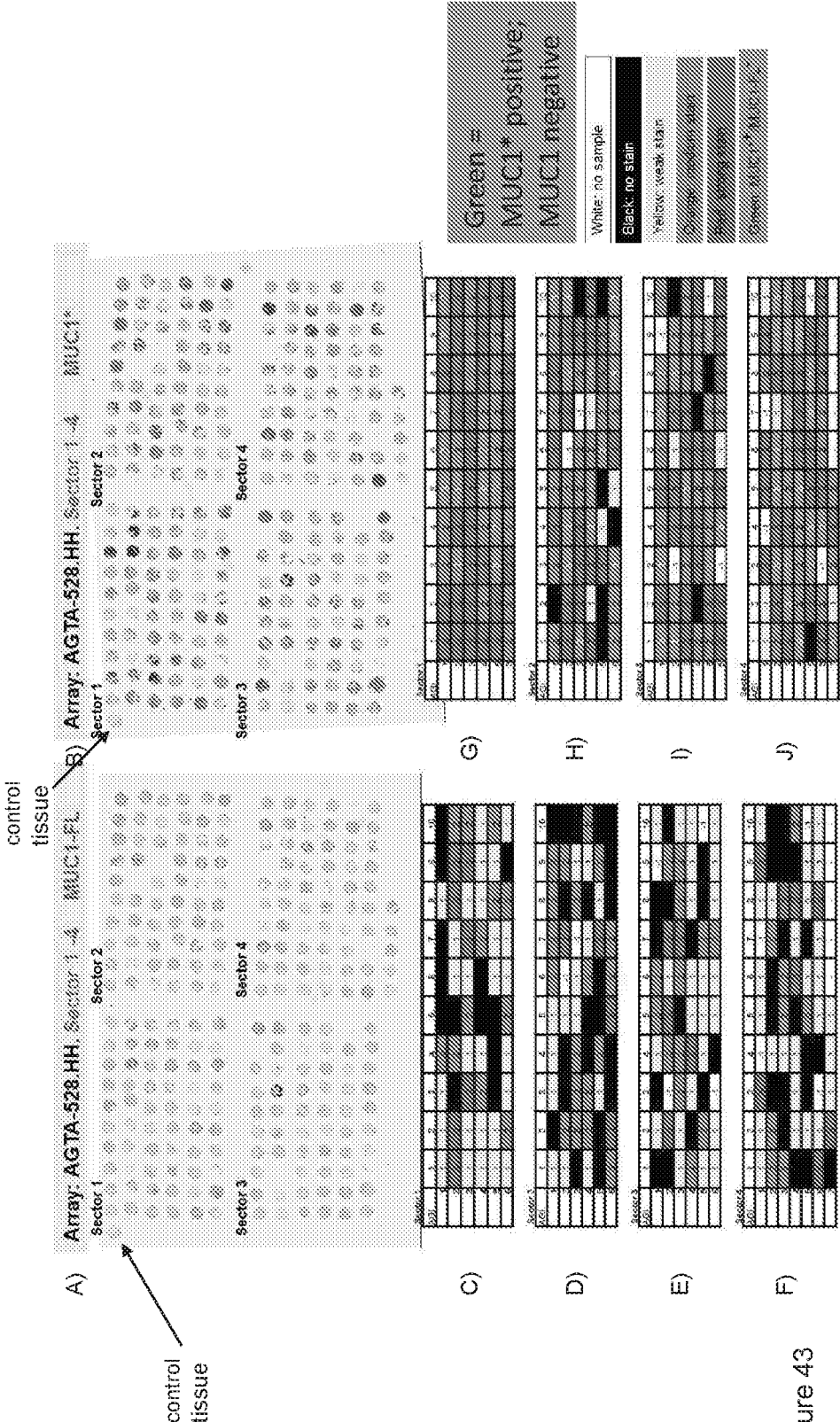


Figure 43

Tissue arrays comprising specimens from 240 breast cancer patients were stained with an antibody (VU4H5) that recognizes full-length MUC1 (left) or stained with an antibody that recognizes MUC1* (MN-C2). The data show that most or all (green boxes) of the MUC1 on cancerous tissue is MUC1* and not MUC1 full-length (MUC1-FL). The data further show that MN-C2 monoclonal antibody binds to cancerous tissue but not the healthy control tissue.

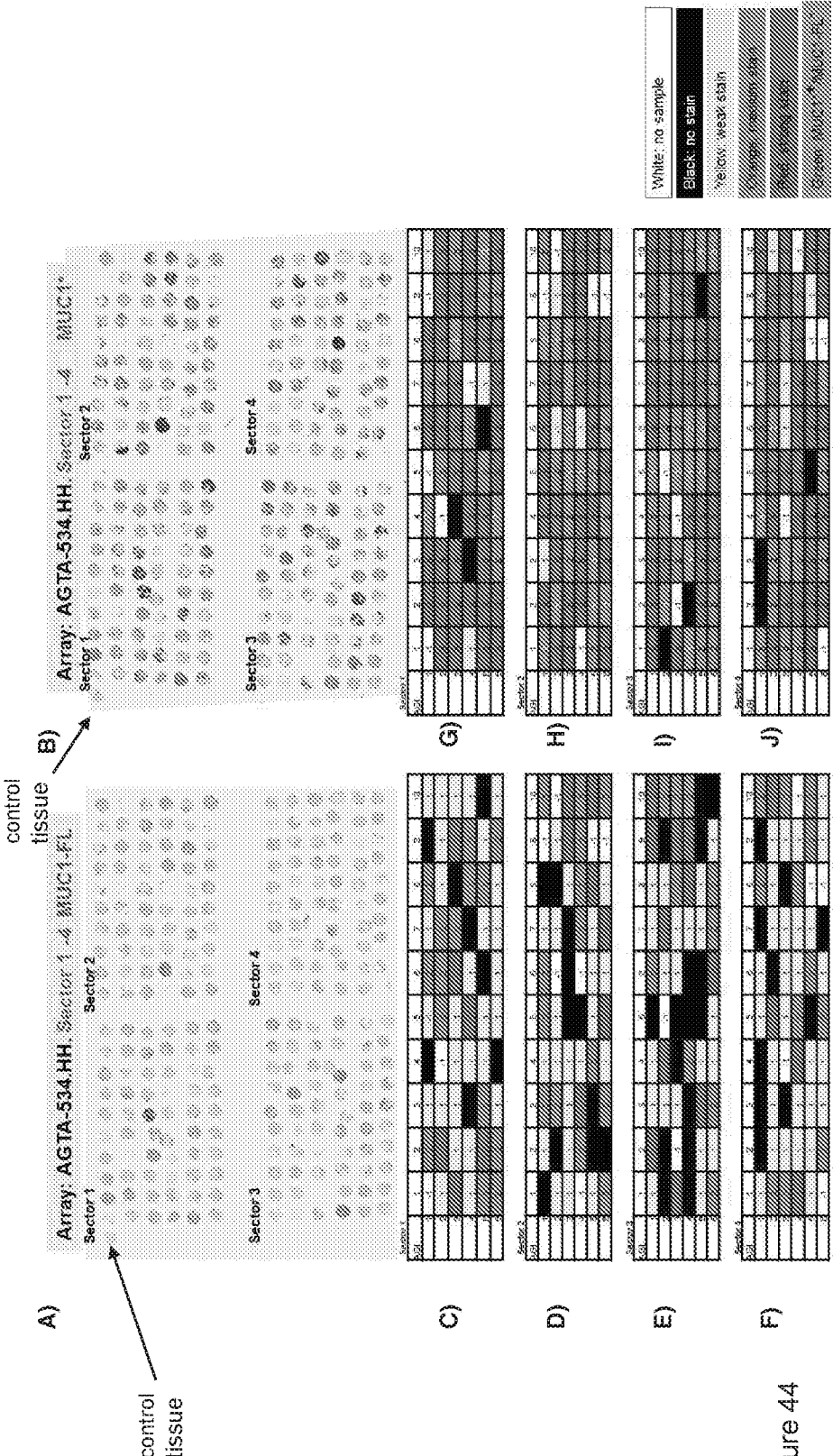


Figure 44

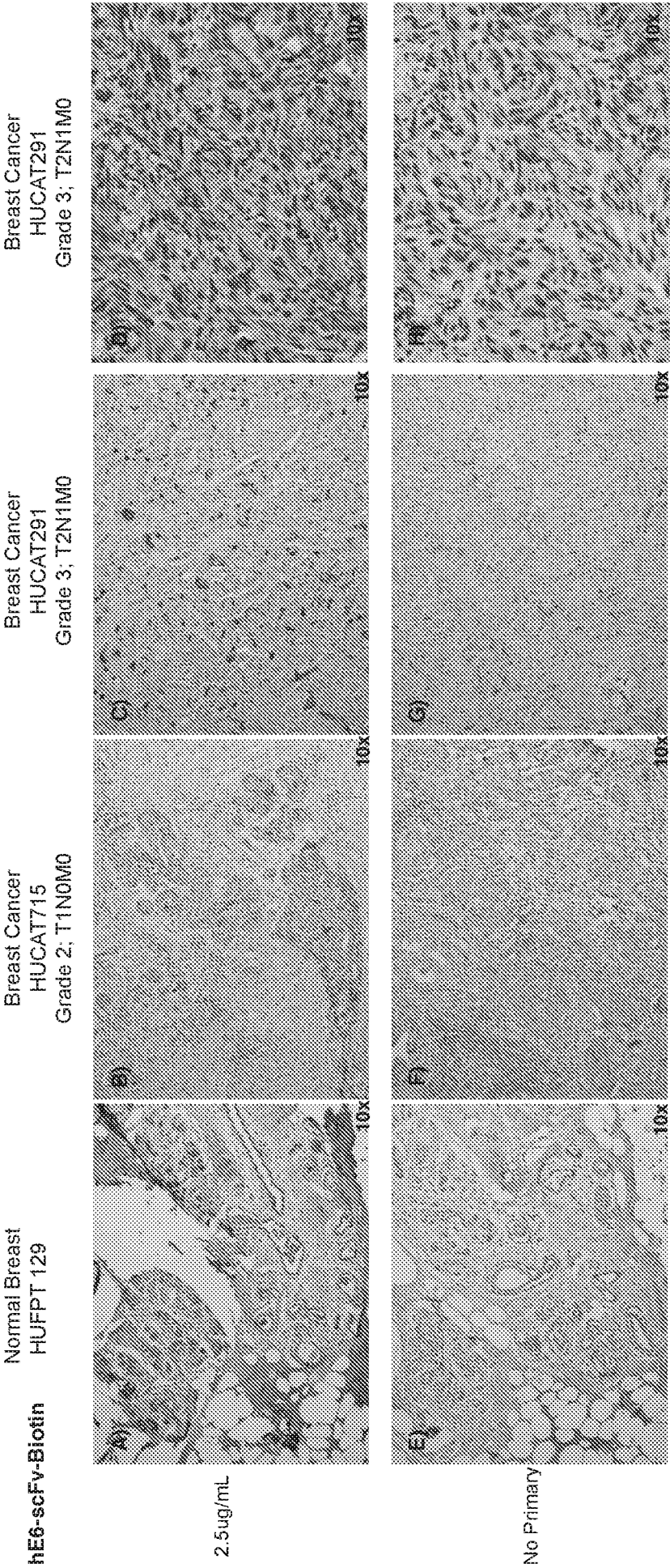


Figure 45

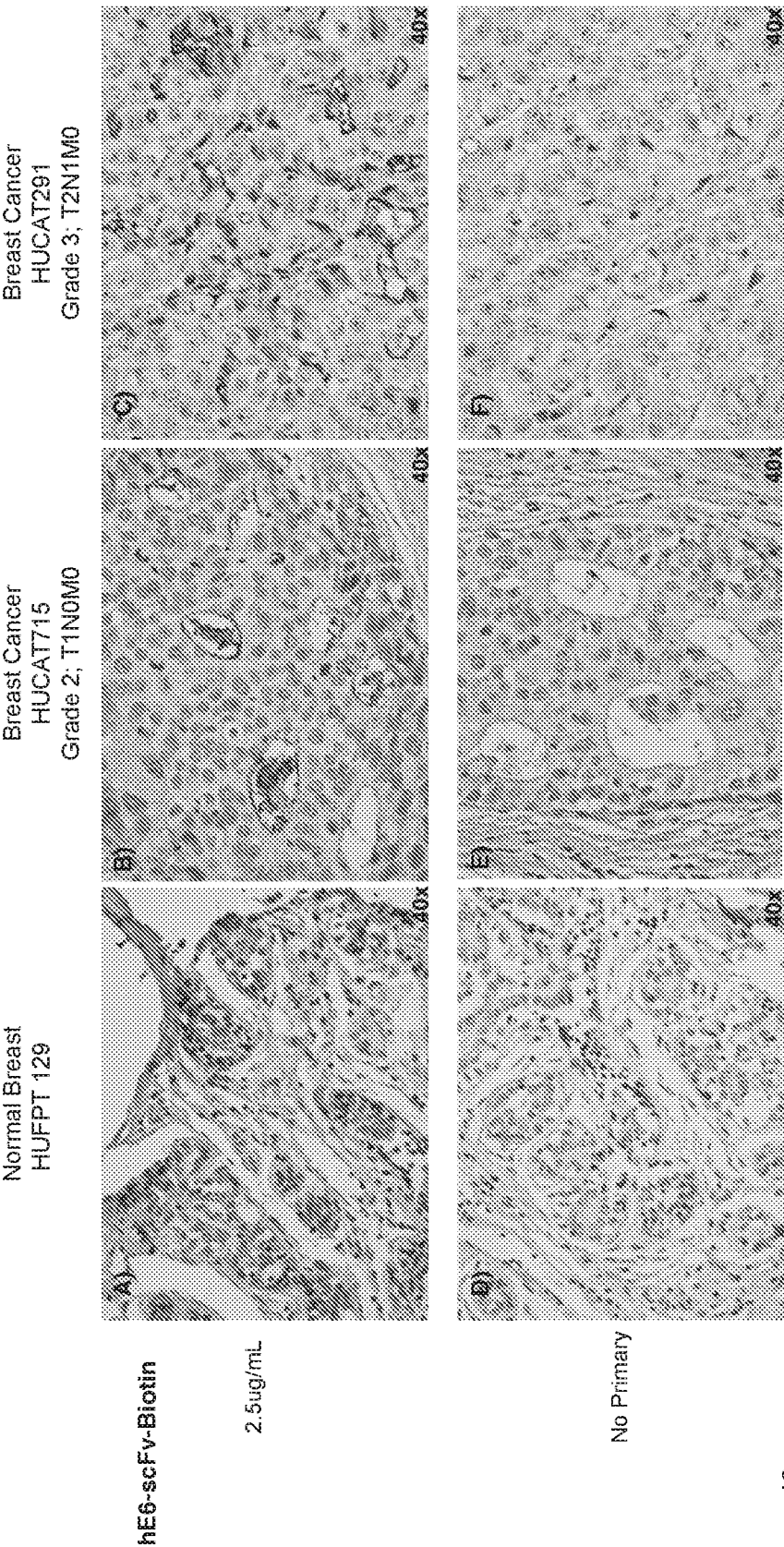


Figure 46

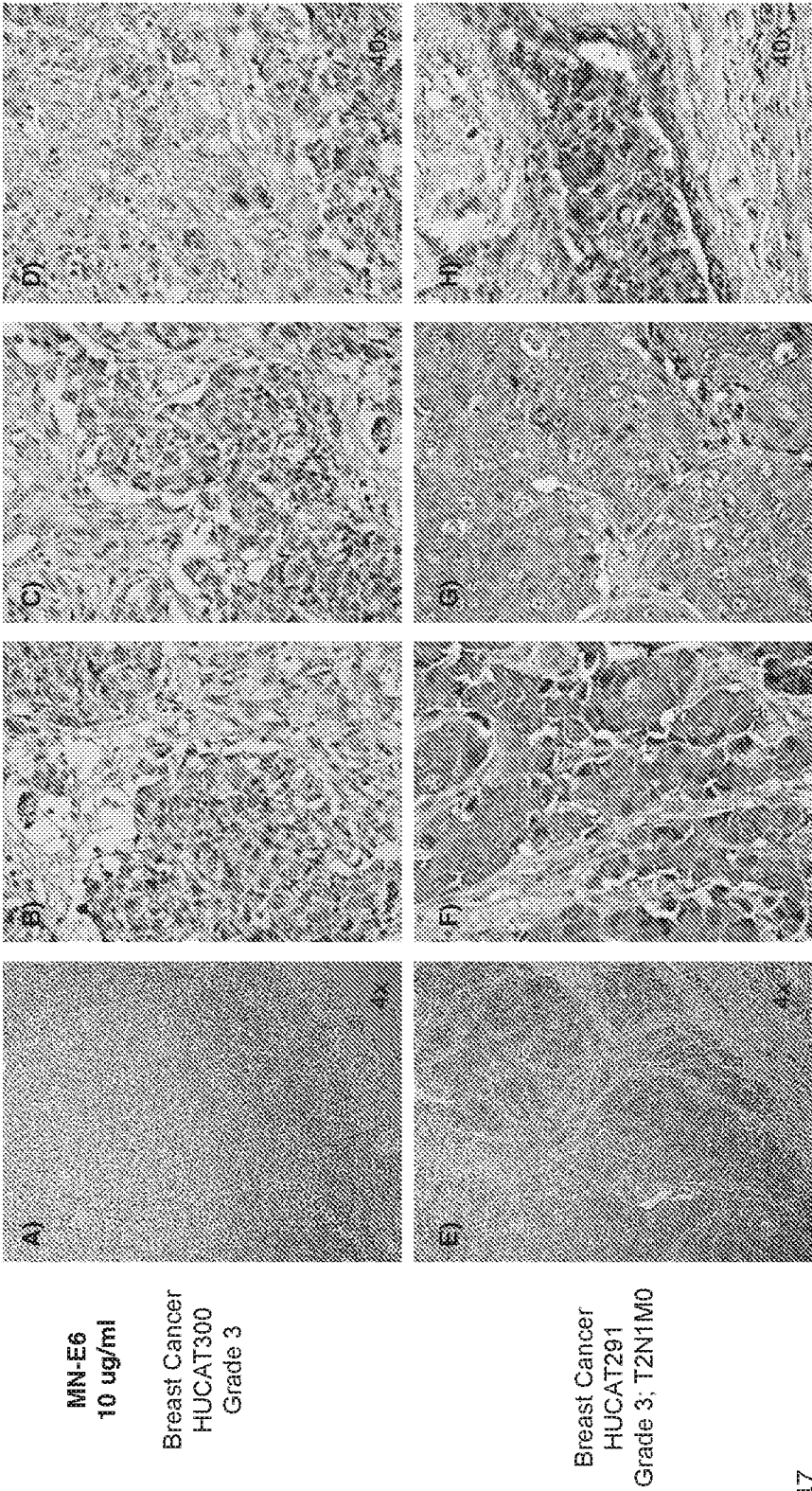


Figure 47

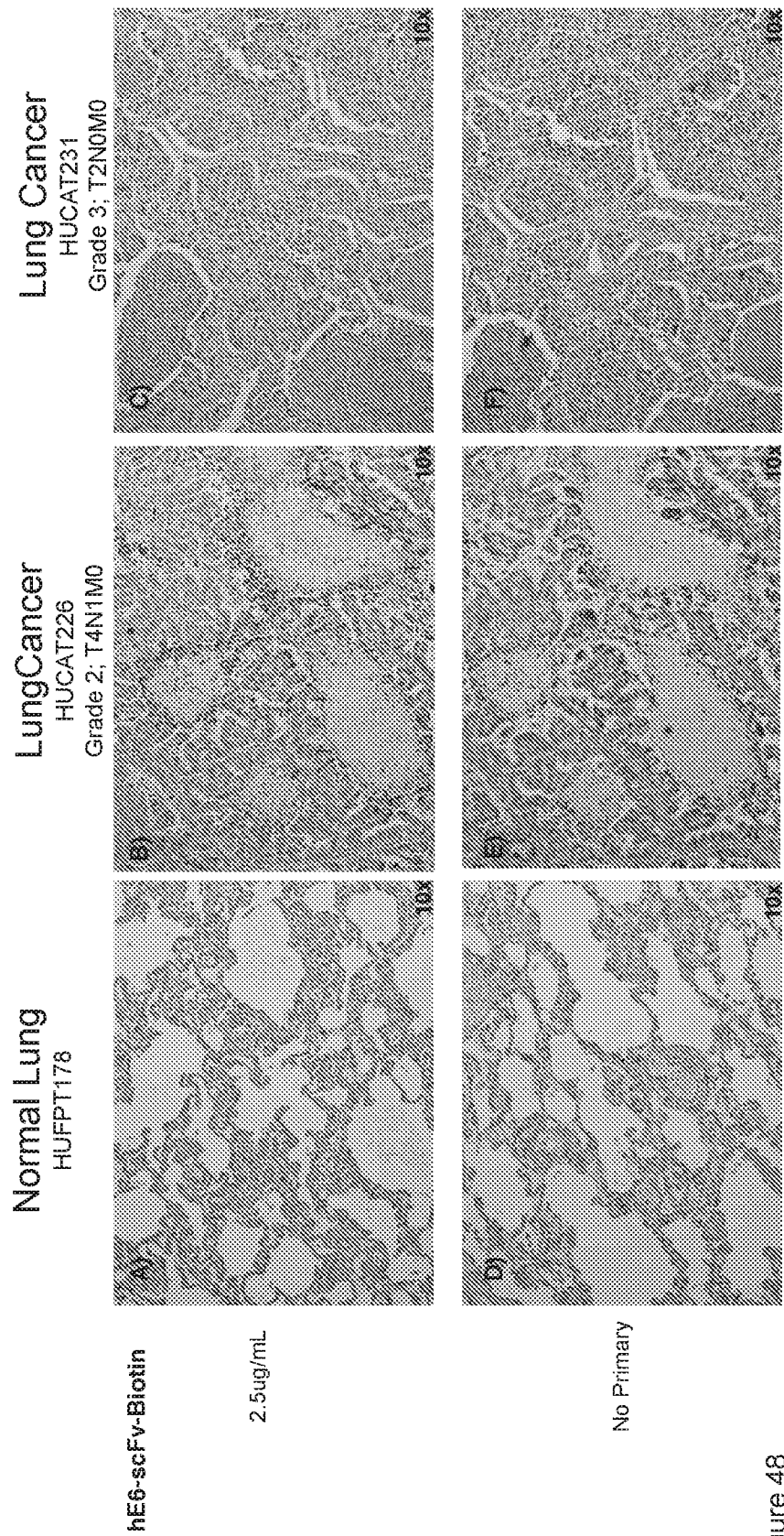


Figure 48

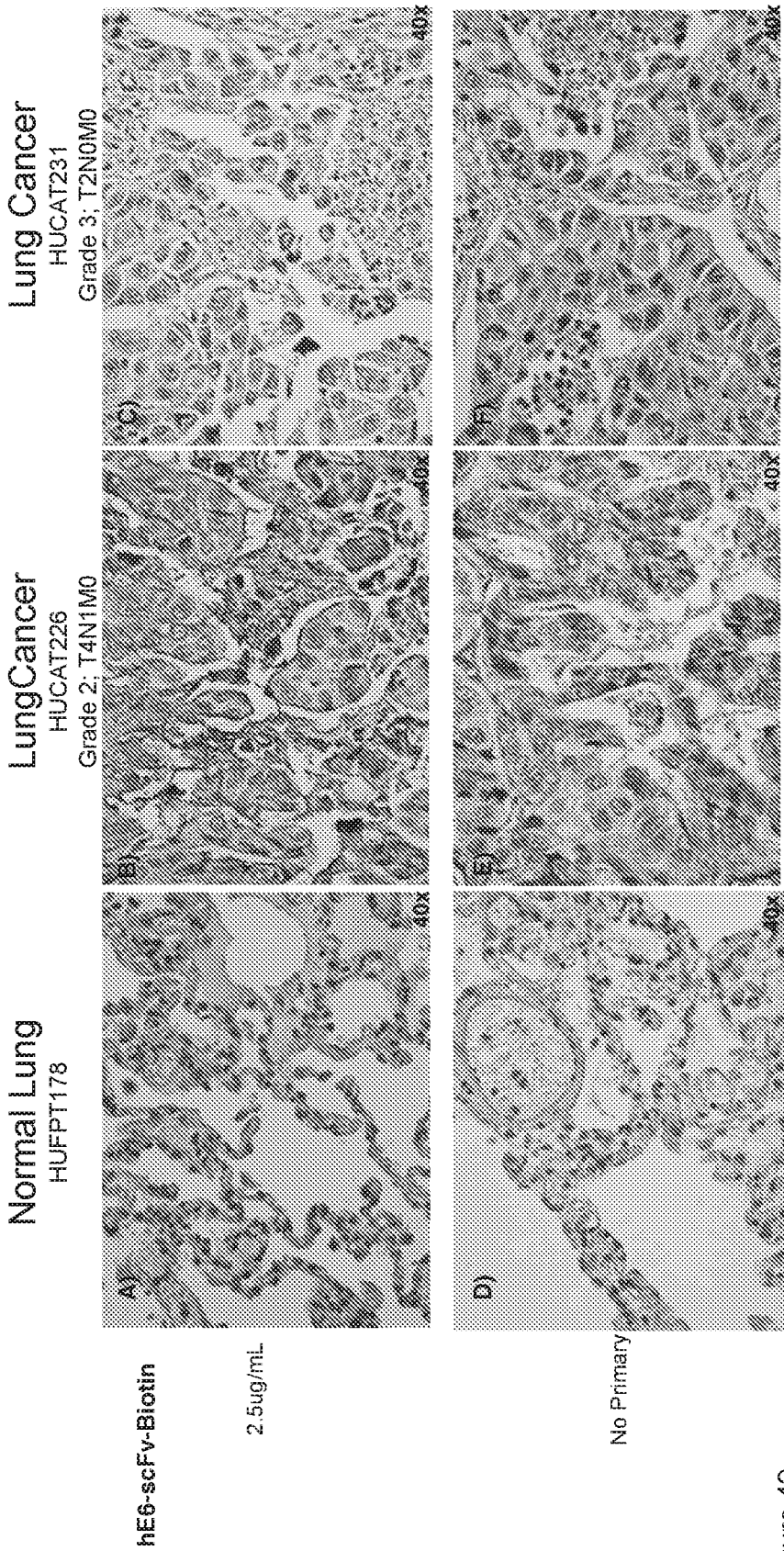


Figure 49

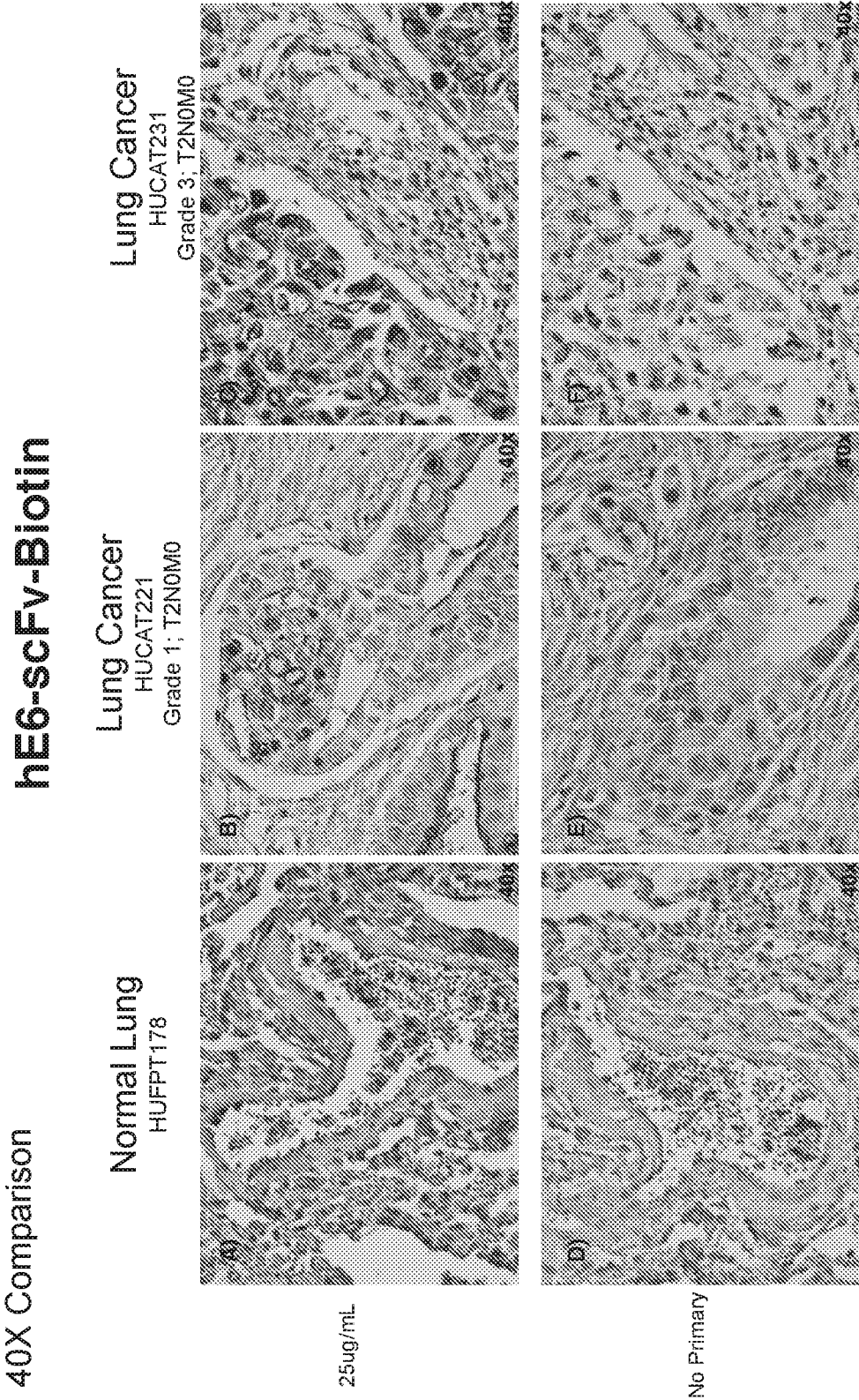


Figure 50

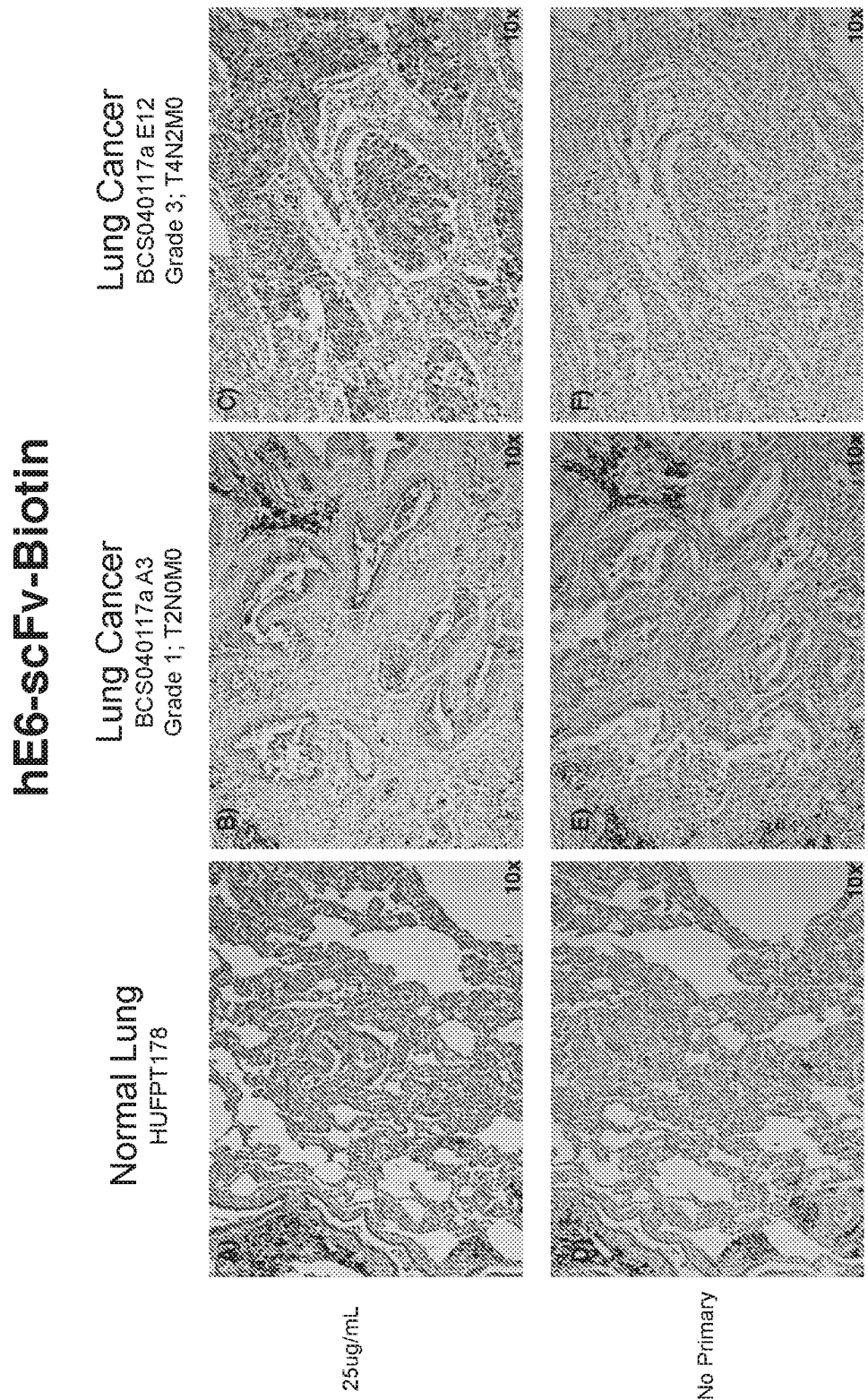
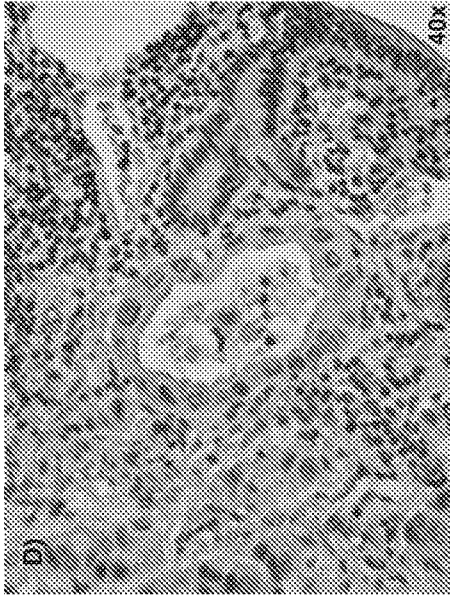
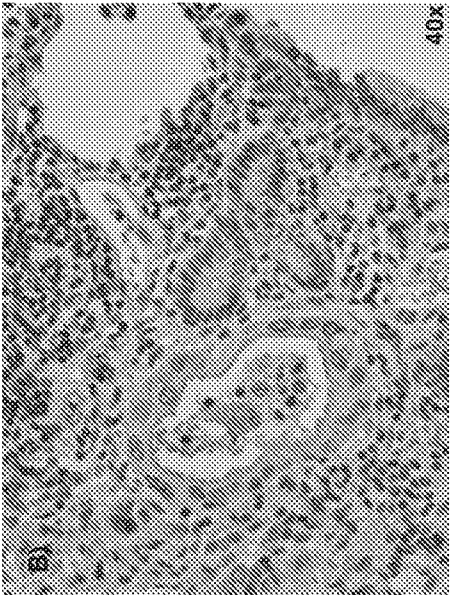
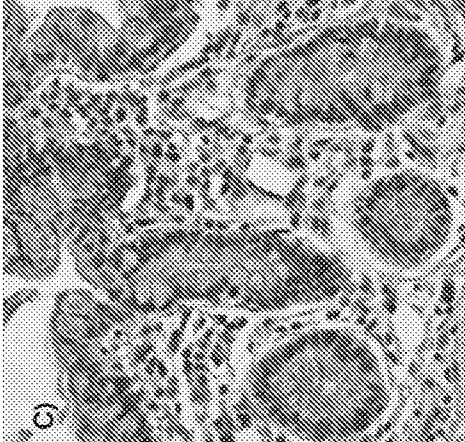


Figure 51

Small Intestine Cancer
HUCAT101m
Grade 3; T3N0M0



Normal Small Intestine
HUFPT081

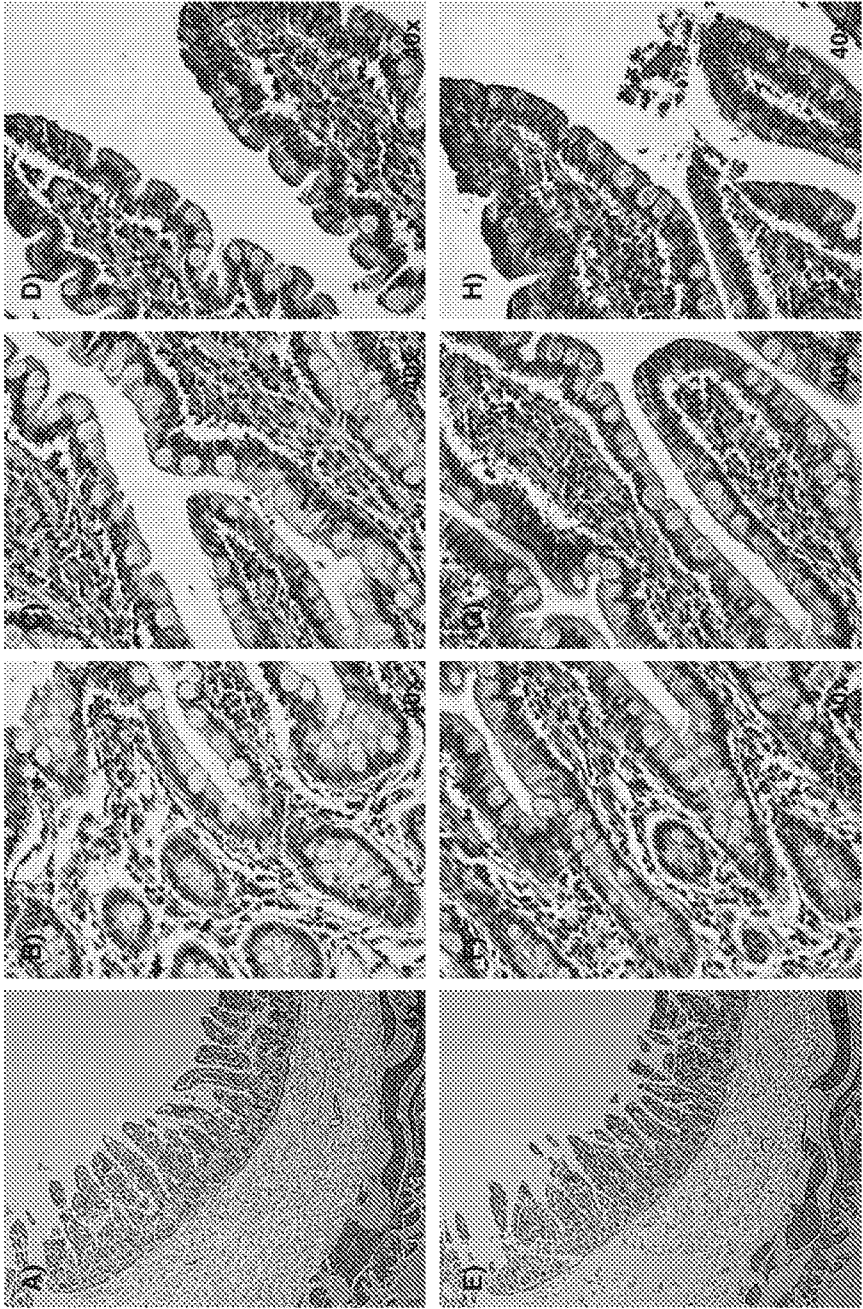


hu-E6-scFv-Fc-
biotin
5ug/mL

No Primary

Figure 52

Small Intestine Normal
HUFPT081



hu-E6
scFv+Fc

50 ug/ml
2' (1:1000)

2' only control
(GaH-HRP)
1:1000

Figure 53

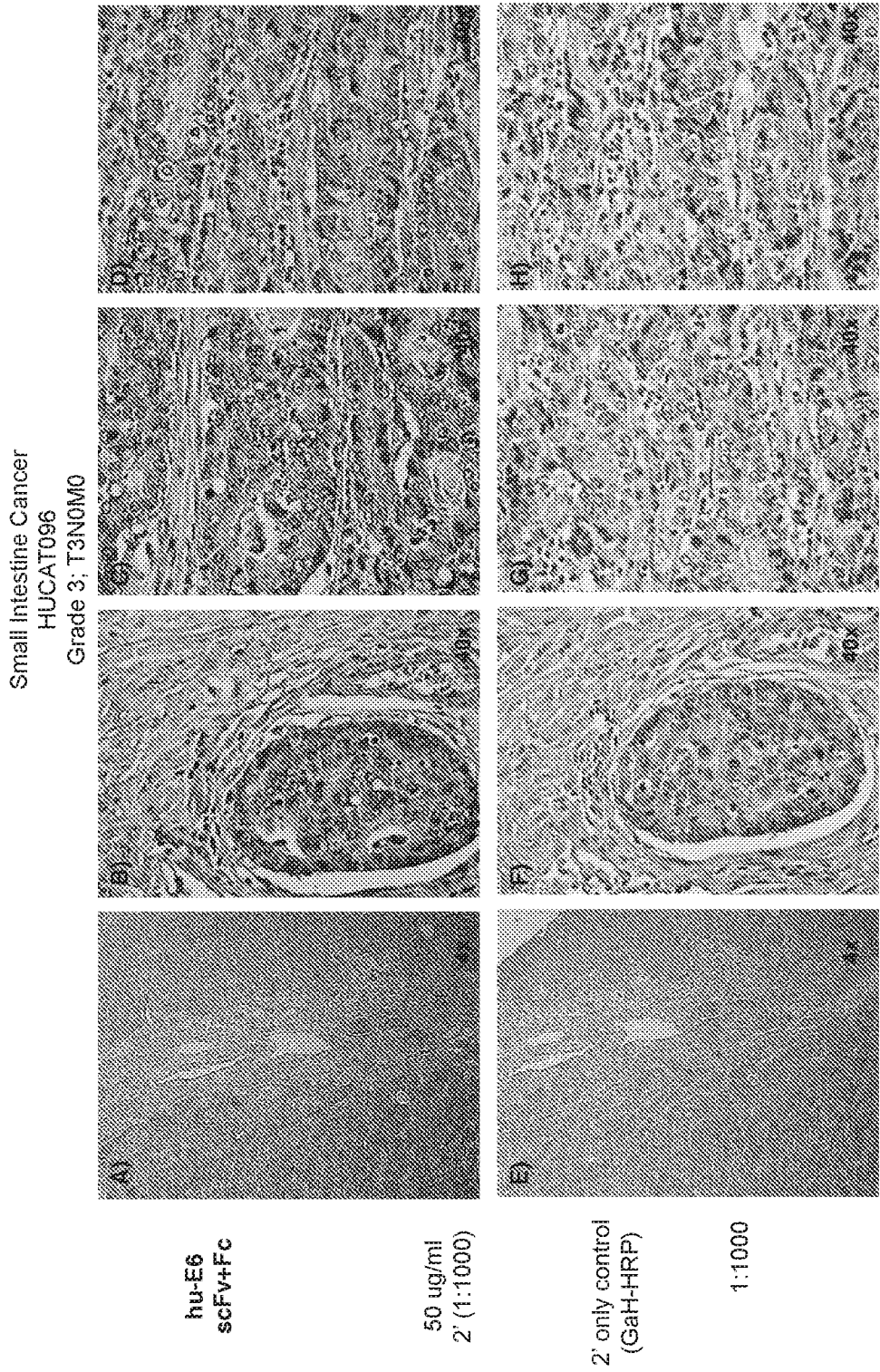


Figure 54

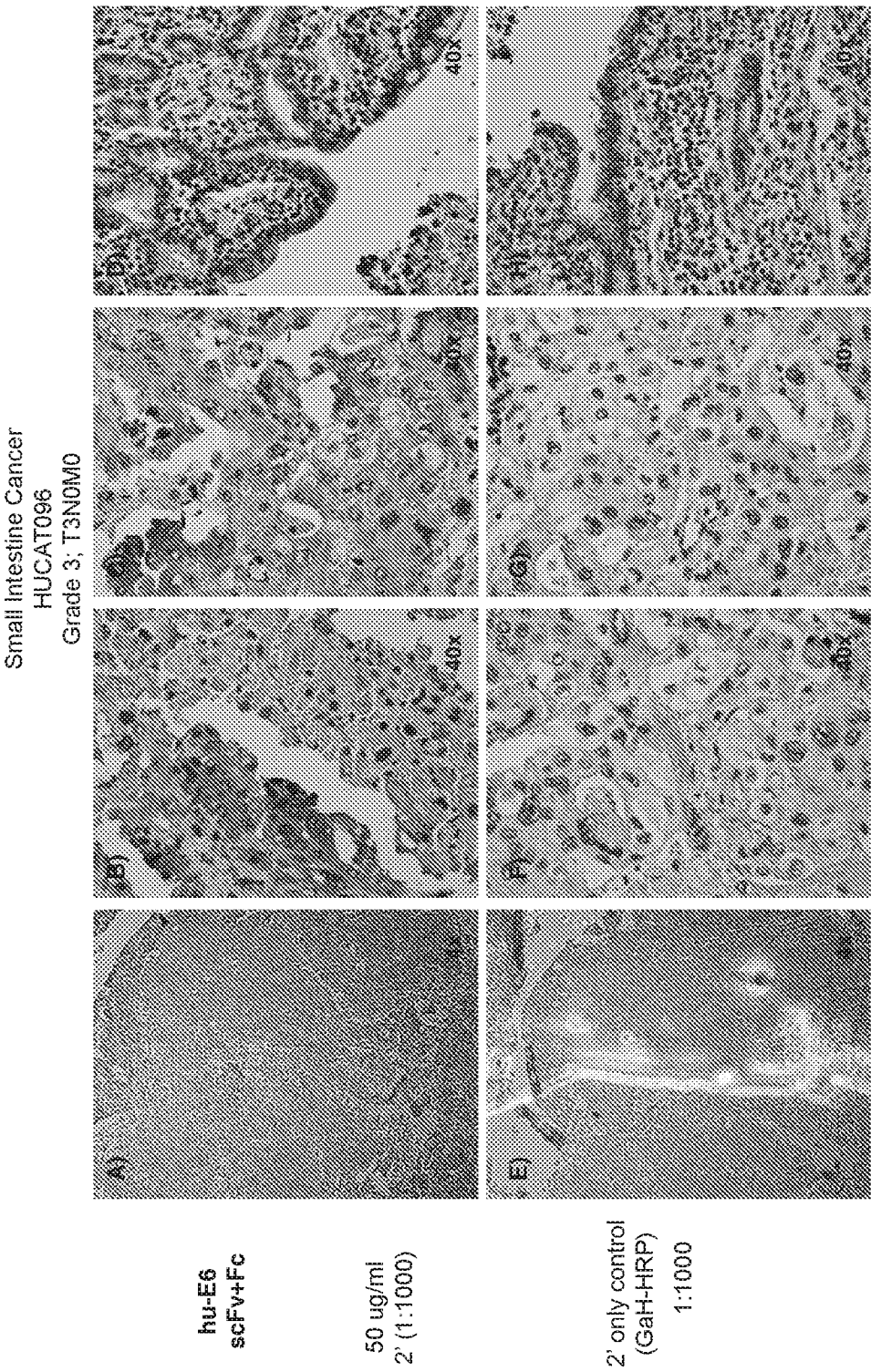


Figure 55

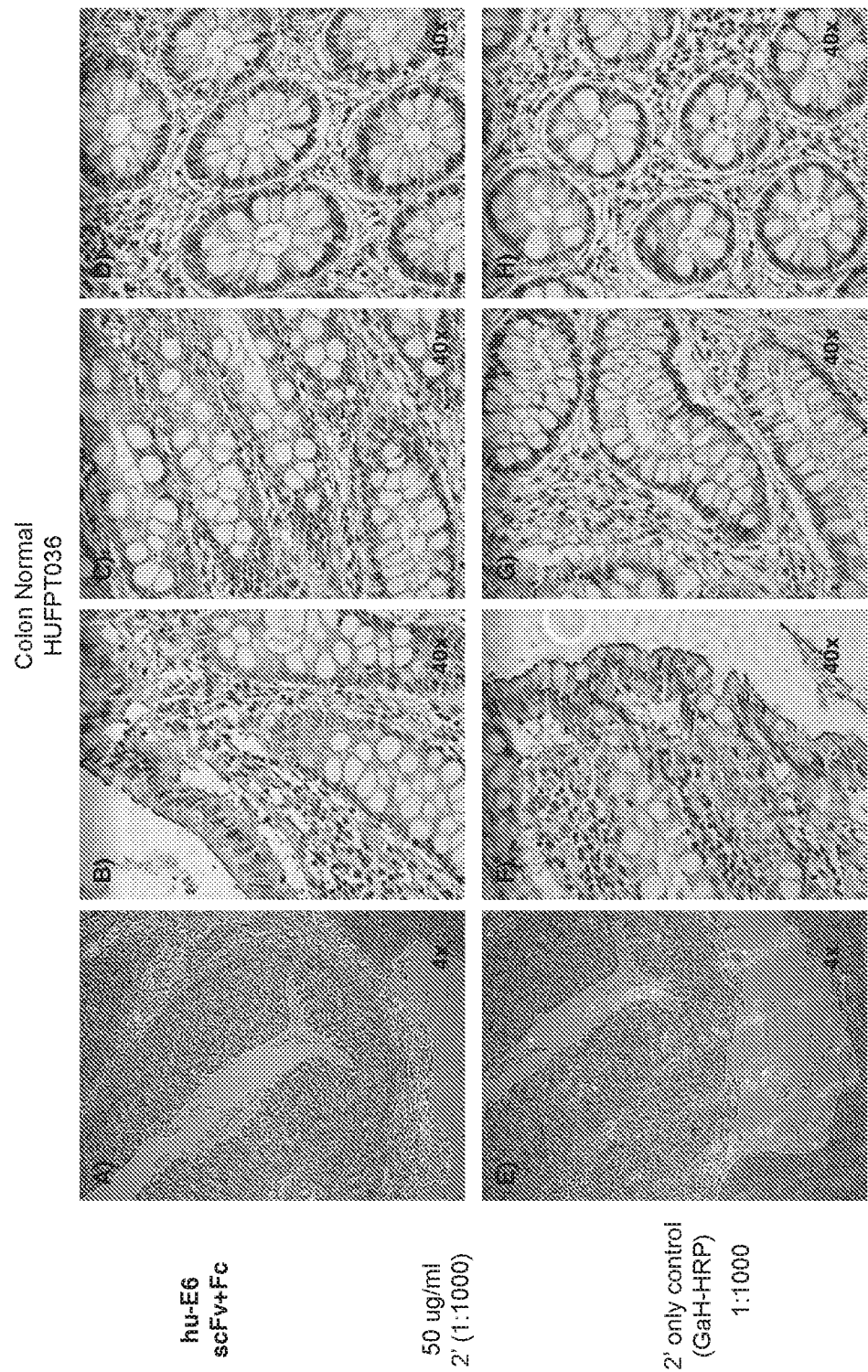


Figure 56

Colon Cancer
HUCAT116
Grade 1; T4N1M1

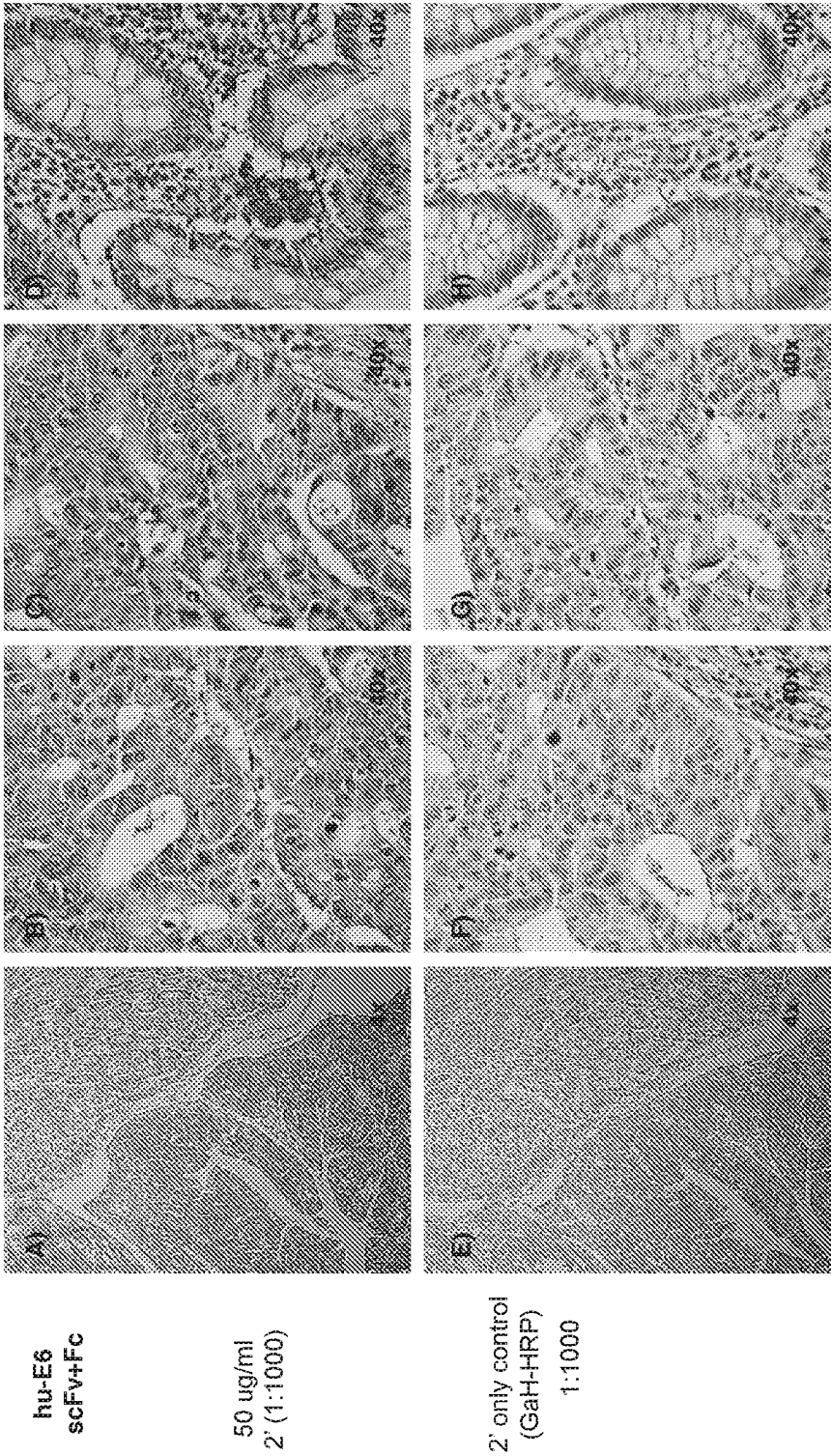


Figure 57

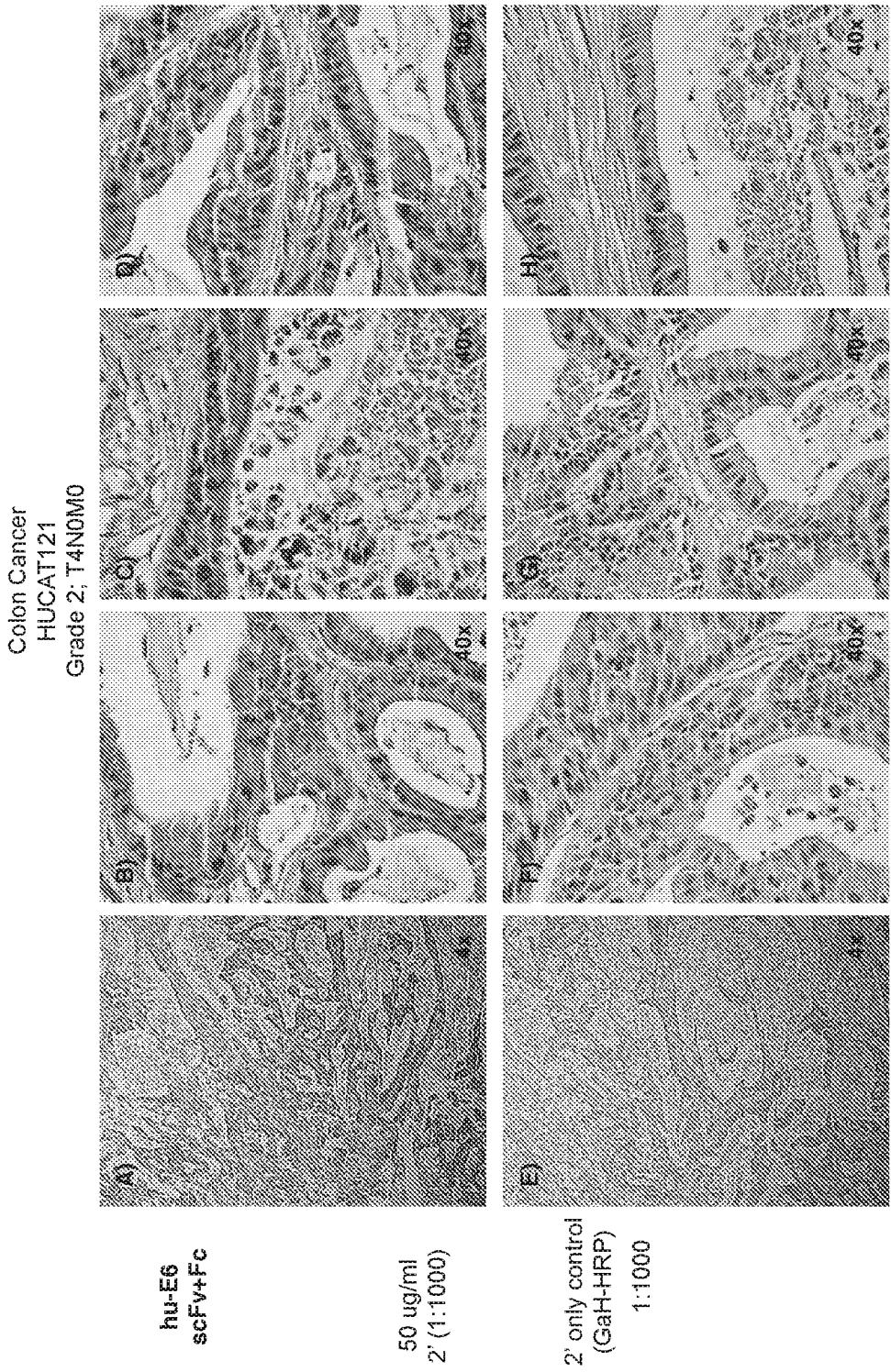


Figure 58

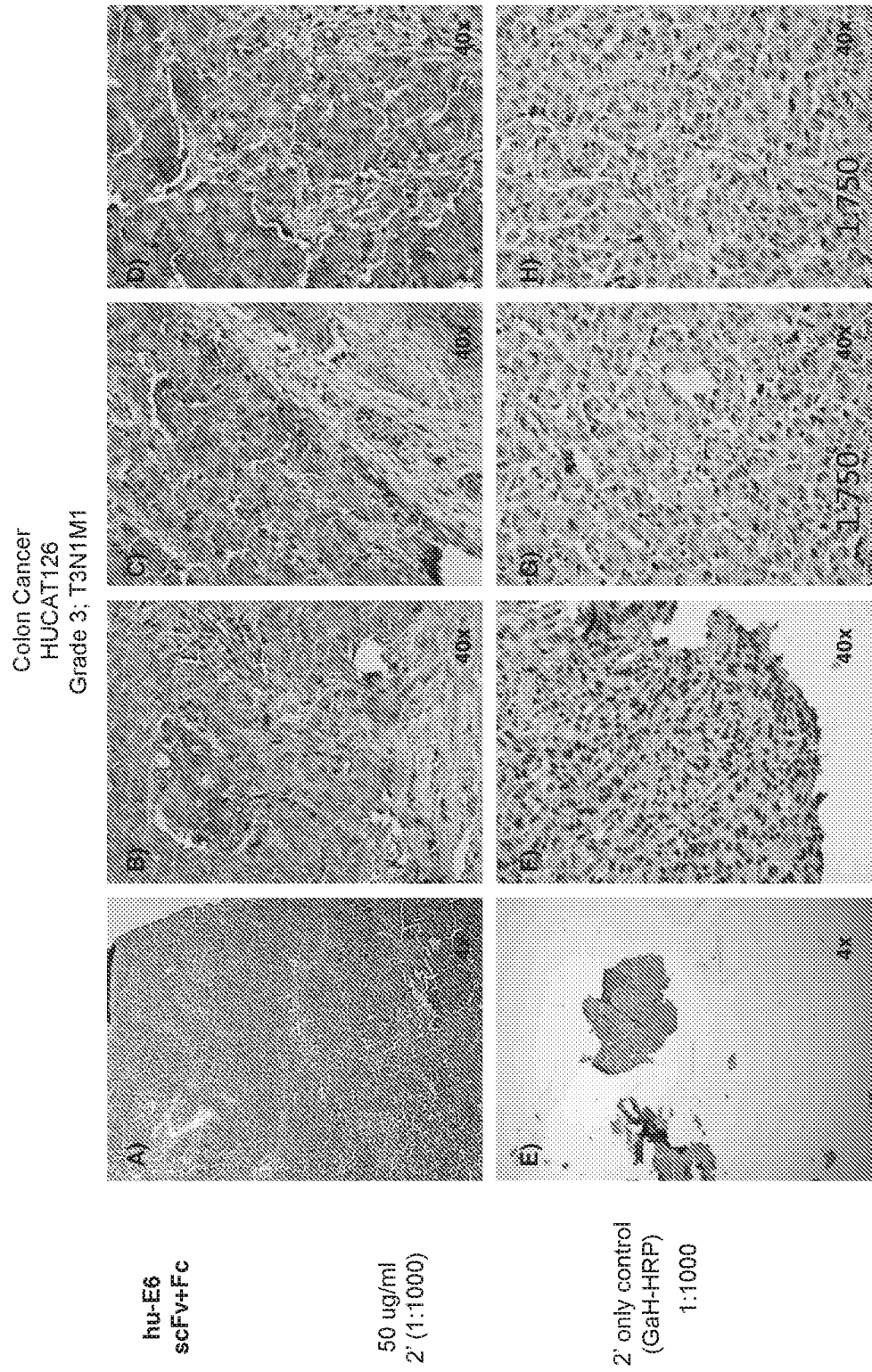


Figure 59

Prostate Cancer
HUCAT361
Grade 1; Gleason 2; 2+2

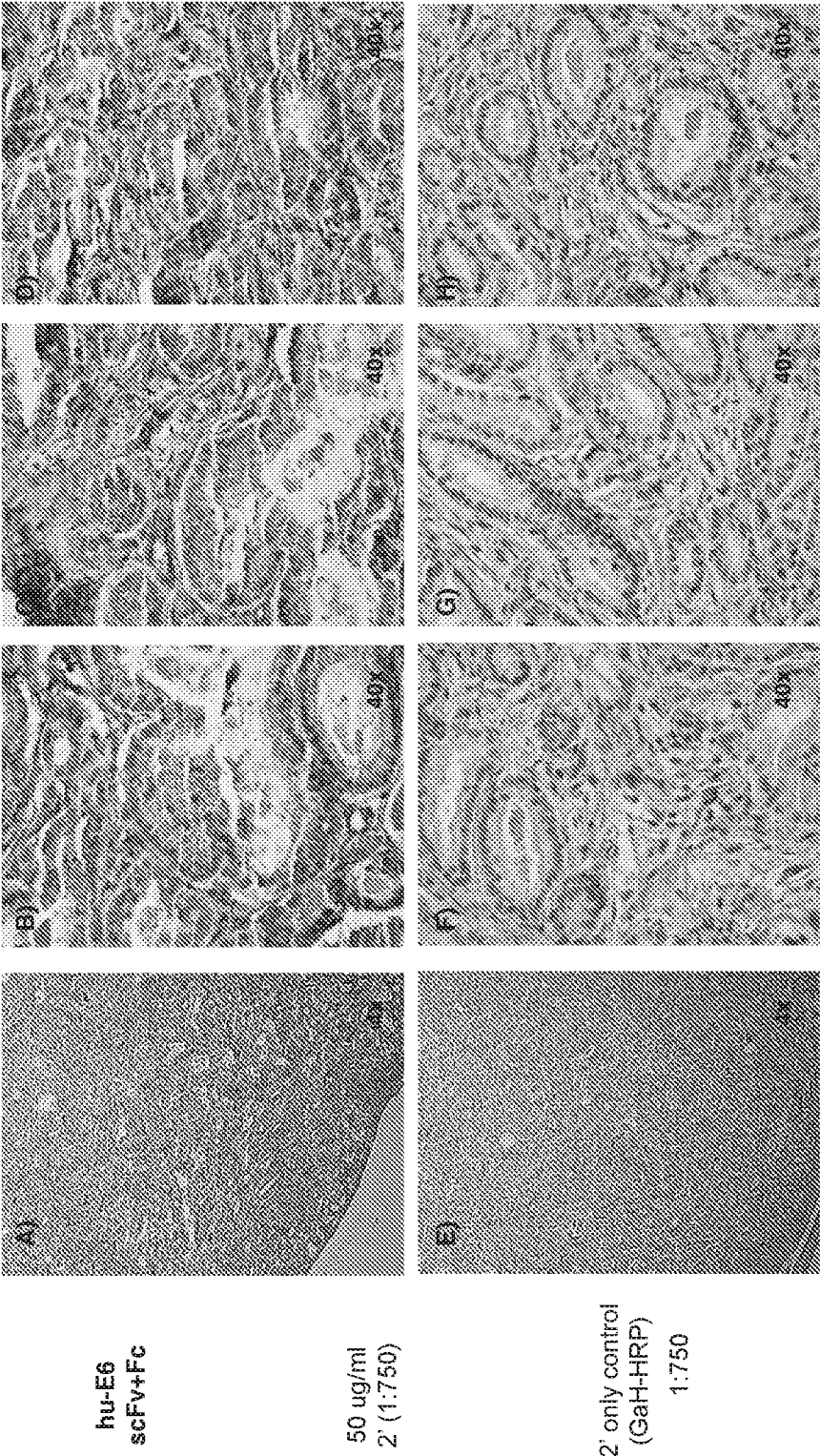


Figure 60

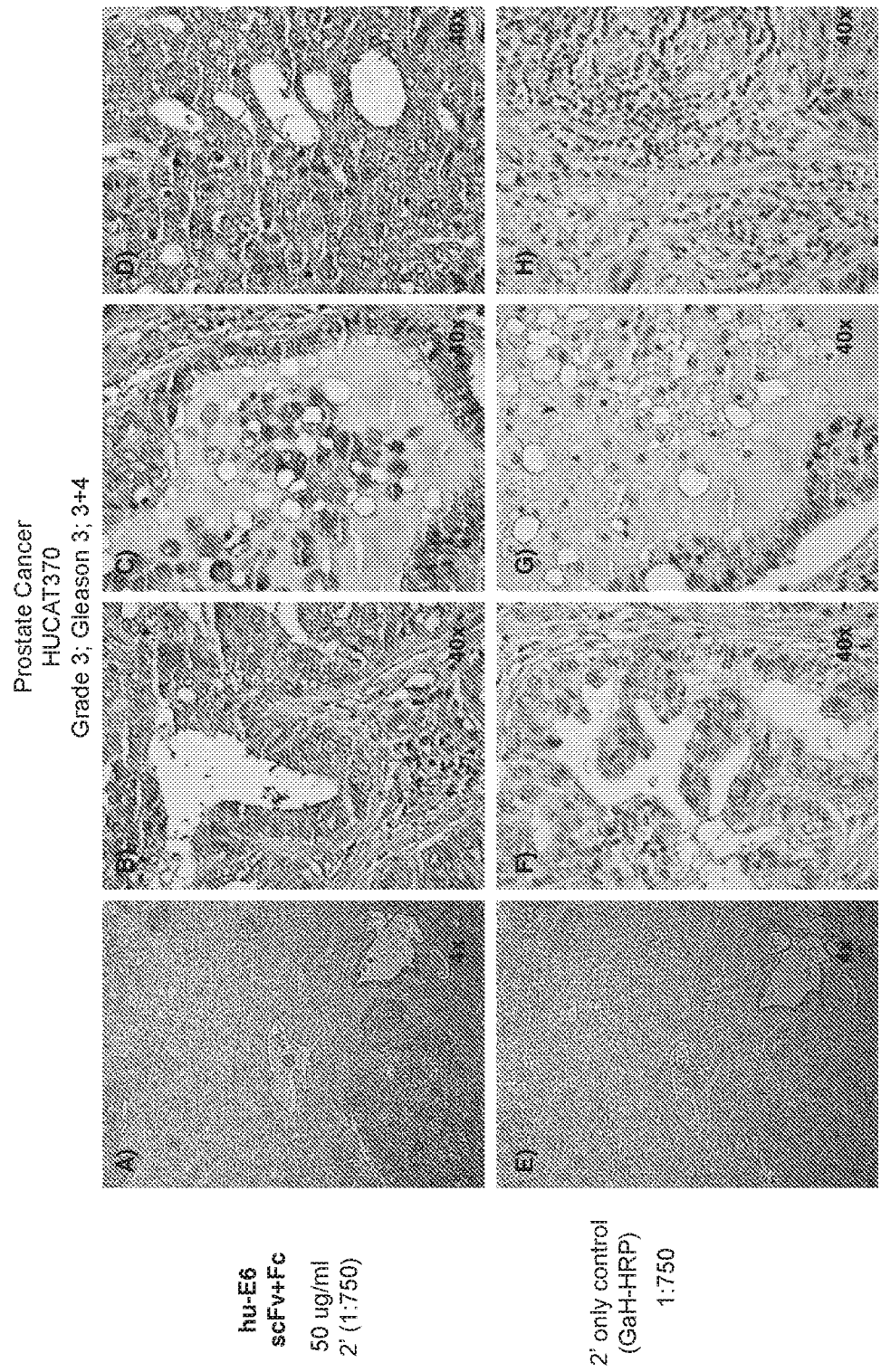


Figure 61

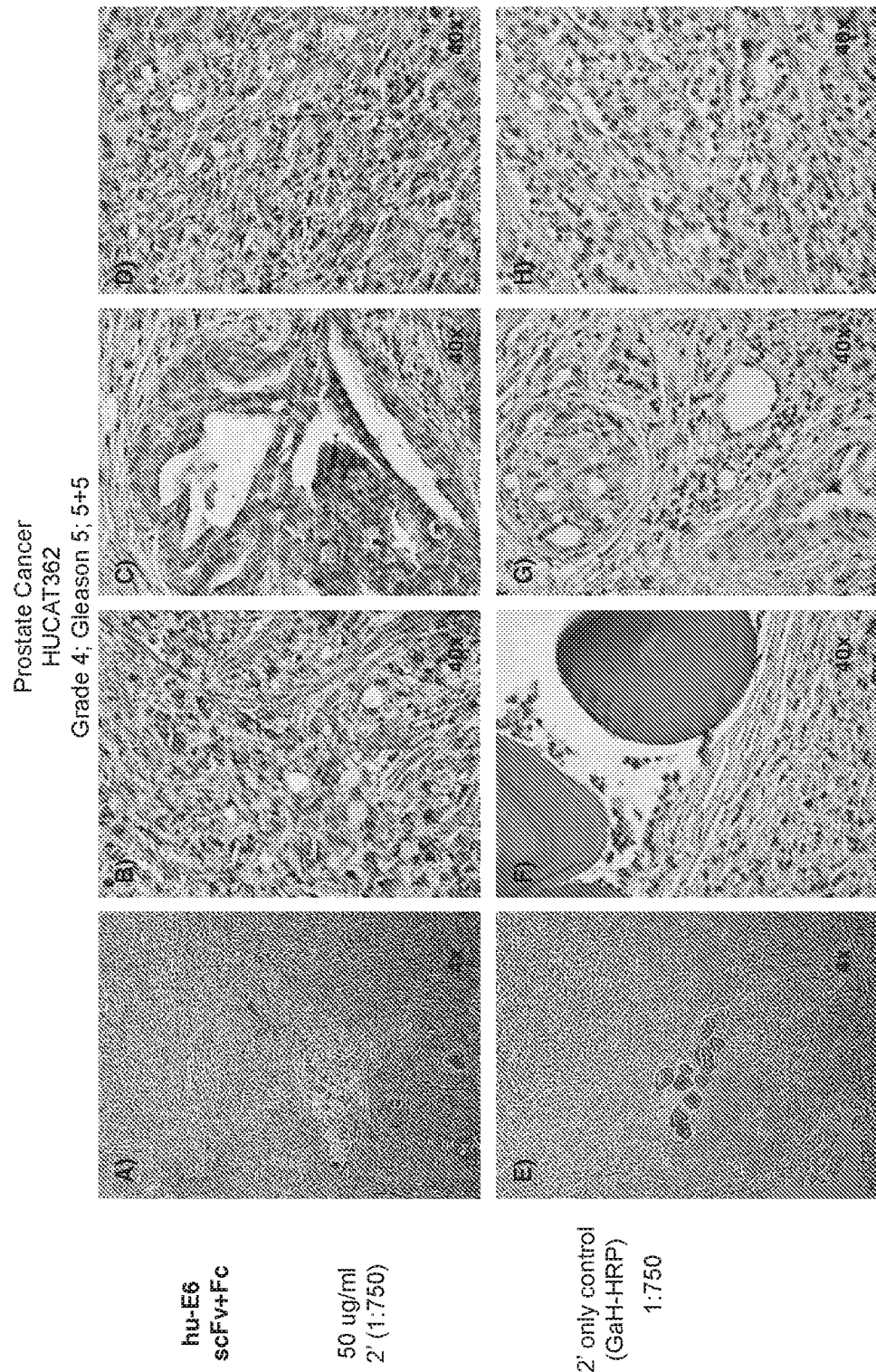


Figure 62

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/17422

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/30, C07K 16/28 (2016.01)

CPC - C07K 16/3092, C07K 16/28, C07K 16/30

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8)- C07K 16/30, C07K 16/28 (2016.01)

CPC- C07K 16/3092, C07K 16/28, C07K 16/30

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched (keyword search, terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (USPT, PGPB, EPAB, JPAB), Google Patents/Scholar

Search Terms Used: MUC1, antibody, extracellular, epitope, PSMGFR, NEM7, NEM6, human, humanized, MN-E6

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y -- A	US 2004/0057952 A1 (PAYNE et al.) 25 March 2004 (25.03.2004) Claim 1, 4, 10, para [0073], [0074], [0112], SEQ 1	1-4, 33-37, 67 ----- 5-9, 25, 40 ----- 26, 41
X -- Y -- A	WO 2014/028668 A2 (MINERVA BIOTECHNOLOGIES CORPORATION) 20 February 2014 (20.02.2014) para [0012], [0049], [0057], [0059], [0060], [0078], [0080], Fig. 27, SEQ ID NOs: 19, 21	38, 39, 76 ----- 5-9, 25, 40, (82-85)/76 ----- 26, 41, 77, (82-85)/77
Y	US 2011/0318757 A1 (Behrens et al.) 29 December 2011 (29.12.2011) abstract, para [0003]	(82-85)/76
Y	US 2013/0177555 A1 (Wilkinson et al.) 11 July 2013 (11.07.2013) para [0005], [0010]	(83-85)/76
Y	US 7,538,088 B2 to (Anderson et al.) 26 May 2009 (26.05.2009) SEQ ID NO: 26	85/76
A	US 2011/0165167 A1 (Pullen) 07 July 2011 (07.07.2011) abstract, para [0027], SEQ ID NO: 22	26

☒ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

25 June 2016 (25.06.2016)

Date of mailing of the international search report

26 JUL 2016

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/17422

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2009/0299039 A1 (Kataoka et al.) 03 December 2009 (03.12.2009) abstract, para [0017], SEQ ID NO: 89	26
A	US 2010/0150918 A1 (Kufer et al.) 17 June 2010 (17.06.2010) SEQ ID NO: 1582	41

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/17422

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 47, 69, 70, 72
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-44, 67, 76-80, 82-85, directed to a human or humanized anti-MUC1* antibody or scFv, that binds to a region on extracellular domain of MUC1 isoform or cleavage product that is devoid of the tandem repeat domains. The anti-MUC1* antibody will be searched to the extent that the humanized antibody is derived from the first named mouse monoclonal MN-E6 antibody (claims 5-7, 77) comprising humanized IgG2 heavy chain SEQ ID NO: 53 and Kappa light chain SEQ ID NO: 108 (claim 25); and scFv of SEQ ID NO: 233 (claim 41). It is believed that claims 1-9, 25, 26, 33-41, 67, 76-77, (82-85)(in part) encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 53, 108, 233. Additional human or humanized anti-MUC1* antibodies will be searched upon the payment of additional fees.

--See extra sheet--

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-9, 25, 26, 33-41, 67, 76-77, (82-85)(in part) limited to SEQ ID NOs: 53, 108, 233.

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/17422

Continuation of:

Box NO III. Observations where unity of invention is lacking

Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be human or humanized anti-MUC1* antibody derived from mouse monoclonal MN-C2 antibody (claims 10-12, 78) comprising humanized IgG1 heavy chain SEQ ID NO: 159 and Lambda light chain SEQ ID NO: 219 (claim 25); and scFv of SEQ ID NO: 239 (claim 42) (claims 1-4, 10-14, 27, 28, 33-40, 42, 67, 76, 78, (82-85)/(in part)).

Group II+: Claims 45-46, 48-60, directed to a chimeric antigen receptor (CAR) comprising a scFv that binds to the extracellular domain of a MUC1 that is devoid of tandem repeats. Group II+ will be searched upon payment of additional fees. The CAR may be searched, for example, to the extent that the CAR encompasses MN-E6 scFv SEQ ID NO: 233 (claim 49); CARMN-E6 CD3z SEQ ID NO: 295 (claim 51); and wherein the extracellular domain unit recognizes peptide of SEQ ID NO: 2 (claim 58); for an additional fee and election as such. It is believed that claims 45-46, 48-58 read on this exemplary invention. Additional scFv, CAR, and peptide will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected CAR. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be MN-C2 scFv SEQ ID NO: 239; CARMN-C2 CD3z SEQ ID NO: 607; and the peptide is SEQ ID NO: 7 (claims 45-46, 48-57, 59-60).

Group III: claims 61-66, drawn to a composition comprising at least two CARs with different extracellular domain units transfected into the same cell.

Group IV: claim 68, drawn to a method of screening a library of antibodies.

Group V: claims 71, 81, 93-97, drawn to a method for treating a disease in a subject.

Group VI: claims 73-75, drawn to a method of isolating stem cell.

Group VII: claims 86-90, drawn to a fusion polypeptide comprising at least two different scFv sequences.

Group VIII: claims 91-92, drawn to a method of detecting presence of a cell that expresses MUC1* aberrantly.

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

Special Technical Features

Group I+ includes the special technical feature of a composition comprising a human or a humanized antibody or scFv, not required by Groups II+, III-VIII.

Group II+ includes the special technical feature of a composition comprising a chimeric antigen receptor, not required by Groups I+ and III-VIII.

Group III includes the special technical feature of a composition comprising a cell with at least two CARs, not required by Groups I+, II+, IV-VIII.

Group IV includes the special technical feature of a method of screening a library of antibodies, not required by Groups I+, II+, III, V-VIII.

Group V includes the special technical feature of a method for treating a disease, not required by Groups I+, II+, III, IV, VI-VIII.

Group VI includes the special technical feature of a method of isolating stem cell, not required by Groups I+, II+, III-V, VII, VIII.

Group VII includes the special technical feature of a composition comprising a fusion polypeptide comprising at least two different scFv sequences, not required by Groups I+, II+, III-VI, VIII.

Group VIII includes the special technical feature of a method of detecting presence of a cell that expresses MUC1* aberrantly, not required by Groups I+, II+, III-VII.

Another special technical feature of the inventions listed as Group I+ is the specific human or humanized antibody or scFv amino acid sequences, recited therein. Each of the inventions of Group I+ requires a unique set of sequences comprising IgG heavy chain, light chain and scFv, not required by the other inventions.

- Please see extra sheet for continuation -

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/17422

Continuation of:

Box NO III. Observations where unity of invention is lacking

Another special technical feature of the inventions listed as Group II+ is the specific scFv, CAR and peptide sequences, recited therein. Each of the inventions of Group II+ requires a unique set of sequences comprising scFv, CAR and peptide, not required by the other inventions.

Common Technical Features

The inventions of Groups I+, II+, III-VIII share the technical feature of MUC1* and anti-MUC1* antibody or scFv.

The inventions of Groups I+, II+, IV, V, VII-VIII share the technical feature of anti-MUC1* that binds to a region on extracellular domain of MUC1 isoform or cleavage product that is devoid of the tandem repeat domains

The inventions of Groups II+, III, V share the technical feature of a chimeric antigen receptor. Groups II+ and V further share MUC1* targeting CAR.

The inventions of Groups I+, V share the technical feature of claim 76.

The inventions of Groups I+, VIII share the technical feature of claim 82.

The inventions of Groups I+ share the technical feature of claims 1, 2, 38, 76, 82, IgG heavy chain and kappa or lambda light chain.

The inventions of Groups II+ share the technical feature of claims 45, 52, 57.

However, these shared technical features do not represent a contribution over prior art in view of US 2004/0057952 A1 to PAYNE et al. (hereinafter 'Payne'). Payne teaches (instant claims 1-2) a human or humanized anti-MUC1 *antibody or antibody fragment or antibody-like protein that binds to a region on extracellular domain of MUC 1 isoform or cleavage product that is devoid of the tandem repeat domains (Claim 1, An isolated monoclonal antibody that specifically binds to an epitope of a non-shed extracellular portion of a shed antigen; para [0073]. Humanized forms of the antibodies are made by substituting the complementarity determining regions of, for example, a mouse antibody, into a human framework domain.), which specifically binds to (iii) a peptide having amino acid sequence of SNIKFRPGSVVQLTLAFREGTINVHDVETQFNQYKTEAASRY (SEQ ID NO:620) (Claim 11, wherein said antibody binds to at least one peptide selected from the group consisting of: a) QLTLAFREGTINVHDVETQFN (SEQ ID NO:8), NOTE, SEQ ID NO: 8 is 100% match to amino acid residues 13-33 of SEQ ID N: 620 of instant claim).

Payne teaches (instant claim 38) a single chain variable fragment (scFv) comprising a heavy and light chain variable regions connected via a linker (para [0074]. These fragments contain at least one fragment of an antibody variable heavy-chain amino acid sequence (VH) tethered to at least one fragment of an antibody variable light-chain sequence (VL) with or without one or more interconnecting linkers.), further comprising CDRs of antibodies (para [0075]. Single-chain antibody fragments contain amino acid sequences having at least one of the variable or complementarity determining regions (CDR's) of the whole antibodies.) that bind to MUC1 * extracellular domain (Claims 1, 11).

Payne teaches (instant claim 76) a scFv comprising variable domain fragments derived from an antibody that binds to a extracellular domain of MUC1 isoform or cleavage product that is devoid of the tandem repeat domains (Claims 1, 11, para [0074], [0075]).

Payne teaches (instant claim 45) a scFv or a humanized variable region that binds to the extracellular domain of a MUC1 that is devoid of tandem repeats, a linker molecule (Claims 1, 11, para [0074], [0075]), but does not specifically teach a chimeric antigen receptor (CAR) comprising said MUC1 targeting scFv. WO 2015/009740 A2 (CELL SIGNALING TECHNOLOGY, INC. et al.) (hereinafter 'CST') teaches a chimeric antigen receptor (CAR) comprising a MUC1 targeting scFv (abstract, anti-MUC1 binding agents (e.g., antibodies and chimeric antigen receptors); pg 35, In 12-32, Examples of chimeric antigen receptors include one or more antibody binding domains (e.g., an scFv that includes one or more sequences disclosed herein) linked by a transmembrane domain to at least one T cell receptor (TCR) signaling domain (e.g., a TCR zeta domain) and, optionally, one or more costimulatory signaling domains (e.g., a CD28, CD37 (4-1BB), or CD134 (OX40) signaling domain).). One of ordinary skill in the art would have prepared a chimeric antigen receptor of CST comprising the scFv as taught by Payne, which would have directed T cell to cancer cell expressing MUC1*.

Payne, in view of CST, teaches (instant claim 52) a cell comprising a CAR with an extracellular domain that binds to MUC1* transfected or transduced cell (Payne, Claims 1, 11, para [0074], [0075]; CST, abstract, pg 35, In 12-32, In some embodiments, T cells expressing such a chimeric antigen receptor can be produced and used for treatment of a tumor that expresses MUC1.).

Payne, in view of CST, teaches (instant claim 57) a CAR molecule in which the extracellular domain unit recognizes a peptide (Payne, Claims 1, 11, para [0074], [0075]; CST, abstract, pg 35, In 12-32).

CST further teaches that anti-MUC1 antibody comprising IgG (pg 26, In 6-13). In addition, it is well known to one of ordinary skill in the art that antibody comprises a heavy chain and a light chain (kappa or lambda).

Payne does not specifically teaches (instant claim 82) a scFv-Fc construct. US 2011/0318757 A1 to Behrens et al. (hereinafter 'Behrens') teaches anti-MUC1 scFv-Fc construct (abstract, para [0003], one or more natural or synthetic antibody domains, e.g. CH1, CH2, CH3, scFv-Fc and other synthetic antibody fusion proteins.).

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

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